Navigating metabolic pathways to enhance antitumour immunity and immunotherapy

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Abstract | The development of immunotherapies over the past decade has resulted in a paradigm shift in the treatment of cancer. However, the majority of patients do not benefit from immunotherapy, presumably owing to insufficient reprogramming of the immunosuppressive tumour microenvironment (TME) and thus limited reinvigoration of antitumour immunity. Various metabolic machineries and nutrient-sensing mechanisms orchestrate the behaviour
of immune cells in response to nutrient availability in the TME. Notably, tumour-infiltrating immune cells typically experience metabolic stress as a result of the dysregulated metabolic activity of tumour cells, leading to impaired antitumour immune responses. Moreover, the immune checkpoints that are often exploited by tumour cells to evade immunosurveillance have emerging roles in modulating the metabolic and functional activity of T cells. Thus, repurposing of drugs targeting cancer metabolism might synergistically enhance immunotherapy via metabolic reprogramming of the TME. In addition, interventions targeting the metabolic circuits that impede antitumour immunity have been developed, with several clinical trials underway.
Herein, we discuss how these metabolic circuits regulate antitumour immunity and the possible approaches to targeting these pathways in the context of anticancer immunotherapy. We also describe hypothetical combination treatments that could be used to better unleash the potential of adoptive cell therapies by enhancing T cell metabolism.

Metabolism involves a network of biochemical reac- tions that convert nutrients into small molecules called metabolites. Through these conversions and the result- ing metabolites, cells generate the energy, redox equiv- alents and macromolecules (including proteins, lipids, DNA and RNA) that they require to survive and sustain cellular functions1. Moreover, metabolic profiles reflect the cellular state, and metabolic pathways are, there- fore, intimately entwined with cell signalling and epi- genetic networks2,3. Thus, metabolism has a central role in cellular homeostasis and adaptation in response to intracellular and extracellular stimuli. The key nutrients available to cells include glucose, amino acids and fatty acids. These nutrients are mostly converted and used in central metabolism, which consists of catabolic glycoly- sis and the tricarboxylic acid (TCA) cycle, as well as the connected anabolic pathways that provide precursors for macromolecule synthesis. Through glycolysis, glucose is broken down to pyruvate, leading to generation of the cellular

energy equivalent ATP. Glucose can also enter the pentose phosphate or glycogenesis pathways for carbon storage and the production of NADPH, nucle- otide sugars and ribose-5-phosphate; in turn, these metabolites support a variety of macromolecule biosyn- thesis, antioxidant production and protein glycosylation pathways. Other glycolytic metabolites, such as glycerol- 3-phosphate and 3-phosphoglycerate, can be diverted into the fatty acid and serine–glycine biosynthesis path- ways, respectively. Pyruvate generated through glycol- ysis can be further metabolized to lactate and alanine. In addition, pyruvate can enter the TCA cycle via con- version to oxaloacetate or acetyl-CoA, which is crucial for the biosynthesis of fatty acids, amino acids and ATP.

Amino acids — many of which can be synthesized within the cell while others, termed essential amino acids, cannot and must be derived from food — are indispensable for nucleotide and protein synthesis. Unsurprisingly, owing to the wide physiological range of available extracellular amino acids, some amino acids are catabolized by cells while others are synthe- sized, depending on the cell type, metabolic state and microenvironment. Glutamine is the most prominent example of a catabolized amino acid. Glutamine can be converted into several other amino acids, such as pro- line and aspartate; used for fatty acid synthesis; or fully oxidized via glutaminolysis, yielding ATP and NADPH4.

Similar to essential amino acids, essential fatty acids derived from food can be taken up by cells and further modified for use in cell membranes and as signalling molecules. Furthermore, the integration of fatty acid β-oxidation (FAO) with the TCA cycle and the electron transport chain results in the production of the important metabolic cofactor acetyl-CoA, NADPH and ATP. In addition to the energy, redox homeostasis and macromolecules resulting from these metabolic con- versions, the intermediary metabolites generated are known to be important regulators of cell signalling and the epigenome2,3,5–11.

Cancer cells rely on the same metabolic networks mentioned above; however, various central metabolism pathways can be dysregulated in cancer cells depending on their genetic landscape12, cellular origin13, micro- environment14,15 and functional phenotype (for example, reflecting dormancy, proliferation, invasion and meta- stasis, drug-resistance or immune evasion16–19). Targeting vulnerabilities of the dysregulated metabolic pathways in cancer cells is, therefore, an attractive therapeutic strategy. In addition, advances made in understanding immunometabolism have emphasized the importance of the metabolic machineries and nutrient-sensing mech- anisms that regulate anticancer immune responses; emerging evidence indicates that cancer cells are able to suppress antitumour immunity by competing for and depleting essential nutrients or otherwise reducing the metabolic fitness of tumour-infiltrating immune cells. Thus, metabolic interventions hold promise for improv- ing the effectiveness of immunotherapies. Importantly, the similar metabolic needs of cancer cells and immune cells might preclude synergistic effects of such combina- tions. However, much potential lies in targeting the meta- bolic pathways that are differently essential to cancer cells and immune cells and, in particular, those that are modulated by cancer cells to evade the immunosurveillance (Fig. 1). Together, these considerations highlight the need for an in-depth understanding and re-evaluation of metabolic approaches to the treatment of cancer. Herein, we summarize the metabolic pathways implicated in tumour immune evasion and escape. We also discuss vulnerabili- ties in these metabolic pathways that could potentially be exploited to enhance anticancer immunotherapy.

