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REVIEW

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Metabolic checkpoints and novel approaches for immunotherapy against cancer

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Abstract

While immunotherapy has achieved unprecedented success in conquering cancer, the majority of patients develop primary or acquired resistance to immunotherapy, largely in part due to the complicated metabolic networks in the tumor microenvironment. The microenvironmental metabolic networks are woven by a set of metabolic checkpoints, and accumulating evidence indicates that these metabolic checkpoints orchestrate antitumor immunity and immunotherapy. Metabolic checkpoints can regulate T cell development, differentiation and function, orchestrate metabolic competition between tumor cells and infiltrating T cells, and respond to the metabolic stress imposed on the infiltrating T cells. Furthermore, metabolic checkpoints and pathways can modulate the expression profiles of immune checkpoint receptors and ligands and vice versa. Therefore, repurposing interventions targeting metabolic checkpoints might synergize with immunotherapy, and promising approaches to reprogram the metabolic environment are much more warranted. In this review, we summarize recent researches on the metabolic checkpoints and discuss how these metabolic checkpoints regulate antitumor immunity and the promising approaches to modulate these metabolic checkpoints in the combination therapy. A comprehensive and objective understanding of the metabolic checkpoints might help the research and development of novel approaches to antitumor immunotherapy.

KEYWORDS
antitumor immunotherapy, immune checkpoints, metabolic checkpoints, T cells

Abbreviations: ACT, adoptive cell transfer; AGK, acylglycerol kinase; AhR, aryl hydrocarbon receptor; AML, acute myeloid leukemia; ATGL, adipose triglyceride lipase; BH4, tetrahydrobiopterin; CAFs, cancer-associated fibroblasts; CAR, chimeric antigen receptor; CCR7, chemokine (C-C motif) receptor 7; COX, cyclooxygenase; CPT1A, carnitine palmitoyltransferase 1A; CRC, colorectal cancer; DUSP2, dual specificity phosphatase 2; EP4, prostaglandin E_2 receptor 4; ER, endoplasmic reticulum; GITR, glucocorticoid-induced TNFR-related protein; Glut1, glucose transporter 1; H3K9, histone 3 lysine 9; HIF-1α, hypoxia-inducible factor-1α; HK2, hexokinase 2; HNSCC, head and neck squamous cell carcinoma; ICDs, intracellular signaling domains; ICIs, immune checkpoint inhibitors; IDH1, isocitrate dehydrogenase 1; IDO, indoleamine 2,3-dioxygenase; Kyn, kynurenine; LAG3, lymphocyte activation gene 3 protein; LC-FAO, long-chain fatty acid oxidation; LDHA, lactate dehydrogenase A; MHC, major histocompatibility complex; MSI-H/dMMR, microsatellite instability-high and/or mismatch repair-deficient; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cells; NKR2B4, natural killer cell receptor 2B4; NSCLC, non-small-cell lung cancer; OXPHOS, oxidative phosphorylation; PAMP, pathogen associated molecular pattern; PAT4, proton-coupled amino acid transporter 4; PCK1, PEP carboxykinase 1; PD-1, programmed cell death 1; PDK1, 3-phosphoinositide-dependent protein kinase-1; PEP, phosphoenolpyruvate; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; PGE_2, prostaglandin E_2; PI3K, phosphoinositide 3-kinase; PIKK, PI3K-related kinase; PTPN2, protein tyrosine phosphatase non-receptor type 2; PUFAs, polyunsaturated fatty acids; RCC, renal cell carcinoma; REDD1, regulated in development and DNA damage response 1; ROS, reactive oxygen species; SERCA, sarco/ER Ca^{2+}-ATPase; SLC3A2, solute carrier family 3 member 2; SLC7A11, solute carrier family 7 member 11; SLC7A8, solute carrier family 7 member 8; T_{CM}, central memory T cells; TCR, T cell receptor; TDO, tryptophan 2,3-dioxygenase; T_{EM}, effector memory T cells; T_H1, $CD4^+$ T helper 1; T_H17, $CD4^+$ T helper 17; T_H2, $CD4^+$ T helper 2; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TILs, tumor infiltrating lymphocytes; TIM3, T cell immunoglobulin mucin receptor 3; TLR8, toll-like receptor 8; TME, tumor microenvironment; TRCs, tumor-repopulating cells; Tregs, regulatory T cells.

1 | INTRODUCTION

Antitumor immunotherapy represents a paradigm-shifting milestone which symbolizes the overwhelming power of unleashing the immune system against tumor. Despite its unprecedented durable response rates, the majority of patients are not responsive to antitumor immunotherapy and many responders relapse eventually.[1,2] Although mechanisms of primary or acquired resistance remain poorly understood, accumulating evidence demonstrates that metabolic checkpoints orchestrate antitumor immunity and immunotherapy.[3,4] Metabolic checkpoints refer to a series of cellular metabolic molecular switches which ensure the timely and accurate conversion of the metabolic status to another proper one, which dynamically orchestrate the metabolic status to regulate immune homeostasis and responses.[5-7] Recent studies show that metabolic checkpoints regulate development, differentiation and function of T cells, and immune cells compete with tumor cells for nutrients in the tumor microenvironment (TME).[8,9] Metabolites in the TME can exert metabolic stress on infiltrating T cells, which contributes to local suppression and immune evasion.[10,11] Furthermore, metabolic checkpoints and pathways can modulate the expression profiles of immune checkpoint receptors and ligands and vice versa.[12,13] Therefore, repurposing of interventions targeting the microenvironmental metabolism might synergistically enhance antitumor immunotherapy by reshaping the metabolic networks within the TME. Besides, interventions aiming at the metabolic checkpoints and circuits that may hinder antitumor immunity or exacerbate immune evasion have been developed, and a large number of clinical trials of metabolic agents combined with immune checkpoint inhibitors (ICIs) are ongoing.[14] In this review, we expound how these metabolic networks regulate antitumor immunity and the potential strategies to reinvigorate immune responses by targeting these metabolic checkpoints and pathways in immunotherapy. We also discuss presumptive combination therapies that might be transferred from bench to bedside in order to better unbridle the brake of adoptive cell therapies by orchestrating T cell metabolism.

2 | METABOLIC CHECKPOINTS IN T CELL DEVELOPMENT, DIFFERENTIATION AND FUNCTION

Metabolism drives T cell development, differentiation and function throughout its life span.[15] Cellular metabolic processes can be dynamically engaged, converted or repressed in the charge of metabolic checkpoints as the demands for energy and substrates soar or plunge, characterized by unique metabolic profiles of each differentiation state and lineage commitment.[16,17] As is acknowledged, the most efficient metabolic way to generate energy is mitochondria-dependent oxidative phosphorylation (OXPHOS), an enzymatic process that produces ATP mediated by a proton and PH gradient across the mitochondrial membrane.[18] The metabolic needs for quiescent or naïve T cells are low, with these cells relying mainly on low levels of OXPHOS of glucose-derived pyruvate or fatty acids to maintain basal physiological functions.[19] Upon activation by encounter with antigen, TCR engagement, interaction with co-stimulatory ligands and cytokine signaling, T cells rapidly alter their metabolic signaling cascades to prioritize proliferation and effector function (eg, production of IL-2, IFN-γ, etc).[20] Metabolically, these activated T cells undergo a shift to aerobic glycolysis (often termed the Warburg Effect in tumor cells), a rather inefficient process with a net gain of 2 ATP molecules for fuel, which requires incremental glucose uptake, provides pyruvate for the TCA cycle and supports anabolic metabolism, thus facilitating the generation of biosynthetic intermediates and cellular proliferation.[19] Nevertheless, OXPHOS still continues and generates reactive oxygen species (ROS) to enhance antigen-induced activation signaling by regulating the nuclear factor of activated T cells (NFAT).[21]

The metabolic switch upon T cell activation is orchestrated by a series of powerful metabolic checkpoints, which are a series of cellular metabolic molecular switches which ensure the timely and accurate conversion of the metabolic status to another proper one (Table 1). Upon antigen-induced activation, cellular glycolysis is boosted by upregulation of nutrient transporters (eg, the glucose transporter glucose transporter 1 [Glut1]) to enhance glucose influx and activation of the mammalian target of rapamycin (mTOR) complex.[3,22] It is reported that Glut1 on the cell membrane can be upregulated by phosphoinositide 3-kinase (PI3K) activation through co-stimulation to augment glucose influx.[23] Notably, concomitant with elevated expression of glucose transporters is upregulated expression of crucial glycolytic enzymes.[8] In addition, this metabolic shift to glycolysis can also be intensified by the mTOR complex, as it controls the expressions of two key metabolic transcription factors c-Myc and the hypoxia-inducible factor-1α (HIF-1α). mTOR has been reported to master the development and differentiation of almost all of the effector subsets, especially $CD4^+$ T helper 1 (T_H1) and T_H17 effector cells, whereas with the exception of peripheral regulatory T cells (Tregs) which utilize OXPHOS and oxidation of fatty acids.[24,25] mTOR is a highly conserved serine/threonine kinase which is a member of the PI3K-related kinase (PIKK) family, integrating cues that guide the sequel of T cell receptor (TCR) engagement from the environment (eg, co-stimulation via CD28, etc) to coordinate multiple cellular activities such as proliferation, metabolism, mobility and macromolecular synthesis.[26] Antigen recognition via the TCR and CD28 engagement by co-stimulatory receptors (ie, CD80 and CD86) activates PI3K, recruits 3-phosphoinositide-dependent protein kinase-1 (PDK1) and AKT, and activates mTOR. The activation of PI3K/AKT/mTOR pathway contributes to the upregulation of surface Glut1 to enhance glucose influx. mTOR is composed of two distinct complexes, namely mTORC1 and mTORC2. The mTORC1 pathway is not only activated by upstream PI3K signaling, but also sensitive to nutrient signaling such as amino acids and fatty acids. Arginine and leucine can prime, activate and sustain TCR-induced mTORC1 in Tregs, accompanied by dynamic lysosomal localization of the mTOR complex.[27] The differentiation of $CD4^+$ T cells is regulated by ω-3 polyunsaturated fatty acids (ω-3 PUFAs) eicosanoid derivatives through mTORC1 inhibition.[28] Of note, Glut1 is only necessary for the differentiation of T_H1, T_H2 and T_H17 cells, but not Tregs or $CD8^+$ T cells.[29] And comparable levels of Glut1 and Glut3 in $CD8^+$ T cells might account for this phenomenon.[30] Augmenting glycolytic flux drives $CD8^+$ T cells towards a terminally

