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Metabolism in tumour-associated macrophages: a *quid pro quo* with the tumour microenvironment

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Tumour-associated macrophages (TAMs) display a high level of functional plasticity and altered metabolism symbolised by high sensitivity to the surrounding tumour microenvironment. The metabolism of TAMs provides novel therapeutic opportunities to treat cancer. https://bit.ly/31OqHhe

ABSTRACT Lung cancer is the leading cause of death from cancer worldwide. Recent studies demonstrated that the tumour microenvironment (TME) is pivotal for tumour progression, providing multiple targeting opportunities for therapeutic strategies. As one of the most abundant stromal cell types in the TME, tumour-associated macrophages (TAMs) exhibit high plasticity. Malignant cells alter their metabolic profiles to adapt to the limited availability of oxygen and nutrients in the TME, resulting in functional alteration of TAMs. The metabolic features of TAMs are strongly associated with their functional plasticity, which further impacts metabolic profiling in the TME and contributes to tumourigenesis and progression. Here, we review the functional determination of the TME by TAM metabolic alterations, including glycolysis as well as fatty acid and amino acid metabolism, which in turn are influenced by environmental changes. Additionally, we discuss metabolic reprogramming of TAMs to a tumouricidal phenotype as a potential antitumoural therapeutic strategy.

Introduction

Lung cancer is the most frequently diagnosed cancer and exhibits the highest mortality rate of all cancers worldwide [1, 2]. However, in the last two decades, lung cancer therapy has been revolutionised from old-fashioned cytotoxic chemotherapies to immune checkpoint therapies, leading to a personalised medicine approach in which the tumour microenvironment (TME) is a key determinant of tumour growth and metastasis rather than a bystander [3, 4].

The lung TME consists of a heterogeneous cell population that includes cancer cells, stromal cells and immune cells [3, 5]. Evolutionary game theory has been applied in the context of cancer, which indicates that cancer cells are the dominant tumour cells. Crosstalk between cancer cells and other non-malignant cells demonstrates a pivotal role in tumourigenesis [6, 7]. Studies from our group clearly demonstrate that tumour-associated macrophages (TAMs) play a central role in lung cancer growth and metastasis, with bidirectional cross-talk between macrophages and cancer cells *via* the C–C chemokine receptor type 2 (CCR2) and CX3C chemokine receptor 1 (CX3CR1) signalling as a central underlying mechanism [5, 8]. In the same line, it has been demonstrated that depletion of TAMs through different approaches such as colony stimulating factor 1 (*Csf1*) inactivation, CSF1 receptor (CSF1R) antibodies and clodronate liposomes diminishes angiogenesis and tumour growth in different tumour models [9, 10]. Interestingly, the combination of TAM-targeted therapies such as CSF1R inhibition with immune checkpoint therapies not only reduces TAMs infiltration and their immunosuppressive phenotypes, but also improves the response to immune checkpoint therapies by completely impaired tumour growth and even regression of the established tumour [11]. In addition, crosstalk between lung cancer cells and T-helper (Th)9/Th17 lymphocytes plays a major role in lung cancer cell epithelial mesenchymal transition, thereby promoting migration and metastasis [12]. It has been shown that other immune cells such as dendritic cells and neutrophils have functional roles in tumourigenesis. In this line, dendritic cells render an immunosuppressive phenotype to TME by modulation of T-cell differentiation and proliferation through secretion of transforming growth factor-β and indoleamine 2,3-dioxygenase (IDO), respectively [13]. Tumour-associated neutrophils are also one of the prominent immune cells within TME that have significant impacts on tumour initiation and progression through production of reactive oxygen species (ROS)/reactive nitrogen species and proteases, which not only support cancer cell transformation but also angiogenesis and metastasis [14]. In the TME, cells demonstrate limited access to oxygen and nutrients, due to disorganised surrounding blood vessels. In this metabolically unstable environment, cells must adapt their metabolic profiles. Therefore, metabolic crosstalk between different cell types for oxygen and nutrients is not only necessary for individual cell survival, but is also a key determinant of cancer cell maintenance and growth and/or modification of the microenvironment to promote cancer cell survival [15].