Glycolysis and lactate production

The aberrant bioenergetic activity that enables tumour cells to use large amounts of glucose and produce lactic acid via glycolysis even in the presence of sufficient oxy- gen (aerobic glycolysis), with a correspondingly low rate of oxidative phosphorylation (OXPHOS), is a phenome- non known as the Warburg effect20. Lactic acid is exported into the extracellular environment via monocarboxylate transporters (MCTs), in particular, monocarboxy- late transporter 4 (MCT4)21, which results in an acidic tumour microenvironment (TME). In the past decade, both aerobic glycolysis and the resultant acidification of the TME have been shown to strongly influence T cell- mediated antitumour immune responses and the activi- ties of tumour-infiltrating myeloid cells (Fig. 1). As a result of high rates of glucose consumption by tumour cells, tumour-infiltrating lymphocytes (TILs) have decreases in mTOR activity, nuclear factor of activated T cells (NFAT) signalling and glycolytic capacity, which lead to impaired production of antitumour effector molecules22,23. Moreover, glycolytic activity in tumours can stimu- late the expression of granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony- stimulating factor (GM-CSF) and thereby contribute to the recruitment of myeloid-derived suppressor cells (MDSCs) to the TME24. Furthermore, glucose depri- vation and the accumulation of lactic acid in the TME interrupt the metabolic programmes and signalling cas- cades that support dendritic cell (DC) maturation and the pro-inflammatory polarization of macrophages, thus promoting the development of pro-tumorigenic myeloid cells, including tolerogenic DCs and M2-like macro- phages25–28. Interestingly, mice harbouring tumours deficient in lactate dehydrogenase A (LDHA), which converts pyruvate to lactate, have a decreased frequency of splenic MDSCs and an improved cytotoxic function of tumour-infiltrating natural killer (NK) cells29, suggesting that production of lactic acid facilitates tumour growth by also impairing innate immune responses. Indeed, lactic acid production by tumour cells might contrib- ute to tumorigenesis by promoting IL-23-mediated and IL-17-mediated inflammation30. In addition to modu- lating immune responses, lactate produced by cancer-associated fibroblasts (CAFs) can be used by tumour cells as an alternative nutrient source31,32. In agreement with findings from murine tumour models, the results of several clinical studies revealed that aerobic glycolytic activity in human tumours is negatively associated with host antitumour immune responses and therapeutic outcomes of anticancer immunotherapy. For example, human tumours refrac- tory to adoptive T cell transfer (ACT) immunother- apy have elevated levels of aerobic glycolytic activity, and glycolytic tumours have lowered levels of T cell tumour infiltration and cytotoxicity compared with less-glycolytic tumours33. In patients with melanoma, tumoural levels of LDHA and lactate negatively correlate with markers of T cell activity and overall survival34; clinical data from 311 patients demonstrated that serum LDH levels >1,000 international units (IU)/l predicted terminal stage, metastatic disease35. Consistent with these findings, LDHA-mediated lactic acid production suppresses IFNγ expression in both tumour-infiltrating T cells and NK cells, thereby promoting tumour growth and immune evasion in mouse models34. Similarly, a negative correlation between intratumoural lactate concentration and overall survival in patients with cer- vical cancer has been reported36. Together, these find- ings suggest that glycolytic activity not only provides an intrinsic growth advantage for tumour cells but also has tumour cell-extrinsic effects that abrogate immuno- surveillance of cancer. Hence, targeting glucose metab- olism and/or lactic acid production and secretion is an appealing strategy for anticancer therapy; however, such approaches, particularly those targeting shared glycolytic pathways that also support T cell function (Fig. 1), might simultaneously blunt immune responses. Conversely, targeting the

glycolytic pathway might sup- press tumour-promoting inflammation mediated by IL-17, IL-6 and IL-23 and thus restrict tumorigenesis30. Therefore, reconsideration of metabolic approaches to anticancer therapy is required in order to ensure that effective antitumour immunity is sustained and to explore whether targeting of the glycolytic pathway at different stages of tumorigenesis leads to distinct therapeutic responses.

PKM2, an enzyme that converts phosphoenol- pyruvate into pyruvate during the final step of glyco- lysis, is often expressed at high levels in tumour cells. Interestingly, PKM2 is less active than PKM1 in con- verting phosphoenolpyruvate into pyruvate, which supports the Warburg effect and thus tumour cell sur- vival and proliferation37,38. Correspondingly, PKM2 activators, such as TEPP-46, DASA-58 and ML-265, decrease tumour cell proliferation and tumour growth in mouse models by increasing the conversion of phos- phoenolpyruvate into pyruvate39. Intriguingly, PKM2 has been demonstrated to promote expression of pro- grammed cell death 1 ligand 1 (PD-L1), a ligand of the inhibitory T cell immune-checkpoint receptor pro- grammed cell death 1 (PD-1), in tumour and immune cells, and, accordingly, increasing PKM2-mediated phosphoenolpyruvate conversion into pyruvate using TEPP-46 reduces the expression of PD-L1 in tumour and myeloid cells in a mouse CT26 colon carcinoma model40. Therefore, PKM2 activators might synergize with PD-1–PD-L1 immune-checkpoint inhibitors (ICIs) by simultaneously reducing metabolic stress and immunosuppression in the TME via abrogation of both aerobic glycolysis and PD-L1 expression in tumour cells (as well as suppressive immune cells).

Phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3) promotes glycolytic activity and lactic acid production in tumour cells41. Inhibitors of PFKFB3 have been shown to abrogate the Warburg effect, tumour pro- gression and metastasis in preclinical models42. Similar to treatment with a PKM2 activator, the PFKFB3 inhibitor PFK-158 has been reported to improve ther- apeutic responses to antibodies targeting the inhibitory immune-checkpoint receptor cytotoxic T lymphocyte antigen 4 (CTLA-4) in a mouse B16 melanoma model43. The precise mechanisms by which inhibitors of glycoly- sis induce synergistic responses with ICIs remain to be delineated, although these findings suggest that modula- tion of cancer metabolism can unleash host antitumour immune responses via reprogramming of the TME.

Inhibition of lactic acid production, and thus the associated acidification of the TME, has also been pro- posed as a strategy to unleash antitumour immunity. Indeed, LDHA inhibitors, such as FX11 and gallofla- vin, have been reported to reduce tumour growth in xenograft models44,45. Whether LDHA inhibitors could be used to enhance immunotherapy remains to be deter- mined; however, treatment of melanoma cells with the LDHA inhibitor GSK2837808A markedly increased cytotoxicity mediated by autologous TILs in an in vitro culture assay33. Moreover, low serum levels of LDH are associated with better therapeutic responses to the anti- PD-1 antibody pembrolizumab in patients with mela- noma46. These findings support the hypothesis that the efficacy of anticancer immunotherapy can be enhanced by reducing the production of lactic acid. In addition to LDHA, the high levels of lactate transporters (MCT proteins) in tumour cells provide therapeutic opportu- nities47; AZD3965, an MCT1 and MCT2 inhibitor, is currently under investigation in a phase I trial involving patients with advanced-stage solid tumours, diffuse large B cell lymphoma or Burkitt lymphoma (NCT01791595). Alternatively, neutralizing the acidity of the TME with bicarbonate has been demonstrated to increase T cell infiltration and improve antitumour immune responses when combined with immune-checkpoint inhibition and ACT in multiple mouse models48.