TABLE 1 Metabolic checkpoints in the TME

Metabolic TME	Metabolic initiator	Metabolic transmitter	Metabolic effector
Nutrient-sparse			
Low glucose	Glucose↓	Glut, MCT4, HK2, PCK1, Enolase 1	Glycolysis↓, AMP:ATP ratio↑
Low amino acids	Glutamine and its metabolites		
	Glutamine↓	GLS, SLC1A5, SLC38A1, SLC38A2	T_H1↓, CTLs↓, T_H17↑
	Glutamate (endogenous)	GLUD1	Convert to αKG and enter TCA cycle
	Glutamate (exogenous)	Glutamate receptors	Costimulatory effects, high concentration can be opposite
	Leucine	SLC7A5, mTORC1 signaling	Effector↓, cytokine-directed differentiation of T_H1, T_H17 and CTLs↓, T_{CM} cells↓
	Arginine↓	Arginase-1 (Mostly expressed in immunosuppressive cells)	T cell activation↓, antitumor immune response↓
Metabolite-excessive			
High lactate	Aerobic glycolysis	CD155-TIGIT signaling, NFAT	Proliferation↓, survival↓, cytotoxicity↓, cytokine production↓
High cholesterol	Cholesterol↑	ACAT1, XBP1	Needs further investigation
High IDO,TDO	Tryptophan↓, kynurenine↑	SLC7A8, PAT4, BH4, AhR	Treg↑, PD-1↑
High potassium	Necrosis↑	PP2A, AKT, ACSS1	Effector↓, stemness↑
High H^+	Aerobic glycolysis	MAPK signaling, NFAT	Proliferation↓, survival↓, cytotoxicity↓, cytokine production↓
Low-oxygen			
High *S*-2-HG	Hypoxic exposure	HIF-1α	Cytokine production↓, cytolytic capacity↓, proliferation↑, long-term survival↑, antitumor immune response↑
High adenosine	CD39, CD73	$A_{2A}R$, $A_{2B}R$	Effector↓, proliferation↓

Abbreviations: $A_{2A}R$, adenosine A_{2A} receptor; $A_{2B}R$, adenosine A_{2B} receptor; ACAT1, acetyl-CoA acetyltransferase 1; ACSS1, acyl-CoA synthetase short-chain family member 1; AhR, aryl hydrocarbon receptor; BH4, tetrahydrobiopterin; CTLs, $CD8^+$ cytotoxic T lymphocytes; GLS, glutaminase 1; GLUD1, glutamate dehydrogenase 1; Glut, glucose transporter; HK2, hexokinase 2; MCT4, monocarboxylate transporter 4; NFAT, nuclear factor of activated T cells; PAT4, proton-assisted amino-acid transporter 4; PCK1, phosphoenolpyruvate carboxykinase 1; PP2A, protein phosphatase 2A; *S*-2-HG, (*S*)-2-hydroxyglutarate; SLC1A5, solute carrier family 1 member 5; SLC38A1, solute carrier family 38 member 1; SLC38A2, solute carrier family 38 member 2; SLC7A5, solute carrier family 7 member 5; SLC7A8, solute carrier family 7 member 8; TCA cycle, tricarboxylic acid cycle; T_{CM} cells, central memory T cells; T_H1, $CD4^+$ T helper 1; T_H17, $CD4^+$ T helper 17; XBP1, X-box binding protein 1; αKG, α-ketoglutarate.

differentiated state, indicating that differentiated $CD8^+$ T cells might rely more on glycolysis.[31] Aerobic glycolysis augments effector T cell inflammatory immune responses, with lactate dehydrogenase A (LDHA) maintaining high levels of acetyl-coenzyme A to foster histone 3 lysine 9 (H3K9) acetylation at the *Ifng* promoter and enhancer, thus promoting its transcription.[32] Also this glycolytic switch alleviates the blockade of *Ifng* mRNA translation into protein by the binding of the glycolytic enzyme GAPDH to AU-rich elements within the 3′ UTR.[33] Therefore, glycolytic dysfunction in T cells might result in immune instability. Loss of immune quiescence and consolidation of effector function can be derived from imposing intensified glycolysis through transgenic expression of Glut1 or genetic modulation of the mTOR pathway.[34,35] Contrariwise, disengaging anabolic metabolic processes in the activated T cells with the genetic deletion of Glut1, restricted uptake or synthesis of fatty acids can lead to compromised effector functions such as decreased T cell numbers and inflammatory cytokine generation.[29,36,37] Treg transcription factor Foxp3 can enhance OXPHOS and nicotinamide adenine dinucleotide oxidation by repressing PI3K-AKT-mTORC1-mediated glycolysis to adapt to the environment with low glucose and high lactate.[38,39] Acylglycerol kinase (AGK) unleashes $CD8^+$ T cell glycolysis through interacting with PTEN and activating PI3K-mTOR signaling.[40] Moreover, inhibiting mTORC1 at the terminal stage of the $CD8^+$ T effector cells facilitates the switch to memory cells that rely more on mitochondrial oxidation after glucose or stimuli withdraw.[35] The transcription factors c-Myc and HIF-1α are also in the charge of the mTOR complex and coordinately regulate the anabolism and effector function of T cells. Generation of effector cells depends on the asymmetric distribution of c-Myc to the proximal daughter cells, which causes asymmetric allocation of metabolic necessities including amino acids and amino acid transporters.[41] In activated T cells, c-Myc promotes glutaminolysis and its connection with polyamine biosynthesis to fulfill the bioenergetic demand of proliferation and function.[42] HIF-1α is a crucial regulator of glucose metabolism in both $CD4^+$ and $CD8^+$ T cells and drives T_H1 and T_H17 differentiation.[43,44] Deletion of HIF-1α leads to loss of effector function in $CD8^+$ T cells.[13] Under hypoxia, HIF-1α also acts as a metabolic switch between glycolytic-driven migration and OXPHOS-driven immunosuppression in Tregs.[45] Blockade of glycolytic flux promotes $CD8^+$ T cell transformation from

effector cells to memory cells.[31] Interestingly, durative HIF-1α activity with constitutive glycolytic flux in virus-specific T cells facilitates the differentiation of effector memory T (T_{EM}) cells, which harbor less mitochondrial respiratory capacity than central memory T (T_{CM}) cells.[46,47] Taken together, the coupling of these metabolic checkpoints with specific metabolic needs of each lineage contributes to the T cell-mediated immune homeostasis.

3 | METABOLIC COMPETITION BETWEEN TUMOR CELLS AND INFILTRATING T CELLS

Tumor infiltrating lymphocytes (TILs) are integral elements of the TME and have been proven to correlate with prognosis and response to therapy.[48,49] Highly active metabolic reactions that are characteristic of tumor cells can impose effect on the immune contexture within the TME. Active metabolic pathways and derangements within the TME contribute to a nutrient-sparse, metabolite-excessive and hypoxic environment where tumor cells and TILs are in fierce competition for limited availability for nutrients and energies such as glucose, amino acids, fatty acids, oxygen and so on. Furthermore, these two counterparts share considerable similarities in metabolic reactions and pathways which direct the malignant proliferation of tumor cells and the clonal expansion and cytokine production of $CD8^+$ effector T cells.[9,50,51] Glucose uptake and immune responses of $CD4^+$ T cells have been proven to be in inverse proportion to glycolytic activity of tumor cells. Moreover, transcriptomic analyses of patients with melanoma from The Cancer Genome Atlas indicate that the mRNA amounts of effector T cell genes (eg, *Ifng* and *Cd40lg*) inversely correlate with those of markers of glycolysis (eg, *Hk2*).[52] Various metabolites accumulate in the TME, show toxic effects and impair antitumor responses of TILs, such as kynurenine, adenosine, potassium, ornithine and ROS. Therefore, TILs become entangled into teeming metabolic networks intertwined within the hostile microenvironment and are compelled to face relentless metabolic competition.