Metabolic crosstalk between cancer cells and immune cells is involved in the immunosuppression of tumours. Cancer cells produce lactic acid and IDO, which inhibit T-cell proliferation and function. Effector T-cells and cytotoxic T-cells compete with cancer cells to reprogram their metabolic profiles towards glycolysis. However, according to Darwinian fitness, only one winner can exist. Thus, cancer cell survival is primarily due to their pro-tumour phenotype and T-cell exhaustion [3]. Additionally, metabolic crosstalk between cancer cells and regulatory T-cells (Tregs) or myeloid-derived suppressor cells leads to modification of immune cell metabolism, which enables immune cells to use cancer cell metabolites [16].

TAMs are one of the most abundant immune cells in the TME of lung cancer and other types of cancer. The abundance of TAMs in the TME correlates with poor prognosis and disease progression in nonsmall cell lung carcinoma, whereas a higher abundance of TAMs in colorectal cancer is associated with better clinical outcomes [17, 18]. These clinical studies are consistent with the protumoural and antitumoural functions of TAMs in *in vivo* and *in vitro* tumour models. In terms of protumoural function, depletion of TAMs by clodronate liposomes and macrophage fas-induced apoptosis transgenic mice showed decreased tumour growth [8]. In addition, protumoural TAMs contribute to tumour progression by supporting angiogenesis and suppressing antitumoural activity in immune cells [19]. Importantly, a growing body of evidence show that TAMs can reduce the efficiency of immune checkpoint therapies; thus, targeting TAMs can potentiate checkpoint inhibitor therapies [20]. In contrast, antitumoural TAMs can impede tumour development by producing pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and reactive oxygen intermediates [21]. However, we do not have a clear picture of TAM metabolism during tumour initiation and progression, and we do not know whether the TAM metabolic profile is associated with pro- and/or antitumoural functions, especially in lung cancer.

Lung macrophages are categorised by their embryogenic origin and anatomical location in adult lungs and can include yolk sac-derived primitive interstitial macrophages, fetal liver-derived alveolar macrophages and bone marrow-derived definitive interstitial macrophages. Alveolar macrophages are key players in the phagocytosis of foreign bodies and catabolism of surfactants, whereas interstitial macrophages exhibit functional roles in tissue remodelling, haemostasis and antigen presentation [22]. In cancer, despite the complexity and high plasticity of TAMs, the dichotomy has been used to categorise TAMs into classically activated macrophages (M1) that exhibit antitumoural functions and alternatively activated macrophages (M2) that possess a protumoural phenotype. In the simplified metabolic view, M1 macrophages are often considered highly glycolytic with a broken tricarboxylic acid (TCA) cycle and substantial production of ROS. In contrast, M2 macrophages possess an intact TCA cycle that is coupled to oxidative phosphorylation

(OXPHOS) for energy production [23]. Although researchers suggested that TAMs are M2-like macrophages, this binary simplified categorisation cannot be indiscriminately applied to the dynamic TME in which TAMs must adopt their metabolic profile based on the availability of oxygen and nutrients [24]. Here, we discuss TAM metabolism and its influence on the TME *via* crosstalk with cancer cells.

TAM metabolism configures the TME and the TME determines the TAM phenotype
Glucose metabolism
TAM glycolysis is associated with TAM recruitment and tumourigenesis. TAMs are mainly derived from circulating monocytes that infiltrate tumour sites in response to chemoattractants, such as chemokines and proinflammatory signals [25]. TAMs encounter a gradual decline in oxygen during extravasation from the blood vessel, which demonstrates a high oxygen availability, to the TME, which exhibits many hypoxic areas [26]. This capacity to migrate is dependent on glycolysis, because inhibition of macrophage glycolysis by dichloroacetic acid strongly diminishes macrophage migration [27]. In addition, dichloroacetic acid profoundly decreases macrophage migration to implanted matrigels in a lung tumour xenograft model [27]. As cellular migration is energetically demanding during this process, adenosine triphosphate (ATP) production by glycolysis can sustain cytoskeletal remodelling [28]. Consistently, pyruvate kinase muscle2, as the key glycolytic enzyme for ATP production, co-localises with F-actin in macrophage filopodia and lamellipodia, which are involved in macrophage migration [27]. Although the TAM phenotype is unknown in cancer cell initiation, it seems that the glycolytic phenotype is one of the basic instincts of TAMs in the early stages of tumourigenesis. Hence, further studies are needed to address how the glycolytic phenotype of TAMs shapes the TME in the initiation step.