Amino acids and their derivatives

Glutamine and glutamate. The metabolic demands of tumour cells can also be fuelled through upregulation of glutamine anaplerosis — via glutaminolysis to gluta- mate — of the TCA cycle intermediate α-ketoglutarate (α-KG). Notably, lactate can promote the expression of both the glutamine transporter ASCT2 (also known as ATB(0)) and glutaminase 1 (GLS) in tumour cells via stabilization of hypoxia-inducible factor 2α (HIF2α; also known as EPAS1), which could potentially reprogramme tumour cells towards increased glutaminolysis49 (Fig. 1). Increased glutamine anaplerosis in tumour cells leads to an increase in the release of ammonia; exposure to ammonia can then activate autophagy in neighbouring cells, such as CAFs. Intriguingly, ammonia-activated autophagy in CAFs has been suggested to further sup- port tumour cell growth by facilitating the release of glutamine from CAFs, which can then be metabolized by tumour cells50. In addition, the products of glu- tamine metabolism — glutamate and α-KG, as well as aspartate — can in turn modulate cellular metabolism, epigenetic landscapes, nucleotide synthesis and redox balance in tumour cells4. Thus, multiple compounds targeting glutamine anaplerosis have been developed as anticancer treatments. Among these agents, the GLS inhibitors BPTES (bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide 3) and compound 968 have been shown to prolong survival in several xenograft tumour models by inhibiting cell proliferation and elicit- ing cell death51,52. Unfortunately, the therapeutic utility of BPTES and compound 968 is limited by their moderate potency, poor metabolic stability and low solubility53. By contrast, CB-839, an allosteric inhibitor of GLS, effec- tively inhibits glutaminolysis and has promising activity in preclinical models of triple-negative breast cancer and haematological malignancies53,54. CB-839 is cur- rently being evaluated in several clinical trials involving patients with solid or haematological malignancies, both as a single agent and in combination with ICIs (Table 1).

In addition to tumour cells, activated T cells and macrophages upregulate glutamine metabolism in order to support cell fate determination and immune responses27,55–57. Glutamine deprivation can suppress T cell proliferation and cytokine production55; however, glutamine restriction during T cell activation in vitro has been shown to promote memory CD8+ T cell dif- ferentiation58. Genetic ablation of GLS expression has also been demonstrated to promote the differentiation and effector function of CD4+ T helper 1 (TH1) cells and CD8+ cytotoxic T lymphocytes (CTLs) but impairs dif- ferentiation of TH17 cells59. Thus, interventions targeting glutamine metabolism in tumour cells are postulated to simultaneously affect the immune state of the TME and antitumour immunity (Fig. 1). In support of this hypoth- esis, CB-839 has been shown to synergize with PD-1 inhibitors in several clinical trials60; however, which immune cells (if any) are responsible for this synergistic antitumour activity remains to be determined. Transient CB-839 treatment augments CTL-mediated antitumour responses in mouse models59, which might explain the synergy between this agent and PD-1 inhibitors and sup- ports the therapeutic potential of GLS inhibition in anti- cancer immunotherapy. Of note, glutamine deprivation has also been shown to hamper TH1 cell differentiation in vitro but favours regulatory T (Treg) cell development in differentiation cultures61. These observations of oppo- site responses in different T cell subsets highlight the need for further investigations to delineate how glu- tamine metabolism is modulated in cells of the TME and the underlying mechanisms by which glutamine orchestrates T cell responses.

In addition to glutamine, glutamate levels can also fine-tune T cell proliferation and cytokine production. Upon T cell receptor (TCR) activation, both CD4+ and CD8+ T cells upregulate

glutamate receptors, which cor- relates with increased expression of activation molecules and production of IFNγ, with evidence of co-stimulatory effects mediated by these voltage-gated potassium chan- nels62. Conversely, however, high concentrations of extracellular glutamate (>100 μM) can suppress T cell activation63. Whether glutamate-mediated signalling can be manipulated in order to enhance anticancer immunotherapy remains unclear.

Arginine. Arginine metabolism also has crucial roles in T cell activation and modulating immune responses. During resolution of inflammatory responses, immuno- modulatory cells promote the degradation of argi- nine via expression of the catabolic enzyme arginase 1 (ARG1)64 (Fig. 1). The accumulation of ARG1-expressing immunomodulatory cells, including M2-like tumour- associated macrophages (TAMs), tolerogenic DCs and Treg cells, in the TME might suppress antitumour immunity by degrading arginine and thus limiting the availability of this amino acid to T cells65. Accordingly, supplementation of arginine stimulates T cell and NK cell cytotoxicity and effector cytokine production in vitro and, in combination with anti-PD-L1 antibody treatment, significantly enhances antitumour immune responses and prolongs the survival of osteosarcoma- bearing mice66. Moreover, arginine supplementation during in vitro expansion of T cells promotes their differentiation to central memory-like T cells with superior antitumour activity67. Thus, replenishment of arginine and prevention of arginine degradation in the TME are attractive strategies to re-invigorate T cell- mediated and NK cell-mediated immune responses. These approaches are currently being tested in a clin- ical trial in which the ARG1 inhibitor INCB001158 is being used in combination with the ICI pembrolizumab (Table 1). In mouse tumour models, INCB001158 treat- ment increases CD8+ T cell and NK cell tumour infil- tration and stimulates the production of inflammatory cytokines in the TME68.
Treatment with PEGylated arginine deiminase (ADI- PEG 20) to deplete the TME of arginine has been shown to suppress growth of arginine auxotrophic cancers, including breast cancer69, small-cell lung cancer70 and acute myeloid leukaemia (AML)71. Intriguingly, ADI-PEG 20 has been reported to enhance T cell activation and tumour T cell infiltration, moderate T cell exhaus- tion and abolish Treg cell accumulation in tumours72. These immune phenotypes form the foundation of ongoing clinical trials in which ICIs are being com- bined with ADI-PEG 20 (Table 1). The tumour types or immune TMEs that are most suited to treatment with an ARG1 inhibitor or ADI-PEG 20 remain unclear. Tryptophan. The resolution of inflammation is also mediated by tryptophan metabolism via enzymes including indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO)64,65. Expression of high levels of these tryptophan-degrading enzymes in tumour cells promotes tumour progression and is cor- related with a worse prognosis in patients with gastric adenocarcinoma73. Furthermore, a variety of stromal cells in the TME, including endothelial cells, TAMs and DCs, also overexpress IDO and TDO74. High levels of IDO and TDO in tumours have been suggested to decrease tryptophan availability in the TME, which in turn suppresses the tumoricidal functions of T cells75,76. In addition to depriving T cells of tryptophan, IDO and TDO catabolize tryptophan to kynurenine, the accumu- lation of which can promote increases in the number of peripheral Treg cells and reduce the proliferation of effector T cells77 (Fig. 1). IDO expression in macrophages also supports their anti-inflammatory and phagocytic activities by producing kynurenine that fuels the synthe- sis of NAD+ (reF.78). This finding suggests that inhibiting IDO might alleviate the M2-like phenotype of TAMs. Intriguingly, findings in a preclinical model of breast cancer