Immune contexture is comprised of various immune cell types, in which effector cells dominate in antitumor immunity. These effector cells exhibiting high proliferation capacities require both vigorous bioenergetic catabolism and concomitant anabolism.[3,50,53] Highly proliferating or expanding cells increase glucose utilization, redirect amino acids such as glycine, arginine and serine to anaplerosis, enhance cholesterol metabolism and acetyl-CoA production from acetate or fatty acids.[51,54-56] Apart from bioenergetic catabolism, the competition for glucose is rather ruthless, as tumor cells largely deprive T cells of these substrates and generate immunosuppressive metabolites. Tumor cells triumph over T cells for glucose, thereby impairing effector function and exacerbating immune evasion. Although the finite glucose pool limits aerobic glycolysis in tumor-infiltrating T cells, the glycolytic metabolite phosphoenolpyruvate (PEP) plays as a metabolic checkpoint, which sustains TCR-mediated Ca^{2+}-NFAT signaling and maintains effector function by inhibiting sarco/ER Ca^{2+}-ATPase (SERCA) activity. Increasing PEP production by overexpressing PEP carboxykinase 1 (PCK1) in T cells reprograms metabolism, restricts tumor growth and extends survival of tumor-bearing mice.[52] Contrarily, overexpressing the key glycolytic enzyme hexokinase 2 (HK2) in tumor cells destructs T cell-mediated immune surveillance. This converse observation demonstrates that ruthless metabolic competition exists between tumor cells and infiltrating T cells, thereby mastering the fate of the titanic struggle between these two counterparts. Moreover, restraining glucose blocks effector function in T cells through inhibiting IFN-γ generation at the posttranscriptional level.[9] In addition to the glycolytic metabolite like PEP, Enolase 1, a critical enzyme in the glycolytic pathway, has been found to inhibit glycolytic metabolism in $CD8^+$ TILs.[57] Another interesting study shows that glutamine deprivation by using antagonism induces divergent metabolic programs in tumor cells and effector T cells, with decreased glycolysis and oxidation in the former and increased oxidative metabolism in the latter. This microenvironmental divergence in metabolic plasticity might exploit glutamine metabolism as a potent metabolic checkpoint for antitumor immunity and immunotherapy.[58] In addition, the enzyme co-factor tetrahydrobiopterin (BH4) can rescue decreased T cell proliferation abrogated by a tryptophan metabolite kynurenine (Kyn).[59] Besides the metabolic enzymes and metabolites that directly regulate metabolism, some critical microRNAs and epigenetic-modifying enzymes are also involved in the metabolism of T cells. Ovarian cancer cells impose glucose restriction on T cells to constrain expression of the methyltransferase EZH2 to epigenetically decrease cytokine generation and curtail survival.[60]

Opposite to effector T cells that require high demand of nutrients to expand rapidly, other T cell subsets might be less vulnerable to metabolic competition for the inadequate nutrients or energy within the TME. For instance, restrained access to glucose and glutamine dampens $CD8^+$ effector T cell expansion but facilitates Treg expansion to promote immune suppression.[38,61] $CD8^+$ memory T cells rely more on metabolic demands for efficient long-chain fatty acid oxidation (LC-FAO) via the enzyme CPT1A at least in large part, as this cell population needs to persist lastingly by homeostatic expansion and convert to an effector phenotype faster than naïve cells vigorously.[62,63] Additionally, Tregs adopt lipid oxidation rather than glycolysis for their expansion at least in part, irrespective of Glut1 expression.[29] Furthermore, toll-like receptor 8 (TLR8) signaling selectively represses glucose uptake and glycolysis in human Tregs, which contributes to reversal of immunosuppression.[64] Collectively, different tumor-infiltrating T cell subsets exhibit distinct metabolic profiles to outcompete in the survival struggle with tumor cells or their counterparts.

4 | METABOLIC STRESS IN THE TME AND ITS IMPACT ON ANTITUMOR IMMUNITY

Abominable conditions in the TME can impose a variety of metabolic stress on infiltrating immune cells, which contributes to local immunosuppression and immune evasion.[65] Tumor cells destruct immune surveillance and deploy immune evasion through forming a hostile metabolic environment characterized by nutrient pillage

and hazardous metabolite accumulation, thus perturbing T cell metabolic homeostasis and effector function.[66] Malignant ascites fluid of ovarian cancer patients inhibits glucose uptake and dampens N-linked protein glycosylation in T cells, thus resulting in metabolic stress cascade IRE1α-XBP1 activation to restrain glutamine influx and mitochondrial respiration.[67] Contrary to the deprived glucose in the TME, other metabolic substrates such as cholesterol and some fatty acids might be redundant in part. In addition to glycometabolic stress, environmental cholesterol also imposes pressure on the effector function of infiltrating T cells. Both tumor tissues enriched with cholesterol and cholesterol content in tumor-infiltrating $CD8^+$ T cells are found to be positively and progressively correlated with upregulation of immune checkpoints including programmed cell death 1 (PD-1), natural killer cell receptor 2B4 (NKR2B4, 2B4 and CD244), T cell immunoglobulin mucin receptor 3 (TIM3) and lymphocyte activation gene 3 protein (LAG3). Cholesterol can induce endoplasmic reticulum (ER) stress in $CD8^+$ T cells via the ER stress sensor XBP1, resulting in T cell exhaustion and tumor immune evasion.[12] In line with this notion, enhancing fatty acid catabolism in $CD8^+$ T cells can preserve $CD8^+$ TIL effector function and inhibit tumor progression.[68]

Immune cells in turn affect tumor cells by imposing metabolic stress. Activated $CD8^+$ T cells can enhance ferroptosis-specific lipid peroxidation in tumor cells, which contributes to the antitumor efficacy of immunotherapy. IFN-γ produced by $CD8^+$ T cells reduces the expression of solute carrier family 3 member 2 (SLC3A2) and SLC7A11, two key subunits of the glutamate-cystine antiporter system x_c^-, and thus dampens cysteine uptake by tumor cells. Impaired cysteine influx results in lipid peroxidation and ferroptosis in tumor cells.[69] In addition, IFN-γ produced by $CD8^+$ T cells also promotes release of high level of Kyn from tumor-repopulating cells (TRCs), with considerable Kyn transferred into adjacent $CD8^+$ T cells via the transporters SLC7A8 and proton-coupled amino acid transporter 4 (PAT4), as a consequence. The entry of Kyn from tumor cells induces aryl hydrocarbon receptor (AhR) activation and thereby upregulates PD-1 expression in $CD8^+$ T cells[70] (Figure 1). Therefore, the metabolic networks of immune cells and tumor cells interweave and influence each other.

Immunosuppressive cells become an important force that cannot be ignored in promoting tumor immune evasion, which can also produce metabolites to affect T cell effector function. Tregs can induce DNA damage in effector T cells via glucose deprivation

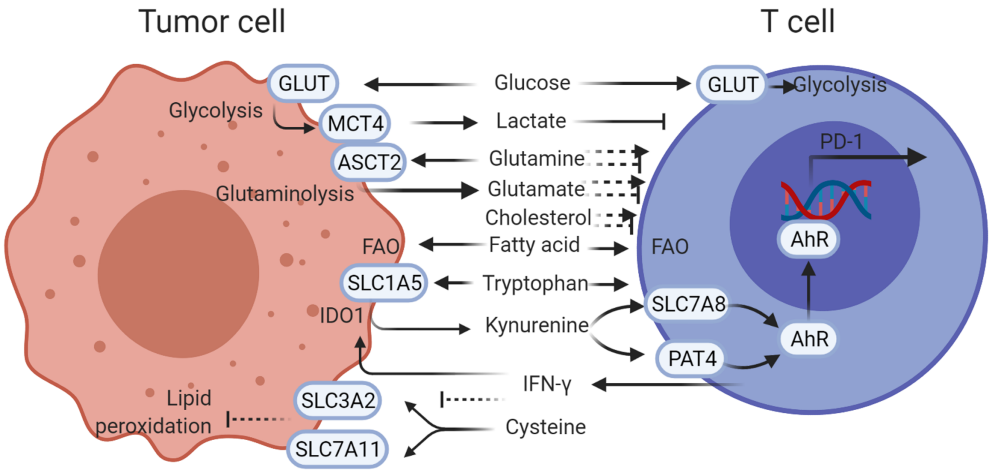

FIGURE 1 Metabolic communication and competition in the tumor microenvironment and its impact on antitumor immunity. Nutrient-mediated and metabolite-mediated communication occurs between tumor cells and infiltrating T cells. Tumor cells prefer aerobic glycolysis (also known as Warburg effect) rather than oxidative phosphorylation (OXPHOS), by constitutively taking up glucose and producing lactate for rapid obtainability of ATP and glycolytic intermediates to proliferate, regardless of the hypoxia in the tumor microenvironment (TME). Tumor cells outcompete the infiltrating T cells by shaping the glucose-deprived, lactate-enriched TME, thereby impairing T cell function and antitumor immunity. Glutamine and its metabolite glutamate stimulate T cell-mediated immune responses but aggravate T cell dysfunction or exhaustion in a context-dependent manner (indicated by dotted lines). The role of cholesterol in T cell activation is controversial. Accumulation of cholesterol in the plasma membrane of $CD8^+$ T cells has been reported to enhance TCR clustering and signaling, and facilitate antitumor immunity. Contrarily, another study shows that cholesterol can induce $CD8^+$ T cell exhaustion in the TME in an ER stress-XBP1-dependent manner. The availability and utilization of fatty acids are also attenuated by tumor cells in the TME. Enhancing fatty acid catabolism in $CD8^+$ T cells within a metabolically challenging TME increases the efficacy of antitumor immunity and immunotherapy. Kynurenine derived from the catabolism of tryptophan by indoleamine 2,3-dioxygenase (IDO) in tumor cells can be transferred into adjacent $CD8^+$ T cells, activate AhR and thereby upregulate PD-1 expression. And IFN-γ produced by $CD8^+$ T cells promotes kynurenine release by tumor cells. However, IFN-γ reduces the expression of two key subunits of the glutamate-cystine antiporter system x_c^-, and thus dampens cysteine uptake by tumor cells. Impaired cysteine influx results in lipid peroxidation and ferroptosis in tumor cells. AhR, aryl hydrocarbon receptor; ASCT2, alanine-serine-cysteine transporter 2; FAO, fatty acid β-oxidation; GLUT, glucose transporter; MCT4, monocarboxylate transporter 4; PAT4, proton-assisted amino-acid transporter 4; PD-1, programmed cell death 1; SLC1A5, solute carrier family 1 member 5; SLC3A2, solute carrier family 3 member 2; SLC7A11, solute carrier family 7 member 11; SLC7A8, solute carrier family 7 member 8. Created with BioRender.com [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Ongoing clinical trials of metabolic interventions combined with immune checkpoint inhibitors