demonstrate that therapeutic vaccination with tumour antigen-loaded, IDO-silenced DCs increases the proliferation and cytotoxic activity of antigen- specific T cells and reduces the abundance of Treg cells, as compared with an IDO-expressing DC vaccine79. Furthermore, systemic IDO inhibition promotes tumour regression by increasing the production of cytokines, including IL-12 and IFNγ, and tumour infiltration of T cells and neutrophils in mouse metastatic liver tumour and bladder tumour models80,81. The first IDO1 inhibi- tor, 1-methyltryptophan (which is a mixture of the two racemic isoforms and a weak IDO inhibitor), has been shown to alleviate immunosuppression in the TME and promote the activation of tumour-specific T cells in pre- clinical models82. Following these encouraging findings, the IDO inhibitor indoximod (the 1-methyl-d-trypto- phan racemer of 1-methyltryptophan, which inhibits IDO1 and IDO2), the IDO1 inhibitor navoximod (also known as GDC-0919 and NLG919) and the dual IDO1– TDO inhibitors HTI-1090 (also known as SHR9146) and DN1406131 have been developed and entered clin- ical testing. The outcomes of combined treatment with indoximod plus the anti-CTLA-4 antibody ipilimumab (NCT02073123), or the anti-PD-1 antibodies nivolumab or pembrolizumab (NCT02073123 and NCT03301636), are being assessed in patients with melanoma (Table 1). In a phase Ib, dose-escalation part of one of these stud- ies (NCT02073123), no dose-limiting toxicities were observed with indoximod plus ipilimumab, and a rec- ommended phase II dose of this combination was deter- mined83. Moreover, indoximod is also being tested in combination with therapeutic anticancer vaccines across several tumour types (NCT02460367, NCT01302821, NCT01042535 and NCT01560923). Navoximod is currently being evaluated in clinical trials, including in combination with the anti-PD-L1 antibody atezolizumab (NCT02471846). HTI-1090 is also being investigated in combination with the anti-PD-1 antibody camre- lizumab (also known as SHR-1210; NCT03491631). In addition to these and other IDO inhibitors that are undergoing clinical testing, mostly in combination with ICIs (Table 1), the highly potent and selective IDO1 inhibitor epacadostat (INCB024360) has progressed the furthest along the clinical development pathway84. This agent is being evaluated in combination with ICIs in phase I–III trials involving patients with various advanced-stage malignancies (Table 1). However, the phase III ECHO-301/KEYNOTE-252 trial of epaca- dostat plus pembrolizumab in patients with unresect- able or metastatic melanoma did not meet its primary objective of an improvement in progression-free sur- vival compared with pembrolizumab plus placebo and was unlikely to meet the co-primary end point of an improvement in overall survival85. Chronic inflamma- tion in tumours could result in local immunosuppres- sion via upregulation of IDO expression and, therefore, the therapeutic benefit of combined treatment with IDO inhibitors and ICIs, such as pembrolizumab, might be affected by the pre-existing inflammatory status of the tumour. Thus, assessment of the expression levels of IDO in tumours and the relationship between IDO expression and T cell infiltration before treatment are likely to be essential to optimizing the use of this thera- peutic approach. Notably, in macrophages, kynure- nine production supports mitochondrial fitness and OXPHOS78; thus, IDO inhibition might impair NAD+ generation not only in immunosuppressive and/or pro- tumour TAMs but also in CD8+ TILs. Importantly, pro- duction of NAD+ is implicated as a crucial event that sustains T cell immune responses86,87.

The oncometabolite 2-hydroxyglutarate. A large per- centage of gliomas express neomorphic mutant forms of isocitrate dehydrogenase 1 (IDH1) or IDH2 that sup- port tumorigenesis. IDH1 mutation has been reported to be detected in 80% of patients with

grade II–III gli- omas and secondary glioblastomas88–90. In addition, IDH2 mutations are detected in gliomas88,91, although much less commonly than IDH1 mutations. IDH1 or IDH2 mutations are also detected in ~20% patients with AML92. Whereas wild-type IDH enzymes convert isocitrate to α-KG, the IDH mutations cause a change in enzymatic activity that results in the conversion of α-KG to the oncometabolite d-2-hydroxyglutarate (D-2-HG), accumulation of which alters epigenetic regulation of gene expression and contributes to mTOR activation93. The discovery of IDH mutations and their role in onco- genesis has led to the development of novel therapeutic strategies predicated on either inhibiting mutant IDH or restoring wild-type IDH function in order to suppress the production of D-2-HG. For example, ivosidenib (also known as AG-120) and enasidenib (AG-221) are first-in-class, oral, selective, potent, reversible, small- molecule inhibitors of the mutant IDH1 and IDH2 enzymes94,95, respectively. Ivosidenib and enasidenib have been approved for treatment of patients with AML harbouring IDH1 or IDH2 mutations, respectively96,97. Interestingly, emerging evidence suggests that D-2-HG also affects immune cell behaviour98,99. A gene expression study of tumour samples from patients with low-grade glioma revealed lower expression of CTL-associated and IFNγ-inducible chemokine genes100. In addition, the results of preclinical modelling studies demonstrated that expression of mutant IDH1 suppressed T cell infil- tration into gliomas, while treatment with IDH-C35, a specific inhibitor of mutant IDH1, restored T cell tumour infiltration100. Moreover, IDH1 mutations have been shown to reduce leukocyte chemotaxis, thus con- tributing to tumour-associated immunosuppression101. One clinical trial (NCT03684811) of FT-2102, a selective inhibitor of mutant IDH1, combined with nivolumab is currently being conducted. However, further inves- tigation is needed to determine whether inhibition of mutant IDH can restore antitumour immunity.

Itaconate

Itaconate, a derivative of citrate, is produced by immune- responsive gene 1 (IRG1)-mediated decarboxylation of the TCA cycle intermediate cis-aconitate in mitochon- dria. Stimulation with lipopolysaccharide strongly pro- motes itaconate production in macrophages, which reduces the production of pro-inflammatory cytokines via inhibition of succinate dehydrogenase and activation of nuclear erythroid 2-related factor 2 (NRF2) and acti- vating transcription factor 3 (ATF3)102–105. These find- ings reveal that itaconate orchestrates metabolic and transcriptomic programmes favouring M2-like macro- phage phenotypes. The phenotypes of macrophages in vivo are more varied and complex than those of in vitro-polarized macrophages106, but M2-like pheno- types have generally been shown to support tumour progression. Thus, the production of itaconate in TAMs might confer them with M2-like phenotypes. In sup- port of this hypothesis, administration of a lentivirus harbouring short hairpin RNAs (shRNAs) against Irg1 (also known as Acod1) to mice harbouring B16 mela- noma or ID8 ovarian carcinoma reduced peritoneal tumour burdens in association with suppression of the M2-like phenotype of TAMs107. Whether re-education of TAMs directly contributes to tumour regression in these models remains unclear, but these findings sug- gest that targeting itaconate production is a promising approach to the treatment of cancer. However, the roles of tumour cells in modulating itaconate production in TAMs and the global effects of itaconate in fostering an immunosuppressive TME remain to be explored.

Adenosine signalling

The concentration of adenosine in tissues is markedly increased within a few hours following tissue injury, as well as in hypoxic tissues and the TME108. The ecto- nucleotidases CD39 (also known as NTPDase 1) and CD73 (5′-NT) are cell surface molecules with pivotal roles in controlling the production of adenosine through the catabolism of ATP to AMP and AMP to adenosine, respectively109. The resulting extracellular adenosine can bind to any of four G protein-coupled purinergic type 1 receptors (adenosine receptor A1 (A1R), A2AR, A2BR or A3R), which activates the PKA signalling cascade by facilitating adenylyl cyclase-mediated production of cAMP. In particular, activation of A2AR and A2BR is associated with profound immunosuppression during inflammatory diseases110. Elevated expression of CD39 and CD73 in tumours is associated with poor progno- sis in patients with gastrointestinal111,112, gynaecologi- cal113 and non-small-cell lung cancers114. In addition to tumour cells, Treg cells can express CD39 and contribute to immunosuppression in the TME via the adenosine– A2AR signalling axis115. Treg cells have also been shown to support dissemination of melanoma cells in mouse models via CD39-dependent abrogation of NK cell- mediated antitumour immune responses, whereas the ecto-nucleoside triphosphate diphosphohydrolase inhib- itor polyoxometalate-1 suppressed tumour growth116. M2-like macrophages also express CD39 and CD73 (reF.117) and thus exert anti-inflammatory functions via the A2AR and A2BR signalling pathways118. In T cells, adenosine signalling via A2AR inhibits the expression of the IL-2 receptor and TCR-stimulated proliferation119 and promotes the expression of inhibitory immune- checkpoint receptors (including PD-1 and CTLA-4)120,121, thereby impeding T cell effector function and proba- bly antitumour immunity. Furthermore, A2AR signal- ling stimulates the expression of PD-L2 (also known as B7-DC) and IL-10 in DCs122, which might increase the capacity of these DCs to suppress T cell antitumour responses. Adenosine signalling via A2AR also facilitates the accumulation of MDSCs in mouse tumours and their production of VEGF, and, accordingly, pharmacological inhibition of A2AR reduced angiogenesis and increased T cell accumulation in the TME123. Thus, targeting CD39 and CD73 activity to inhibit adenosine production is an attractive strategy for enhancing antitumour immunity. Indeed, Cd39-deficient mice have decreased pulmonary metastasis in a melanoma engraftment model compared with their Cd39-wild-type counterparts124,125. In addi- tion, genetic ablation or pharmacological inhibition of CD73 reduced the migratory capacity of breast cancer cells in mice in an adenosine-dependent manner126–128. A number of preclinical studies have revealed that treat- ment with antagonistic anti-CD73 antibodies improves the outcomes of ICI therapy in preclinical models120,129. Similarly, combined treatments with A2AR antagonists and ICIs can also elicit synergistic antitumour responses in mouse models130,131. Several clinical trials have been initiated to test the safety and efficacy of targeting the adenosinergic signalling pathway using various different classes of agent in combination with ICIs in patients with cancer (Table 1). Initial evidence from one phase I/Ib trial (NCT02655822) has revealed that the A2AR inhib- itor CPI-144 is associated with a high rate of disease control in patients with refractory renal cell carcinoma (RCC), as a monotherapy (disease control rate of 60%) and as a combined treatment with the anti-PD-L1 antibody atezolizumab (disease control rate of 100%)132.

The cyclooxygenase and PGE2 pathway Prostaglandin E2 (PGE2) is a bioactive lipid metabolite derived from cyclooxygenase-mediated arachidonic acid metabolism that elicits a wide range of biological effects associated with inflammatory diseases133–135. In contrast to cyclooxygenase 1 (COX1), which is con- stitutively expressed in nonmalignant tissues, COX2 is overexpressed in numerous cancers and strongly associated with

immunosuppression and production of a high level of PGE2 in the TME136. Preclinical studies have revealed that PGE2 overproduction promotes the development and differentiation of Treg cells137–139, inhib- its IL-2 and IFNγ production in human T cells140 and skews activated T cells towards a phenotype associated with the generation of high levels of anti-inflammatory cytokines, including IL-4, IL-10 and IL-13 (reF.141). PGE2 also promotes M2-like differentiation of TAMs142 and the immunosuppressive functions of MDSCs143. In mouse models, the abundance of PGE2 in the TME further impedes T cell infiltration by abrogating NK cell- mediated recruitment of conventional type I DCs, thus contributing to cancer immune evasion144. Moreover, PGE2 signalling has been shown to inhibit CTL sur- vival and function145. Together, these findings suggest that inhibition of PGE2 production and signalling cas- cades could improve multiple facets of the antitumour immune response.

In support of this theory, aspirin (acetylsalicylic acid) inhibits COX1 and COX2 and thus PGE2 biosynthesis, with considerable evidence supporting the potential of this agent to suppress tumorigenesis, particularly of colorectal cancer (CRC)146,147. Celecoxib, a selective COX2 inhibitor, induces synergistic antitumour immune responses when combined with anti-PD-1 antibody therapy in mouse tumour models148. Clinical trials of COX inhibitors in combination with ICIs have been ini- tiated (Table 1). In addition to antagonizing the activity of COX2, extensive effort has been devoted to elucidating PGE2 signalling pathways of tumours, with the goal of identifying selective inhibitors of PGE2 receptors for use in anticancer therapy. Notably, expression of PGE2 recep- tor 4 (EP4) is associated with poor prognosis in patients with cancer and suppressive features of the TME149–154. Several EP4 antagonists (AH23848, ONO-AE3-208 and GW627368X) have been developed and tested in preclinical animal models of cancer155,156. Grapiprant, a selective antagonist of EP4, is currently being evaluated in patients with non-small-cell lung adenocarcinoma or microsatellite-stable CRC, both as a monotherapy and combined with pembrolizumab (Table 1).

Fatty acids and cholesterol

Tumour cells often have increased rates of de novo fatty acid synthesis to divert energy production into anabolic pathways for the generation of plasma membrane phos- pholipids and signalling molecules157. Moreover, adipo- cytes and adipocyte-derived fibroblasts can be identified in the TME and contribute to the increased lipid content of the TME158,159. Lipid accumulation in tumour-infiltrating myeloid cells, including MDSCs, DCs and TAMs, has been shown to skew these immune cells towards immuno- suppressive and anti-inflammatory phenotypes via metabolic reprogramming160–163. CD8+ TILs with high levels of PD-1 expression isolated from patients with non-small-cell lung carcinoma also have an increased lipid content compared with CD8+ TILs with lower or no PD-1 expression164. Notably, however, these PD-1-high TILs had a higher capacity to recognize tumour cells and were predictive of favourable survival after treatment with PD-1 inhibitors164. These findings suggest that lipid metabolism in tumour cells and immune cells in the TME has a crucial role in orchestrating immunosuppression and warrant targeting of these metabolic pathways as an approach to enhancing antitumour immunity. Most pharmacological inhibitors of fatty acid and cholesterol metabolism were developed to dampen autoimmunity in the context of autoimmune diseases including systemic lupus erythematosus165 and graft-versus-host disease166, but strategies to increase the activity of these pathways might improve antitumour immunity.