Metabolic agent	Immune checkpoint inhibitor combination partner	Cancer types	Study phase	Recruitment status	ClinicalTrials.Gov reference
Glucose metabolism					
Metformin (a first-line oral hypoglycemic drug for the treatment of diabetes)	Nivolumab (anti-PD-1 antibody)	Unresectable or metastatic NSCLC	II	Active, not recruiting	NCT03048500
		Refractory metastatic microsatellite stable CRC	II	Active, not recruiting	NCT03800602
	Pembrolizumab (anti-PD-1 antibody)	Advanced-stage melanoma	I	Recruiting	NCT03311308
	Sintilimab (anti-PD-1 antibody)	SCLC	II	Recruiting	NCT03994744
Glutamine and glutamate metabolism					
CB-839 (GLS1 inhibitor)	Nivolumab (anti-PD-1 antibody)	Advanced-stage clear cell RCC, melanoma or NSCLC	I/II	Completed	NCT02771626
Trigriluzole	Nivolumab or Pembrolizumab (anti-PD-1 antibodies)	Metastatic or unresectable solid malignancies or lymphoma	II	Completed	NCT03229278
One-carbon metabolism					
Carboplatin, pemetrexed	Pembrolizumab (anti-PD-1 antibody)	NSCLC	III	Recruiting	NCT03793179
Oxaliplatin, leucovorin, 5-FU	Durvalumab (anti-PD-L1 antibody)	Localized unresectable esophageal cancer	II	Recruiting	NCT03777813
Arginine metabolism					
INCB001158 (ARG1 inhibitor)	Pembrolizumab (anti-PD-1 antibody)	Advanced-stage solid tumors	I/II	Active, not recruiting	NCT02903914
ADI-PEG 20 (pegylated arginine deiminase)	Pembrolizumab (anti-PD-1 antibody)	Advanced-stage solid tumors	I	Active, not recruiting	NCT03254732
Adenosine metabolism					
Oleclumab (MEDI9447; anti-CD73 antibody)	Durvalumab (anti-PD-L1 antibody) + paclitaxel and carboplatin	Inoperable locally recurrent or metastatic TNBC	I/II	Recruiting	NCT03616886
	Durvalumab (anti-PD-L1 antibody)	Ovarian cancer	II	Recruiting	NCT03267589
	Durvalumab (anti-PD-L1 antibody)	Advanced-stage solid tumors	I	Completed	NCT02503774
BMS-986179 (anti-CD73 antibody)	Nivolumab (anti-PD-1 antibody)	Advanced-stage solid tumors	I/II	Active, not recruiting	NCT02754141
NZV930 (SRF373; anti-CD73 antibody) ± PBF-509	Spartalizumab (anti-PD-1 antibody)	Advanced-stage solid tumors	I	Recruiting	NCT03549000
CPI-006 (anti-CD73 antibody)	Pembrolizumab (anti-PD-1 antibody)	Advanced-stage cancers	I	Recruiting	NCT03454451
CPI-444 (A_{2A}R antagonist)	Atezolizumab (anti-PD-L1 antibody)	Advanced-stage solid tumors	I	Recruiting	NCT02655822
PBF-509 (NIR178; A_{2A}R antagonist)	Spartalizumab (PDR001; anti-PD-1 antibody)	NSCLC	I/II	Active, not recruiting	NCT02403193
AZD4635 (A_{2A}R antagonist)	Durvalumab (anti-PD-L1 antibody)	Advanced-stage solid tumors	I	Active, not recruiting	NCT02740985
COX enzymes and/or PGE_2 metabolism					
Aspirin (COX1 and/or COX2 inhibitor) or celecoxib (COX2 inhibitor)	BAT1306 (anti-PD-1 antibody)	Advanced-stage MSI-H/dMMR cancers	II	Recruiting	NCT03638297
Aspirin (COX1 and/or COX2 inhibitor)	Pembrolizumab (anti-PD-1 antibody) + clopidogrel (P2Y12 inhibitor)	Recurrent or metastatic HNSCC	I	Recruiting	NCT03245489

TABLE 2 (Continued)

Metabolic agent	Immune checkpoint inhibitor combination partner	Cancer types	Study phase	Recruitment status	ClinicalTrials.Gov reference
Grapiprant (EP4 antagonist)	Pembrolizumab (anti-PD-1 antibody)	Advanced-stage or progressive microsatellite-stable CRC	I	Recruiting	NCT03658772
IDO metabolism					
Epacadostat (INCB024360; IDO1 inhibitor)	Pembrolizumab (anti-PD-1 antibody)	Advanced-stage imatinib-refractory gastrointestinal stromal tumors	II	Completed	NCT03291054
	Pembrolizumab (anti-PD-1 antibody) + INCAGN01876 (agonistic anti-GITR antibody)	Advanced or metastatic malignancies	I/II	Completed	NCT03277352
	Pembrolizumab (anti-PD-1 antibody)	Cisplatin-ineligible urothelial carcinoma	III	Completed	NCT03361865
Linrodostat (BMS-986205; IDO1 inhibitor)	Relatlimab (anti-LAG3 antibody) and Nivolumab (anti-PD-1 antibody)	Advanced-stage cancers	I/II	Recruiting	NCT03459222
	Nivolumab (anti-PD-1 antibody)	Advanced-stage cancers	I	Recruiting	NCT03335540
HTI-1090 (SHR9146; dual IDO1-TDO inhibitor)	Camrelizumab (SHR-1210; anti-PD-1 antibody) ± Apatinib (VEGFR TKI)	Advanced-stage solid tumors	I	Unknown	NCT03491631
Navoximod (GDC-0919; IDO1 inhibitor)	Atezolizumab (anti-PD-L1 antibody)	Locally advanced or metastatic solid tumors	I	Completed	NCT02471846
Indoximod (IDO1 and IDO2 inhibitor)	Ipilimumab (anti-CTLA-4 antibody),Nivolumab or Pembrolizumab (anti-PD-1 antibodies)	Metastatic melanoma	I/II	Completed	NCT02073123
Mutant IDH enzymes					
FT-2102 (inhibitor of mutant IDH1)	Nivolumab (anti-PD-1 antibody)	Advanced solid tumors and gliomas with an IDH1 mutation	Ib/II	Active, not recruiting	NCT03684811
Immunometabolism					
Imprime PGG (β-glucan)	Pembrolizumab (anti-PD-1 antibody)	Advanced-stage melanoma or metastatic TNBC	II	Completed	NCT02981303
		Metastatic NSCLC	I/II	Active, not recruiting	NCT03003468
	Atezolizumab (anti-PD-L1 antibody) + Bevacizumab (anti-VEGFA antibody)	Metastatic CRC	I/II	Recruiting	NCT03555149

Abbreviations: $A_{2A}R$, adenosine A_{2A} receptor; COX, cyclooxygenase; CRC, colorectal cancer; EP4, prostaglandin E_2 receptor 4; HNSCC, head and neck squamous cell carcinoma; IDH, isocitrate dehydrogenase; IDO, indoleamine 2,3-dioxygenase; LAG3, lymphocyte activation gene 3 protein; MSI-H/dMMR, microsatellite instability-high and/or mismatch repair-deficient; NSCLC, non-small-cell lung cancer; P2Y12, P2Y purinoceptor 12; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; PGE_2, prostaglandin E_2; RCC, renal cell carcinoma; TDO, tryptophan 2,3-dioxygenase; TNBC, triple-negative breast cancer; VEGFR TKI, VEGF receptor tyrosine kinase inhibitor.

during crosstalk, thereby leading to effector T-cell senescence.[71] Lactate released by glycolytic cancer-associated fibroblasts (CAFs) can decrease the percentage of the antitumoral T_H1 subset but increase that of Tregs.[72] Another powerful immunosuppressive population, neutrophils, has been found to deploy oxidative mitochondrial metabolism to maintain ROS generation and T cell suppression under the glucose-deprived condition.[73] Taken together, suppressive immune cells also impose metabolic stress on antitumoral immune cells.

In addition to direct or indirect "education" which changes the functional status of immune cells, microenvironmental metabolism also regulates the migration or recruitment of immune cells, thereby altering the inflamed niche to orchestrate tumor progression. For instance, abrogating glycolysis dampens maintenance of elongated morphology, chemokine (C-C motif) receptor 7 (CCR7) oligomerization and dendritic cell migration to draining lymph nodes.[74] Taken together, the complex networks woven by a set of metabolic checkpoints orchestrate metabolic stress between tumor cells and

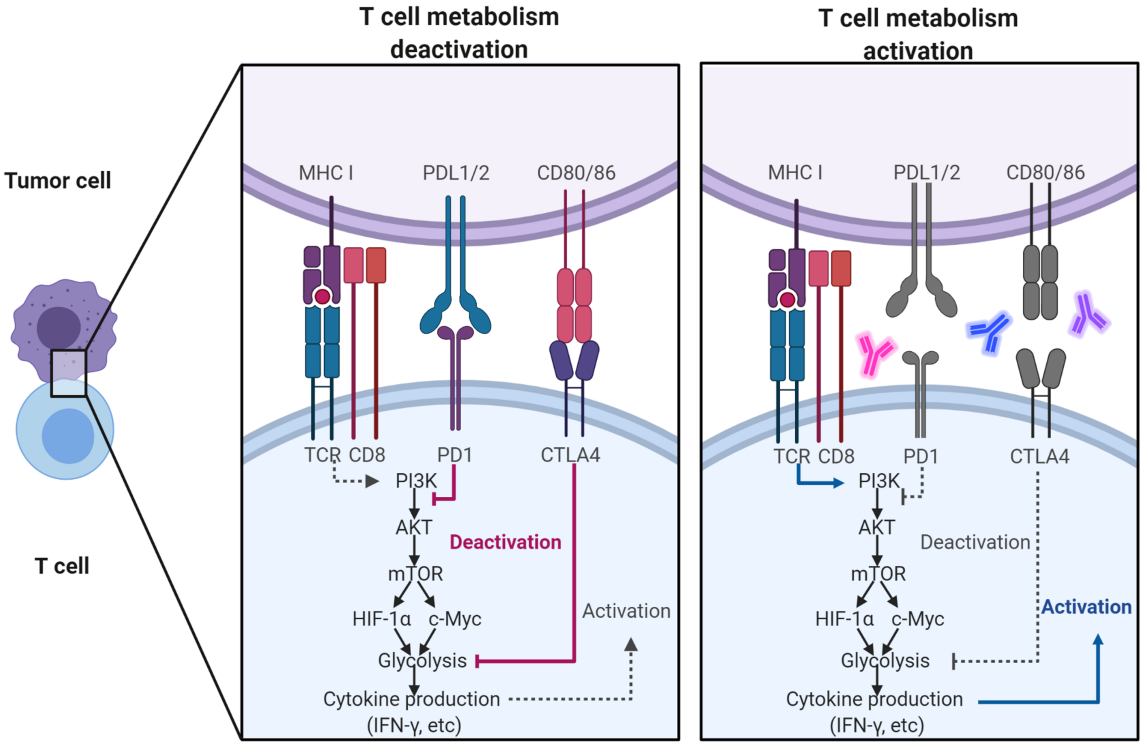

FIGURE 2 Regulation of metabolic pathways by immune checkpoint receptors and ligands. T cell receptor (TCR) signals along with costimulation augment cellular glycolysis, which requires a process dependent on phosphoinositide-3 kinase (PI3K)/AKT/mTOR signals. In addition, TCR signals induce c-Myc and HIF-1α, which enact transcription of metabolic genes critical for T cell activation. However, activation of the inhibitory immune checkpoint receptors programmed cell death 1 (PD-1) or cytotoxic T lymphocyte antigen 4 (CTLA-4) in T cells dampens glycolysis. And antibodies against PD-1 or CTLA-4 can release the brake of the exhausted T cells in the TME. HIF-1α, hypoxia-inducible factor-1α; MHC, major histocompatibility complex; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; TCR, T cell receptor. Created with BioRender.com. Adapted from "T-cell Deactivation vs. Activation", by BioRender.com (2021). https://app.biorender.com/biorender-templates [Color figure can be viewed at wileyonlinelibrary.com]

immune cells, and exert profound impact on antitumor immunity and immunotherapy.

5 | INTERPLAY BETWEEN METABOLIC CHECKPOINTS AND IMMUNE CHECKPOINTS

Accumulating evidence indicates that metabolic alterations can unleash or imprison the power of the host's immune system to conquer tumor. PD-1 signaling impairs glycolysis and amino acid metabolism but enhances FAO in activated T cells. This FAO depends on upregulation of carnitine palmitoyltransferase 1A (CPT1A) and adipose triglyceride lipase (ATGL) that promotes lipolysis.[75] CB-839, a glutaminase 1 inhibitor, is being evaluated in combination with Nivolumab in patients with clear cell renal cell carcinoma (RCC), melanoma and non-small-cell lung cancer (NSCLC) (Table 2). In addition, T cell immunoreceptor with Ig and ITIM domains (TIGIT) signaling in tumor-infiltrating $CD8^+$ T cell dampens glycolysis, expression of glycolytic enzymes Glut1 and HK2, and lactate production, which can be rescued by TIGIT blockade.[76] Contrariwise, agonizing glucocorticoid-induced TNFR-related protein (GITR), a costimulatory receptor, can enhance glycolysis of tumor-infiltrating $CD8^+$ T cells in a mouse tumor model.[77] Metabolic alterations can also in turn affect expression profiles of immune checkpoints. For instance, both extracellular and intracellular cholesterol can induce tumor-infiltrating $CD8^+$ T cell exhaustion by upregulating expression of PD-1 and 2B4.[12] Moreover, glucose consumption by tumor cells can suppress glycolysis of T cells, and immune checkpoint blockade with antibodies against PD-1/PD-L1 and CTLA-4 can attenuate tumor cell glycometabolism, thereby preserving the glucose for the infiltrating T cells[9] (Figure 2). Metformin, a first-line oral hypoglycemic drug for the treatment of diabetes, is currently being assessed in combination with anti-PD-1 antibodies in several clinical trials involving patients with solid tumors (Table 2). Additionally, agonizing peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α)/transcription factor complexes can promote oxidative phosphorylation and glycolysis in $CD8^+$ T cells, thereby fostering their effector function and synergistically facilitating the antitumor effect of PD-1 blockade.[78] Inspiringly, the folate pathway inhibitor pemetrexed enhances antitumor immunity combined with ICIs through promoting $CD8^+$ T cell mitochondrial biogenesis, infiltration and activation.[79] In addition, deletion of dual specificity phosphatase 2 (DUSP2, PAC1) can inhibit PD-1 expression on the tumor infiltrating $CD8^+$ T cells, and rescue the host's antitumor immunity.[80] Besides, loss of protein tyrosine phosphatase non-receptor

type 2 (PTPN2) can unleash the antitumor capacities of $CD8^+$ T cells, and enhance the efficacy of immune checkpoint blockade.[81] These findings suggest that agents targeting the interweaving and competing metabolic pathways in the TME might synergize with ICIs by alleviating metabolic stress imposed on tumor-infiltrating T cells.

6 | METABOLIC COMMUNICATION IN METASTATIC TME

Metastasis is the leading cause of cancer-related mortality.[82] Accumulating evidence shows that microenvironmental metabolism plays an important role in tumor metastasis.[83] Since different tissues or organs exhibit different metabolic microenvironments due to specific structures, unique cell subsets or physiological functions, disseminated or circulating tumor cells which metastasize might make metabolic communication or adaptation in the metastatic TME.[84] Brain metastatic cancer cells exploit the polyunsaturated fatty acids derived from astrocytes to activate PPARγ signaling to promote brain metastasis.[85] Adipocytes within the omentum can promote ovarian cancer cells to preferably metastasize to the omentum, as adipocytes rich in lipids transfer fatty acids to ovarian cancer cells for growth.[86] In addition, metabolic regulation based on immune cells has been gradually disclosed. Regulated in development and DNA damage response 1 (REDD1) deficiency in tumor-associated macrophages outcompetes endothelial cells for glucose uptake, stabilizes tumor endothelial cell junctions and prevents tumor metastasis.[87] In a murine lung metastatic model of breast cancer, lung-infiltrating neutrophils offer neutral lipids to fuel disseminated tumor cells in the metastatic niche.[88]

7 | METABOLIC APPROACHES TO IMPROVING ADOPTIVE CELL TRANSFER

Adoptive cell transfer (ACT) has been proven to be an effective and promising approach to enhancing the host's immune capacity against cancer. By now, ACT mainly includes two forms, in vitro-expanded autologous TILs and genetically engineered T cells.[89,90] Among various strategies for ACT, in vitro-expanded autologous TILs with high-dose IL-2 might have embraced multiple clinical trials within the longest period, and showed durable remission rates. A significant advantage of TILs is the extensive spectrum of T cell recognition of defined or undefined tumor antigens, without the limited inclusion of finite major histocompatibility complex (MHC). However, the inferiority of TILs is also obvious. The TILs isolated from patients are terminally differentiated and most of them are functionally exhausted, indicating that phenotypic transformation from effector to memory might better maintain the effective immune response. As mentioned above, PI3K-AKT-mTOR signaling endows T cells with more potent effector function though enhancing glycolysis, and targeting this metabolic cascade might help the formation of memory T cells. In line with this notion, pharmacologic inhibition of AKT has been proven to promote immunologic memory after viral infection. Consistently, TILs isolated from patients can also be expanded and transformed by pharmacologic inhibition of AKT to memory ones with distinctive transcriptional, metabolic and functional features.[91] These findings sponsor more efforts to combine metabolic interventions with existing approaches to improve ACT. Maybe more valuable biomarkers will be warranted for precise stratification of the patients and better prediction of outcome of ACT combining metabolic interventions and immunotherapy.