Effective TCR clustering and formation of the immuno- logical synapse are essential for T cell function and are dependent on the lipid composition of cell mem- branes167,168. Accordingly, disrupting cholesterol ester- ification using the sterol O-acyltransferase 1 inhibitor avasimibe has been reported to increase the fraction of cholesterol in the plasma membranes of CD8+ TILs and to improve T cell effector function and proliferation169. Of note, avasimibe synergizes with PD-1 inhibitors in eradicating melanoma in mouse models169. Drugs that increase FAO through activation of peroxisome proliferator-activated receptor (PPAR) signalling have similar effects170,171. Indeed, the reported lipid accumu- lation in CD8+ TILs164 could be suggestive of defective utilization of fatty acids, thus warranting the develop- ment of approaches to stimulate the antitumour T cell responses by promoting FAO. Interestingly, different fatty acids drive the differentiation and proliferation of CD4+ T cells belonging to certain lineages. Specifically, the long-chain fatty acid lauric acid supports the dif- ferentiation of pro-inflammatory TH1 cells and TH17 cells172, whereas the short-chain fatty acid propionate promotes the development of Treg cells172,173. Thus, the lipid species present in the TME are likely to orches- trate the infiltration pattern of effector CD4+ T cells and might determine the outcomes of targeting lipid metabolism for the treatment of cancer.

Lipid metabolism programmes also differ between M1-like and M2-like macrophages. Specifically, fatty acid synthesis predominates in M1-like macrophages, whereas M2-like macrophages are dependent on FAO to fuel their bioenergetic demands5. To date, whether inhibiting FAO or augmenting fatty acid synthesis improves the anti- tumour activity of macrophages has not been established. Interestingly, limiting flux through the cholesterol bio- synthetic pathway in macrophages induces type I inter- feron responses that drive antiviral immunity via both autocrine and paracrine signalling174; however, whether the same responses could be exploited to enhance the antitumour activity of macrophages remains unclear. In contrast to type I interferon responses, inhibiting ATP- binding cassette transporter G1 (ABCG1), which medi- ates cholesterol secretion, shifted macrophages from an M2-like towards an M1-like phenotype, thereby increas- ing their capacity to kill cancer cells in vitro175. Thus, how cholesterol metabolism fine-tunes macrophage behaviour under different conditions remains to be determined. Finally, macrophages of Map3k8-null mice showed impaired M2 polarization, which was associated with decreased lipid catabolism176. Hence, interfering with lipid metabolism in macrophages, including inhibition of CD36-mediated lipid uptake and FAO177, might enhance antitumour immunity.

Metabolic programmes of trained immunity

Trained immunity is a specialized form of immune response, in which training stimuli, such as β-glucan, stimulate a long-term enhancement of the activity of innate immune cells through metabolite-orchestrated epigenetic reprogramming178,179. For example, by edu- cating innate immune cells to produce high levels of pro-inflammatory cytokines, training stimuli can pro- vide protection from a variety of infections178. Training stimuli have been tested in various trials with the aim of eliciting antitumour responses179 (Table 1). These trials are largely supported by the fact that the engagement of trained immunity through Bacillus Calmette–Guérin (BCG) vaccination in patients with non-muscle- invasive bladder cancer180. Given that innate immune cells have important roles in forming the immuno- suppressive TME, stimulating trained immunity in TAMs and/or tumour-infiltrating DCs might synergize with other immunotherapies by reprogramming the TME to become more immunostimulatory. Given that metabolites, including α-KG, acetyl-CoA, succinate,

fumarate and NAD+, are key orchestrators of trained immunity178,181,182, other metabolic interventions dis- cussed herein might synergize with training stimuli in augmenting antitumour immunity.

Metabolic effects of immune checkpoints

ICIs are an outstanding advance in the treatment of can- cer. These therapies were initially developed to enhance the signalling pathways for T cell activation; however, emerging evidence indicates that ICIs also affect the met- abolic fitness of T cells (Fig. 2). Indeed, the findings of several studies further suggest that immune-checkpoint ligation or inhibition influences the metabolic commu- nication and competition between tumour and T cells in the TME. For example, interaction of PD-1 with PD-L1 or PD-L2 impairs metabolic reprogramming, including upregulation of aerobic glycolysis and glutaminolysis, in T cells via suppression of the PI3K–AKT–mTOR pathway183. By contrast, PD-1 signalling promotes FAO in T cells by stimulating AMPK activity and inducing the expression of carnitine palmitoyltransferase 1A (CPT1A), a rate-limiting enzyme of the FAO pathway184. In addition to modulating the metabolic profile of TILs, immune checkpoints can also directly affect metabolism in tumour cells. Expression of PD-L1 and B7-H3 (also known as CD276) in tumour cells has been shown to stimulate aerobic glycolysis by activating the PI3K–AKT–mTOR pathway22,185. Thus, inhibition of the PD-1–PD-L1 axis might have synergistic anticancer effects by promot- ing the reinvigoration and metabolic fitness of TILs while simultaneously suppressing aerobic glycolysis in tumour cells. Consequently, PD-1–PD-L1 inhibition might also increase the amount of glucose available to TILs, which could ameliorate the nutrient stress imposed on TILs by metabolic conditions in the tumour. In support of this hypothesis, a preclinical study has revealed that PD-1– PD-L1 inhibition indeed increases glucose availability in the TME and enhances the glycolytic activity of T cells33.

Other inhibitory immune-checkpoint receptors have been reported to affect the metabolic programmes of T cells. CTLA-4 signalling inhibits CD28-mediated co-stimulation at least in part by reducing AKT phos- phorylation and activation186 and might therefore impair the increased glucose metabolism and mitochondrial remodelling that occurs following T cell activation — similar to the effects of PD-1 signalling. In contrast with the PD-1 pathway, however, CTLA-4 signalling does not augment FAO184. T cell immunoglobulin mucin recep- tor 3 (TIM3; also known as HAVCR2), which is another inhibitory immune-checkpoint receptor that is highly expressed in dysfunctional exhausted T cells, has also been demonstrated to alter T cell metabolism via inter- ruption of PI3K–AKT–mTOR signalling187,188. Moreover, lymphocyte activation gene 3 protein (LAG3)-deficient CD4+ T cells have a substantially increased rate of basal respiration and aerobic glycolysis as well as excess respiratory capacity compared with wild-type CD4+ T cells189, suggesting that LAG3 reduces the metabolic fitness of T cells. Clinical testing of anti-LAG3 or anti- TIM3 agents is underway in numerous trials, including in combination with other ICIs (a list of these trials is beyond the scope of this Review). However, most of the ongoing trials were not designed on the basis of the rationale of reprogramming the immunometabolic pathways of T cells.