Another promising ACT is chimeric antigen receptor T (CAR T) cell therapy, which has so far achieved unprecedented success in clinical antitumor immunotherapy. Accumulating evidence demonstrates that pharmacologic interventions of metabolic processes through targeting PI3K-AKT signaling can also enhance the antitumor effect of CAR T cells, similar to that of in vitro-expanded TILs. Pharmacologic inhibition of AKT during the in vitro expansion of anti-CD19 CAR T cells has been found to reprogram their metabolism, promote the shift to memory cells and facilitate superior antitumor efficacy.[92] Moreover, treatment with a PI3K inhibitor can preserve a less differentiated state of CAR T cells without impairing expansion, extend their survival time and repress tumor progression in an acute myeloid leukemia (AML) model.[93] Besides pharmacologic interventions, specific intracellular signaling domains (ICDs) are also deployed to regulate metabolism in designing the CAR. Addition of a CD28 domain promotes glycolysis and effector function of CAR T cells, and inclusion of a 4-1BB costimulatory domain facilitates mitochondrial oxidation and subsequent central memory-like characteristics, which enhances the persistence of CAR T cells[94] (Figure 3). Taken together, navigating the metabolic pathways of ACT might shed light on high efficacy and long duration in antitumor immunity and immunotherapy.

8 | CLINICAL TRIALS OF METABOLIC INTERVENTIONS COMBINED WITH IMMUNE CHECKPOINT INHIBITORS

Based on the discovery of the above molecular mechanisms, the corresponding drugs have been developed. Some clinical trials of metabolic interventions combined with ICIs are already ongoing. CB-839, a selective inhibitor of glutaminase 1, has been proven to inhibit glutaminolysis and promote antitumor activity in triple-negative breast cancer and some hematological malignancies.[95,96] CB-839 has been involved in an open-label phase I/II study including RCC, NSCLC and melanoma, which evaluates the safety and efficacy of its combination with Nivolumab (Table 2). Several chemotherapeutic agents regulating one-carbon metabolism have also been involved in the combination therapy with ICIs. Carboplatin and pemetrexed are involved in testing the timing of Pembrolizumab alone or with chemotherapy as the first line treatment and maintenance in NSCLC. Oxaliplatin, leucovorin, 5-FU are involved in a randomized, multicenter, comparative phase II trial assessing the efficacy of Durvalumab in combination with radiochemotherapy (FOLFOX4 simplified and IMRT 50 Gy) and as maintenance therapy for patients with localized unresectable esophageal cancer. INCB001158 has been proven to inhibit T cell proliferation and tumor growth in mouse models of cancer in combination with

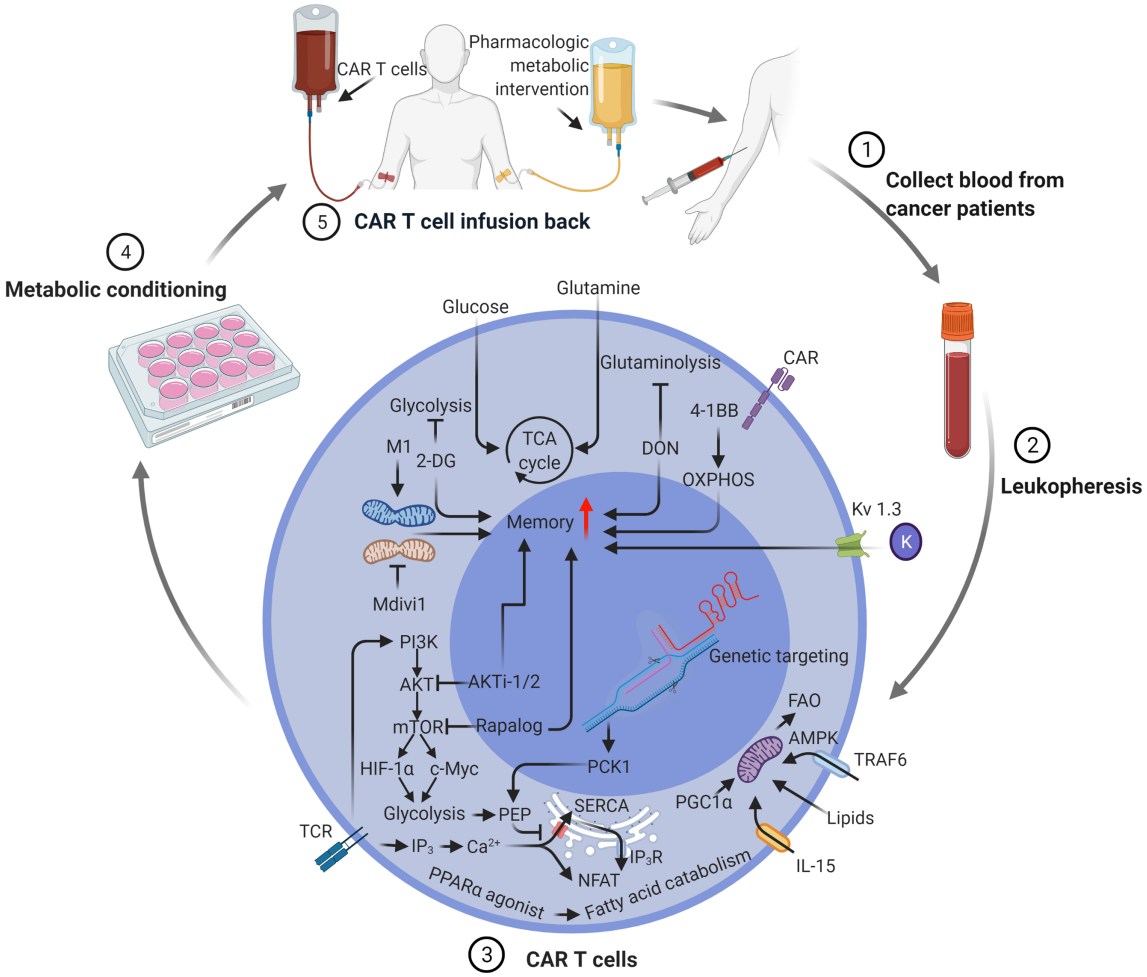

FIGURE 3 Highly flexible metabolic interventions enhance adoptive cell transfer (ACT) through ex vivo culture conditioning or genetic editing of metabolic processes. During ACT, T cells transduced with chimeric antigen receptors (CARs) that redirect them to recognize and eradicate tumor cells expressing a cognate target ligand are expanded ex vivo before reinfusion. This regimen enables a metabolically conditioned window for the use of chemical agents, metabolically engineered media or genetic editing. Furthermore, pharmacologic treatment after T cell reinfusion also facilitates metabolic conditioning. Metabolic interventions for priming more potent antitumor T cells could either be aimed at driving T cell differentiation towards an enhanced memory phenotype or at inducing a metabolic signature that improves T cell persistence and antitumor response in the nutrient-deprived TME. 2-DG, 2-deoxyglucose; AKTi-1/2, AKT inhibitor VIII; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; CAR, chimeric antigen receptor; DON, 6-diazo-5-oxo-l-norleucine; FAO, fatty acid β-oxidation; IP_3, inositol triphosphate; IP_3R, inositol triphosphate receptor; M1, mitochondrial fusion promoter; Mdivi1, mitochondrial division inhibitor 1; NFAT, nuclear factor of activated T cells; PCK1, PEP carboxykinase 1; PEP, phosphoenolpyruvate; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; PPARα, peroxisome proliferator-activated receptor-α; SERCA, sarco/ER Ca^{2+}-ATPase; TCA, tricarboxylic acid; TCR, T cell receptor; TRAF6, TNF receptor associated factor 6. Created with BioRender.com [Color figure can be viewed at wileyonlinelibrary.com]

immunotherapy.[97] And INCB001158 has been involved in an open-label phase I/II evaluation as a single agent and in combination with ICIs in patients with advanced or metastatic solid tumors. ADI-PEG 20, a pegylated arginine deiminase targeting arginine metabolism, has also been involved in a phase Ib, open-label trial in combination with Pembrolizumab for assessment of safety and tolerability of drug combination. The ectonucleotidases CD39 and CD73 account for production of adenosine through the catabolism of ATP to AMP and AMP to adenosine, respectively. MEDI9447 or Oleclumab, an anti-CD73 antibody, has been involved in several clinical trials with Durvalumab in some solid tumors. BMS-986179, another anti-CD73 antibody, has been involved in a phase I/IIa study in combination with Nivolumab to assess the safety and tumor-shrinking ability of experimental medication in patients with advanced solid tumors. In addition, a phase I/Ib, open-label, multicenter study of NZV930 (SRF373, anti-CD73 antibody) as a single agent and in combination with PDR001 and/or NIR178 in patients with advanced tumors is ongoing, in order to evaluate the safety, tolerability and preliminary antitumor activity of the above experimental medications. Furthermore, CPI-006, a humanized monoclonal antibody against CD73, is being under evaluation in a phase I/Ib, open-label, dose escalation and dose expansion study in adults with advanced tumors, as a single agent or in combination with Pembrolizumab. The ensuing secreted adenosine binds to any of four G protein-coupled purinergic type 1 receptors, namely, adenosine

receptor A1 (A_1R), $A_{2A}R$, $A_{2B}R$ or A_3R, and subsequently promotes the production of cAMP. CPI-444 or ciforadenant, an $A_{2A}R$ antagonist, is being under evaluation in a phase I/Ib study to evaluate its safety and tolerability and/or in combination with Atezolizumab in advanced tumors. Other $A_{2A}R$ antagonists, PBF-509 and AZD4635 also get involved in clinical trials in combination with ICIs in cancer patients. Cyclooxygenase (COX)-mediated arachidonic acid metabolism and its metabolite prostaglandin E_2 (PGE_2) also play important roles in antitumor immunity. Aspirin, a common COX1 and/or COX2 inhibitor, may exert synergistic effect in combination with ICIs.[98] Several clinical trials are ongoing to assess the efficacy and safety of combination of PD-1 antibody and Aspirin in patients with advanced tumors, such as microsatellite instability-high and/or mismatch repair-deficient (MSI-H/dMMR) or high tumor mutation burden colorectal cancer (CRC), and recurrent or metastatic head and neck squamous cell carcinoma (HNSCC). PGE_2 signaling through the PGE_2 receptor 4 (EP4) can promote tumorigenesis, and grapiprant, a selective EP4 antagonist is being under evaluation both as a monotherapy and combined with Pembrolizumab in a phase Ib, multicenter, open-label study in patients with microsatellite stable CRC. Overexpression of tryptophan-degrading enzymes including indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) can reduce tryptophan abundance in the TME, subsequently inhibiting the antitumor capacities of T cells. Epacadostat, an IDO inhibitor, is under evaluation in combination with Pembrolizumab in patients with advanced-stage Imatinib-refractory gastrointestinal stromal tumors. Linrodostat, an IDO1 inhibitor, is under evaluation in combination with ICIs in patients with advanced cancers. HTI-1090, a dual IDO1-TDO inhibitor, has been involved in a phase I study in combination with PD-1 inhibitor SHR-1210 plus VEGFR inhibitor Apatinib or not in patients with advanced solid tumors. Isocitrate dehydrogenase 1 (IDH1) or IDH2 mutation can lead to the conversion of α-KG to the oncometabolite D-2-hydroxyglutarate (D-2-HG), which activates the mTOR signaling pathway.[99] FT-2102, an inhibitor of mutant IDH1, is involved in a phase Ib/II study in combination therapy in patients with advanced solid tumors and gliomas with an IDH1 mutation. In addition, imprime PGG, a soluble yeast β-glucan, which can act as a pathogen associated molecular pattern (PAMP) and elicit synergistic effect with anti-PD1 antibody, has been under evaluation in combination with ICIs in patients with advanced/metastatic cancers.

9 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Manifestly, the metabolic checkpoints regulate the immune response in antitumor immunity and immunotherapy through orchestrating the metabolic interaction between tumor cells and tumor-infiltrating immune cells. In aggregate, while the immunotherapy approaches such as ICIs and ACT have achieved unprecedented success on the expedition to conquer cancer, the genuinely effective metabolic interventions that can synergize with immunotherapy are still far behind the clinical demands. Therefore, novel and promising metabolic checkpoints are much more warranted to be discovered in antitumor immunity and immunotherapy, with more basic investigations and clinical trials on the way.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*. 2017;168:707-723.
2. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359:1350-1355.
3. Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic instruction of immunity. *Cell*. 2017;169:570-586.
4. Chang CH, Pearce EL. Emerging concepts of T cell metabolism as a target of immunotherapy. *Nat Immunol*. 2016;17:364-368.
5. Geltink RIK, Kyle RL, Pearce EL. Unraveling the complex interplay between T cell metabolism and function. *Annu Rev Immunol*. 2018;36:461-488.
6. Kishton RJ, Sukumar M, Restifo NP. Metabolic regulation of T cell longevity and function in tumor immunotherapy. *Cell Metab*. 2017;26:94-109.
7. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol*. 2012;13:907-915.
8. Pollizzi KN, Powell JD. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nat Rev Immunol*. 2014;14:435-446.
9. Chang CH, Qiu J, O'Sullivan D, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell*. 2015;162:1229-1241.
10. Lim S, Liu H, Madeira da Silva L, et al. Immunoregulatory protein B7-H3 reprograms glucose metabolism in cancer cells by ROS-mediated stabilization of HIF1alpha. *Cancer Res*. 2016;76:2231-2242.
11. Cascone T, McKenzie JA, Mbofung RM, et al. Increased tumor glycolysis characterizes immune resistance to adoptive T cell therapy. *Cell Metab*. 2018;27:977-987.
12. Ma X, Bi E, Lu Y, et al. Cholesterol induces CD8(+) T cell exhaustion in the tumor microenvironment. *Cell Metab*. 2019;30:143-156.
13. Palazon A, Tyrakis PA, Macias D, et al. An HIF-1alpha/VEGF-A axis in cytotoxic T cells regulates tumor progression. *Cancer Cell*. 2017;32:669-683.
14. Yue EW, Sparks R, Polam P, et al. INCB24360 (Epacadostat), a highly potent and selective Indoleamine-2,3-dioxygenase 1 (IDO1) inhibitor for Immuno-oncology. *ACS Med Chem Lett*. 2017;8:486-491.
15. Bantug GR, Galluzzi L, Kroemer G, Hess C. The spectrum of T cell metabolism in health and disease. *Nat Rev Immunol*. 2018;18:19-34.
16. Hang S, Paik D, Yao L, et al. Bile acid metabolites control TH17 and Treg cell differentiation. *Nature*. 2019;576:143-148.
17. Bailis W, Shyer JA, Zhao J, et al. Distinct modes of mitochondrial metabolism uncouple T cell differentiation and function. *Nature*. 2019;571:403-407.

18. Harel M, Ortenberg R, Varanasi SK, et al. Proteomics of melanoma response to immunotherapy reveals mitochondrial dependence. *Cell*. 2019;179:236-250.
19. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol*. 2013;31:259-283.
20. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol*. 2016;16:553-565.
21. Sena LA, Li S, Jairaman A, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity*. 2013;38:225-236.
22. Patel CH, Powell JD. Targeting T cell metabolism to regulate T cell activation, differentiation and function in disease. *Curr Opin Immunol*. 2017;46:82-88.
23. Frauwirth KA, Riley JL, Harris MH, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity*. 2002;16:769-777.
24. Delgoffe GM, Pollizzi KN, Waickman AT, et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol*. 2011;12:295-303.
25. Delgoffe GM, Kole TP, Zheng Y, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity*. 2009;30:832-844.
26. Waickman AT, Powell JD. mTOR, metabolism, and the regulation of T-cell differentiation and function. *Immunol Rev*. 2012;249:43-58.
27. Shi H, Chapman NM, Wen J, et al. Amino acids license kinase mTORC1 activity and Treg cell function via small G proteins rag and Rheb. *Immunity*. 2019;51:1012-1027. e7.
28. Bi X, Li F, Liu S, et al. Omega-3 polyunsaturated fatty acids ameliorate type 1 diabetes and autoimmunity. *J Clin Invest*. 2017;127:1757-1771.
29. Macintyre AN, Gerriets VA, Nichols AG, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab*. 2014;20:61-72.
30. Sanin DE, Pearce EJ. The cell identity of cytotoxic T lymphocytes. *Nat Immunol*. 2016;17:45-46.
31. Sukumar M, Liu J, Ji Y, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest*. 2013;123:4479-4488.
32. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science*. 2016;354:481-484.
33. Chang CH, Curtis JD, Maggi LB Jr, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell*. 2013;153:1239-1251.
34. Jacobs SR, Herman CE, Maciver NJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol*. 2008;180:4476-4486.
35. Pollizzi KN, Patel CH, Sun IH, et al. mTORC1 and mTORC2 selectively regulate CD8(+) T cell differentiation. *J Clin Invest*. 2015;125:2090-2108.
36. Nakaya M, Xiao Y, Zhou X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity*. 2014;40:692-705.
37. Berod L, Friedrich C, Nandan A, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med*. 2014;20:1327-1333.
38. Gerriets VA, Kishton RJ, Johnson MO, et al. Foxp3 and toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat Immunol*. 2016;17:1459-1466.
39. Angelin A, Gil-de-Gomez L, Dahiya S, et al. Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metab*. 2017;25:1282-1293.
40. Hu Z, Qu G, Yu X, et al. Acylglycerol kinase maintains metabolic state and immune responses of CD8(+) T cells. *Cell Metab*. 2019;30:290-302.
41. Verbist KC, Guy CS, Milasta S, et al. Metabolic maintenance of cell asymmetry following division in activated T lymphocytes. *Nature*. 2016;532:389-393.
42. Wang R, Dillon CP, Shi LZ, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity*. 2011;35:871-882.
43. Shehade H, Acolty V, Moser M, Oldenhove G. Cutting edge: hypoxia-inducible factor 1 negatively regulates Th1 function. *J Immunol*. 2015;195:1372-1376.
44. Shi LZ, Wang R, Huang G, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med*. 2011;208:1367-1376.
45. Miska J, Lee-Chang C, Rashidi A, et al. HIF-1alpha is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of Tregs in glioblastoma. *Cell Rep*. 2019;27:226-237.
46. Phan AT, Doedens AL, Palazon A, et al. Constitutive glycolytic metabolism supports CD8(+) T cell effector memory differentiation during viral infection. *Immunity*. 2016;45:1024-1037.
47. Graef P, Buchholz VR, Stemberger C, et al. Serial transfer of single-cell-derived immunocompetence reveals stemness of CD8(+) central memory T cells. *Immunity*. 2014;41:116-126.
48. Fridman WH, Galon J, Pages F, Tartour E, Sautes-Fridman C, Kroemer G. Prognostic and predictive impact of intra- and peritumoral immune infiltrates. *Cancer Res*. 2011;71:5601-5605.
49. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12:298-306.
50. Galluzzi L, Kepp O, Vander Heiden MG, Kroemer G. Metabolic targets for cancer therapy. *Nat Rev Drug Discov*. 2013;12:829-846.
51. Salazar-Roa M, Malumbres M. Fueling the cell division cycle. *Trends Cell Biol*. 2017;27:69-81.
52. Ho PC, Bihuniak JD, Macintyre AN, et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell*. 2015;162:1217-1228.
53. O'Sullivan D, Sanin DE, Pearce EJ, Pearce EL. Metabolic interventions in the immune response to cancer. *Nat Rev Immunol*. 2019;19:324-335.
54. Geiger R, Rieckmann JC, Wolf T, et al. L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell*. 2016;167:829-842.
55. Comerford SA, Huang Z, Du X, et al. Acetate dependence of tumors. *Cell*. 2014;159:1591-1602.
56. Jain M, Nilsson R, Sharma S, et al. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science*. 2012;336:1040-1044.
57. Gemta LF, Siska PJ, Nelson ME, et al. Impaired enolase 1 glycolytic activity restrains effector functions of tumor-infiltrating CD8(+) T cells. *Sci Immunol*. 2019;4:eaap9520.
58. Leone RD, Zhao L, Englert JM, et al. Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. *Science*. 2019;366:1013-1021.
59. Cronin SJF, Seehus C, Weidinger A, et al. The metabolite BH4 controls T cell proliferation in autoimmunity and cancer. *Nature*. 2018;563:564-568.
60. Zhao E, Maj T, Kryczek I, et al. Cancer mediates effector T cell dysfunction by targeting microRNAs and EZH2 via glycolysis restriction. *Nat Immunol*. 2016;17:95-103.
61. Klysz D, Tai X, Robert PA, et al. Glutamine-dependent alpha-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. *Sci Signal*. 2015;8:ra97.
62. Pearce EL, Walsh MC, Cejas PJ, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature*. 2009;460:103-107.
63. Raud B, McGuire PJ, Jones RG, Sparwasser T, Berod L. Fatty acid metabolism in CD8(+) T cell memory: challenging current concepts. *Immunol Rev*. 2018;283:213-231.