In contrast to the metabolic impairments caused by inhibitory immune-checkpoint receptors, co-stimulatory molecules support T cell activation by stimulating sig- nalling pathways that control transcriptional reprogram- ming as well as metabolic switches. For example, CD28 signalling enhances the metabolic fitness of T cells by simultaneously stimulating aerobic glycolysis190 and facilitating mitochondrial fusion, which enables effec-

tive production of acetyl-CoA191 (Fig. 2). Similarly, 4-1BB (also known as TNFRSF9) signalling, which strongly enhances CD8+ T cell proliferation, activates glucose and fatty acid metabolism192. Furthermore, 4-1BB and OX40 (also known as TNFRSF4) dual co-stimulation augments glycolysis in CD8+ T cells as a result of robust induction of glucose transporters193. Activation of glucocorticoid- induced TNFR-related protein (GITR; also known as TNFRSF18) upregulates nutrient uptake, lipid storage, glycolysis and oxygen consumption in CD8+ T cells194. Furthermore, inducible T cell co-stimulator (ICOS) co-stimulation can drive glycolysis in activated T cells via activation of the PI3K–AKT–mTOR pathway195,196. Taken together, a growing body of evidence indicates that ICIs and stimulators of co-stimulatory receptors (such as agonistic anti-OX40 or anti-GITR antibodies) have a major impact on T cell metabolism. Many tri- als of such combinations have been initiated (and are too numerous to list herein). In addition to the many clinical trials in which agonists of co-stimulatory recep- tors are being combined with ICIs alone, a GITR ago- nist is also being tested in combination with PD-1 and IDO inhibition in patients with advanced-stage cancers (NCT03277352; Table 1). Furthermore, a 4-1BB agonist combined with PD-1 inhibition is currently being eval- uated in the context of adoptive cell therapy in patients with metastatic melanoma (NCT02652455). Moreover, an OX40 and a 4-1BB agonist are being combined with a PD-L1 inhibitor in the JAVELIN Medley trial involv- ing patients with various advanced-stage solid tumours (NCT02554812).

Notably, the TME imposes a variety of metabolic stresses on TILs; therefore, the combined use of ICIs and/or co-stimulatory receptor agonists together with metabolic treatments to alleviate glucose deprivation, such as LDHA, MCT1 and/or MCT4 or PFKFB3 inhib- itors, might improve the efficacy of immunotherapy. Thus, more detailed investigations to elucidate the meta- bolic regulatory networks of antitumour immunity are warranted.

Mitochondrial regulation of T cells

TCR stimulation induces mitochondrial biogenesis and remodelling (Fig. 2), which are necessary to fulfil the metabolic requirements of T cell activation197. During CD8+ T cell differentiation, mitochondrial fusion and fission also instruct metabolic programming via cur- rently undefined signalling pathways87. Furthermore, mitochondria physically associate with the immune synapse early after productive TCR activation, which leads to local generation of ATP and stabilizes the immune synapse by modulating calcium signalling198. TCR activation also stimulates production of mito- chondrial reactive oxygen species (ROS), which in turn drives the cell expansion phase of T cell activation199. Intriguingly, most CD8+ TILs have an effector mem- ory phenotype. Following cognate antigen stimulation, CD8+ effector memory T cells are characterized by a rapid mTOR complex 2 (mTORC2)–AKT-dependent upregulation of aerobic glycolysis200. Interestingly, acti- vated mTORC2 and AKT colocalize with and inhibit GSK3β at mitochondria–endoplasmic reticulum junctions; subsequent recruitment of hexokinase 1 to voltage-dependent anion channels on mitochondria promotes pyruvate oxidation, thereby supporting the metabolic requirements and reprogramming necessary for efficient acquisition of effector function by memory T cells201. However, TILs are characterized by a decreased mitochondrial mass compared with peripheral blood T cells and subsequently show a limited respiratory capacity86. Therefore, one can assume that these meta- bolic processes centred on mitochondrial activity and dynamics are compromised in the TME, thus abrogating CD8+ T cell function. The loss of mitochondrial mass in TILs might result from persistent AKT activation in turn leading to progressively decreasing expression of PPARγ co-activator 1α (PGC1α), which co-

activates sev- eral transcription factors, such as PPARγ, NRF1 and/or NRF2 and ERRα, and thereby stimulates mitochondrial biogenesis and FAO86. In experimental models, over-expression86 or induction of PGC1α through 4-1BB stimulation202 rescues mitochondrial function and avoids metabolic exhaustion of TILs, resulting in enhanced antitumour activity. Moreover, combination treatment of tumour-bearing mice with an anti-PD-1 antibody and the pan-PPAR–PGC1α agonist bezafibrate increases mitochondrial biogenesis and OXPHOS in CD8+ T cells isolated from tumour-draining lymph nodes, in associ- ation with increases in the abundance and antitumour activity of effector memory T cells171. By contrast, TILs from patients with RCC have been reported to contain small, punctate, fragmented mitochondria with poorly defined membranes and cristae and an increased inner membrane mass203. These mitochondria are hyper- polarized, leading to excessive production of ROS that suppress T cell antitumour function203. Indeed, in vitro treatment with scavengers of mitochondrial ROS improves the activation and proliferation of TILs from patients with RCC203.

Despite the evidence that the augmentation of mito- chondrial biogenesis and fitness improves antitumour function of T cells86,202,203, whether this strategy stimu- lates OXPHOS in TILs remains unknown. Notably, the hypoxic conditions of the TME might limit the oxidative capacity of TILs and, in combination with low glucose levels, could force TILs to rely on alternative energy sources. Indeed, compared with their counterparts in the circulation, CD8+ TILs have increased expression of the transcription factor PPARα and its target genes involved in fatty acid uptake, triglyceride turnover and peroxisomal and mitochondrial FAO170. Furthermore, enhancement of this metabolic signature using a PPARα agonist enables TILs to maintain efficient antitumour activity in the TME despite being deprived of oxygen and glucose170. Autophagy in CD8+ T cells also has a role in providing lipid substrates necessary for FAO, thus supporting memory CD8+ T cell differentiation and sur- vival204,205. Similarly, autophagy of mitochondria them- selves has been found to be important for the survival of memory CD4+ T cells, mainly by limiting the toxic effects of mitochondrial activity and lipid overload206. This selective autophagy of mitochondria, known as mitophagy207, is also crucial for the clearance of dys- functional mitochondria in NK cells and the induc- tion of trained NK cell-mediated immunity upon viral infection208. Clearly, intact mitochondrial function is essential to mounting an efficient antitumour response. Investigations are therefore warranted to clarify whether autophagy and/or mitophagy in TILs contribute to sup- plying lipids for FAO or to mitochondrial homeostasis and whether pharmacological activation of these pro- cesses could enhance TIL metabolic fitness, survival and antitumour activity.

Metabolic interventions in ACT

Tumour-infiltrating lymphocyte expansion and immunotherapy. ACT of in vitro-expanded autologous TILs has successfully been used to treat patients with cancer209. The isolated TILs are, however, terminally differentiated and might therefore have limited long- term activity. Interestingly, the infusion of T cells with a self-renewing, memory phenotype confers a stronger and more sustained antitumour response in mouse models210 (Fig. 3). Moreover, exposure to IL-15 during in vitro culturing polarizes tumour-reactive CD8+ T cells to a central memory phenotype that is associated with more potent antitumour activity after adoptive transfer to tumour-bearing mice211. IL-15 actively drives a meta- bolic shift towards oxidative metabolism via mitochon- drial biogenesis and expression of the key regulatory enzyme of FAO, CPT1A212. Of note, IL-15-generated memory CD8+ T cells have a

fused mitochondrial net- work, in contrast to the punctate mitochondria of effec- tor cells87. Accordingly, culturing CD8+ T cells with the promoter of mitochondrial fusion M1 and the inhibitor of mitochondrial fission mDivi1 has shown potential as a therapeutic strategy to drive T cells towards a mem- ory phenotype and thereby improve efficacy of ACT immunotherapy87.

An early indication of the crucial role of meta- bolic modulation in activated T cells during a primary immune response came from the observation that mTORC1 inhibition with rapamycin leads to the gener- ation of increased numbers of memory T cells after viral clearance213. In this study213, silencing of the mTORC1 component Raptor phenocopied the effect of rapamycin. Alternatively, inhibition of mTORC2–AKT signalling or glycolysis (the metabolic signature of effector CD8+ T cells) during in vitro expansion of CD8+ T cells can also endow the cells with a memory phenotype and increased antitumour activity214,215. Interestingly, not only naive but also tumour-reactive TILs isolated from patients can be metabolically manipulated with AKT inhibitors during in vitro expansion, resulting in a memory-like phenotype and increased antitumour activity upon allo- geneic transplantation into immunodeficient, multiple myeloma-bearing mice214. These data encourage efforts to integrate such metabolic interventions into current clinical protocols for ACT immunotherapy.

A better understanding of the processes inducing metabolic T cell exhaustion in the TME might also reveal new therapeutic targets. For example, glucose depriva- tion in the TME might cause phosphoenolpyruvate (PEP) insufficiency in TILs23. Correspondingly, increas- ing PEP production in melanoma antigen-specific T cells via overexpression of phosphoenolpyruvate carboxykinase 1 (PCK1) suppressed the activity of sarcoplasmic/endoplasmic reticulum calcium ATPase 3 (SERCA), thereby enabling sustained intratumoural TCR-mediated calcium–NFAT signalling and T cell effector function upon ACT into B16 melanoma- bearing mice23. Tumours can also contain high levels of extracellular potassium, derived from necrotic cells216. TILs consequently have higher intracellular levels of potassium, which inhibits TCR-driven AKT–mTOR signalling and antitumour activity216. Accordingly, increasing potassium efflux via overexpression of the potassium channel Kv1.3 in tumour antigen-specific CD8+ T cells during in vitro expansion can improve the outcomes of ACT in melanoma-bearing mice216. A trial designed to investigate how interventions that alter metabolic processes of T cells could be used in combination with TIL-based ACT immunotherapy is ongoing (NCT02489266).

Chimeric antigen receptor T cell therapy. ACT using autologous T cells genetically modified to express chi- meric antigen receptors (CARs) targeting a specific tumour antigen is a promising therapeutic strategy, with clinical successes resulting in regulatory approvals: the anti-CD19 CAR T cell products tisagenlecleucel and axicabtagene ciloleucel are indicated for the treatment of selected patients with B cell acute lymphoblastic leu- kaemia (B-ALL) or large B cell lymphoma. Similar to the effects observed with ACT of TILs214, AKT inhibition during the ex vivo expansion of anti-CD19 CAR T cells has been shown to alter their metabolism, increase their differentiation towards a memory phenotype and improve their therapeutic activity against B-ALL in immunodeficient mice217. Likewise, treatment of CAR T cells with a PI3K inhibitor in vitro resulted in less- differentiated cells with improved in vivo persistence and antitumour activity in mice218. These findings are in keeping with the roles of AKT–mTOR signalling in promoting a terminally differentiated effector phenotype and increasing glycolytic flux upon T cell activation219. Indeed, inhibition of mTOR or the

glycolytic pathway (using 2-deoxyglucose) also favours T cell differentia- tion towards naive and memory phenotypes, although with a dramatic reductive effect on cell proliferation213,220, thereby potentially limiting the utility of such metabolic interventions in the context of in vitro CAR T cell expan- sion. Intriguingly, PI3K inhibition skews T cell differen- tiation towards naive and memory phenotypes without suppressing CAR T cell proliferation, thus suggesting that memory T cell differentiation does not always reduce proliferative capacity218. The mechanisms under- lying these disparate effects of inhibiting PI3K versus mTOR or glycolysis remain to be determined.

In addition to pharmacological intervention, the co- stimulatory domain used in the CAR construct has also been shown to determine metabolic fitness and persis- tence of the resulting T cell product. Whereas inclusion of a CD28 domain stimulates CAR T cell glycolysis and effector differentiation, use of a 4-1BB co-stimulatory domain induces mitochondrial biogenesis, OXPHOS and subsequent memory T cell differentiation, thus resulting in better in vivo persistence221. Together, these findings strongly suggest that immunometabolism is a key determinant of the outcomes of CAR T cell ther- apy and other ACT approaches. Importantly, the clin- ical protocols of ACT therapy are well suited to rapid elucidation and manipulation of the crucial metabolic machineries of T cells owing to the ability to study can- didate compounds during the in vitro T cell engineering and expansion phases (Fig. 3).

Conclusions

Evidently, targeting of cancer and/or immune cell metabolism can synergize with immunotherapy. Understanding and harnessing metabolic crosstalk in the TME has the potential to increase the often low response rates achieved with immunotherapies. While various combinations of metabolic agents and immuno- therapies are already in clinical trials (Table 1), efforts to better understand the metabolic mechanisms of tumour immune evasion and the metabolic demands of immune cells are essential to fully exploiting the thera- peutic potential of combination therapies. Notably, metabolic programmes also influence antigen presenta- tion and recognition222. Thus, metabolic interventions might not only improve immune cell responses against highly immunogenetic cancers but also increase the immunogenicity of cancer cells, thereby broadening the spectra of cancers that can be effectively treated with immunotherapy.

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Author contributions
All authors made substantial contributions to researching the data, discussions of content and writing of the manuscript and reviewed and edited the manuscript.

Competing interests
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