64. Li L, Liu X, Sanders KL, et al. TLR8-mediated metabolic control of human Treg function: a mechanistic target for cancer immunotherapy. *Cell Metab*. 2019;29:103-123.
65. Biswas SK. Metabolic reprogramming of immune cells in cancer progression. *Immunity*. 2015;43:435-449.
66. Speiser DE, Ho PC, Verdeil G. Regulatory circuits of T cell function in cancer. *Nat Rev Immunol*. 2016;16:599-611.
67. Song M, Sandoval TA, Chae CS, et al. IRE1alpha-XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. *Nature*. 2018;562:423-428.
68. Zhang Y, Kurupati R, Liu L, et al. Enhancing CD8(+) T cell fatty acid catabolism within a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. *Cancer Cell*. 2017;32:377-391.
69. Wang W, Green M, Choi JE, et al. CD8(+) T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature*. 2019;569:270-274.
70. Liu Y, Liang X, Dong W, et al. Tumor-repopulating cells induce PD-1 expression in CD8(+) T cells by transferring kynurenine and AhR activation. *Cancer Cell*. 2018;33:480-494.
71. Liu X, Mo W, Ye J, et al. Regulatory T cells trigger effector T cell DNA damage and senescence caused by metabolic competition. *Nat Commun*. 2018;9:249.
72. Comito G, Iscaro A, Bacci M, et al. Lactate modulates CD4(+) T-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via TLR8/miR21 axis. *Oncogene*. 2019;38:3681-3695.
73. Rice CM, Davies LC, Subleski JJ, et al. Tumour-elicited neutrophils engage mitochondrial metabolism to circumvent nutrient limitations and maintain immune suppression. *Nat Commun*. 2018;9:5099.
74. Guak H, Al Habyan S, Ma EH, et al. Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. *Nat Commun*. 2018;9:2463.
75. Patsoukis N, Bardhan K, Chatterjee P, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun*. 2015;6:6692.
76. He W, Zhang H, Han F, et al. CD155T/TIGIT signaling regulates CD8(+) T-cell metabolism and promotes tumor progression in human gastric cancer. *Cancer Res*. 2017;77:6375-6388.
77. Sabharwal SS, Rosen DB, Grein J, et al. GITR agonism enhances cellular metabolism to support CD8(+) T-cell proliferation and effector cytokine production in a mouse tumor model. *Cancer Immunol Res*. 2018;6:1199-1211.
78. Chowdhury PS, Chamoto K, Kumar A, Honjo T. PPAR-induced fatty acid oxidation in T cells increases the number of tumor-reactive CD8(+) T cells and facilitates anti-PD-1 therapy. *Cancer Immunol Res*. 2018;6:1375-1387.
79. Schaer DA, Geeganage S, Amaladas N, et al. The Folate pathway inhibitor Pemetrexed Pleiotropically enhances effects of cancer immunotherapy. *Clin Cancer Res*. 2019;25:7175-7188.
80. Dan L, Liu L, Sun Y, et al. The phosphatase PAC1 acts as a T cell suppressor and attenuates host antitumor immunity. *Nat Immunol*. 2020;21:287-297.
81. LaFleur MW, Nguyen TH, Coxe MA, et al. PTPN2 regulates the generation of exhausted CD8(+) T cell subpopulations and restrains tumor immunity. *Nat Immunol*. 2019;20:1335-1347.
82. Ganesh K, Massague J. Targeting metastatic cancer. *Nat Med*. 2021;27:34-44.
83. Elia I, Doglioni G, Fendt SM. Metabolic hallmarks of metastasis formation. *Trends Cell Biol*. 2018;28:673-684.
84. Schild T, Low V, Blenis J, Gomes AP. Unique metabolic adaptations dictate distal organ-specific metastatic colonization. *Cancer Cell*. 2018;33:347-354.
85. Zou Y, Watters A, Cheng N, et al. Polyunsaturated fatty acids from astrocytes activate PPARgamma signaling in cancer cells to promote brain metastasis. *Cancer Discov*. 2019;9:1720-1735.
86. Nieman KM, Kenny HA, Penicka CV, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med*. 2011;17:1498-1503.
87. Wenes M, Shang M, Di Matteo M, et al. Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis. *Cell Metab*. 2016;24:701-715.
88. Li P, Lu M, Shi J, et al. Lung mesenchymal cells elicit lipid storage in neutrophils that fuel breast cancer lung metastasis. *Nat Immunol*. 2020;21:1444-1455.
89. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science*. 2018;359:1361-1365.
90. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. *Nat Rev Immunol*. 2012;12:269-281.
91. Crompton JG, Sukumar M, Roychoudhuri R, et al. Akt inhibition enhances expansion of potent tumor-specific lymphocytes with memory cell characteristics. *Cancer Res*. 2015;75:296-305.
92. Klebanoff CA, Crompton JG, Leonardi AJ, et al. Inhibition of AKT signaling uncouples T cell differentiation from expansion for receptor-engineered adoptive immunotherapy. *JCI Insight*. 2017;2(23):e95103.
93. Zheng W, O'Hear CE, Alli R, et al. PI3K orchestration of the in vivo persistence of chimeric antigen receptor-modified T cells. *Leukemia*. 2018;32:1157-1167.
94. Kawalekar OU, O'Connor RS, Fraietta JA, et al. Distinct signaling of Coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity*. 2016;44:380-390.
95. Gross MI, Demo SD, Dennison JB, et al. Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Mol Cancer Ther*. 2014;13:890-901.
96. DeLaBarre B, Hurov J, Cianchetta G, Murray S, Dang L. Action at a distance: allostery and the development of drugs to target cancer cell metabolism. *Chem Biol*. 2014;21:1143-1161.
97. Steggerda SM, Bennett MK, Chen J, et al. Inhibition of arginase by CB-1158 blocks myeloid cell-mediated immune suppression in the tumor microenvironment. *J Immunother Cancer*. 2017;5:101.
98. Hamada T, Cao Y, Qian ZR, et al. Aspirin use and colorectal cancer survival according to tumor CD274 (programmed cell death 1 ligand 1) expression status. *J Clin Oncol*. 2017;35:1836-1844.
99. Han CJ, Zheng JY, Sun L, et al. The oncometabolite 2-hydroxyglutarate inhibits microglial activation via the AMPK/mTOR/NF-kappaB pathway. *Acta Pharmacol Sin*. 2019;40:1292-1302.

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