Following the settling of TAMs and tumour establishment, the TME transits to a Th2-type environment in which TAMs are polarised and acquire a protumoural function by being subjected to various factors including interleukin (IL)-4 produced by cancer cells and CD4$^+$ T-cells, colony-stimulating factor-1, and granulocyte-macrophage colony-stimulating factor produced by cancer cells [29, 30]. Based on the hypoxic and nutrient limitation of the TME, TAMs maintain their glycolytic phenotype after migration. A comprehensive proteomic analysis of tumour extract-stimulated bone marrow-derived macrophages (TES-TAMs) shows upregulation of aerobic glycolysis in comparison with bone marrow-derived macrophages [31]. Moreover, the key regulatory glycolytic enzymes, hexokinase 2 (HK2), phosphofructokinase, and enolase1 (ENO1) are upregulated in mouse mammary tumor virus-polyoma virus middle T antigen (MMTV-PyMT) murine tumour model-derived TES-TAMs and primary TAMs [31].

Activated TAM glycolysis is also involved in angiogenesis and tumour metastasis [32]. Co-culture experiments on human peripheral blood monocytes with two human cancer cell types, including pancreatic ductal adenocarcinoma (PDAC) cells and medullary thyroid carcinoma cells, reveal a strong glycolytic profile in TAMs with an increase in the transcription of glycolysis-associated genes and a shift towards aerobic glycolysis in an mammalian target of rapamycin (mTOR)-dependent manner, respectively [33, 34]. In these systems, a reduction in the pro-metastatic capabilities of TAMs by HK2 inhibition reveals that the glycolytic profile of TAMs is a necessary tool that determines the metastatic capabilities of cancers. However, astonishing cooperation occurs in the hypoxic areas of the TME, where TAMs reside in large numbers [35]. Hypoxic TAMs exhibit an increased glycolysis rate accompanied by upregulation of growth factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor, which can sustain tumour growth by inducing angiogenesis and metastasis [35]. A high glycolytic profile is considered a protumoural feature of hypoxic TAMs; however, in lung cancer, modification of the mTOR-REDD1 axis in hypoxic TAMs reprogrammes metabolism towards a higher glycolytic state that can reduce the metastatic burden by inducing glucose competition between TAMs and endothelial cells [36]. This means that the high glycolysis rate is precisely fine-tuned in hypoxic TAMs in a way that not only supports the prometastatic capacity of TAMs, but also maintains the glucose concentration in the perivascular space at a level that can induce endothelial hyperactivation and disorganise the tumour vasculature. Interestingly, TAMs also directly contribute to the induction of tumour hypoxia and glycolysis in cancer cells, which could be induced by activation of 5'-adenosine monophosphate-activated protein kinase, which increases glucose uptake and glycolysis flux, and enhances the mitochondrial oxygen consumption rate in TAMs in lung cancer (figure 1) [37, 38]. Therefore, TAM metabolism can force cancer cells to adopt glycolysis as their primary metabolic pathway, thereby rendering an invasive cancer cell phenotype [39].

Our group recently identified an increase in the transcription of glycolysis-associated genes in fluorescence-activated cell sorting of TAMs from human lung tumours compared with counterparts from matched non-tumour tissues. Intriguingly, the TAM glycolytic gene profile was similar between TAMs derived from invasive margins and tumour central compartments (unpublished data). These data suggest that the glycolytic phenotype of TAMs is an independent variable in the status of the TME. Overall, the glycolytic phenotype of TAMs can be considered an innate feature that confers protumoural functions in
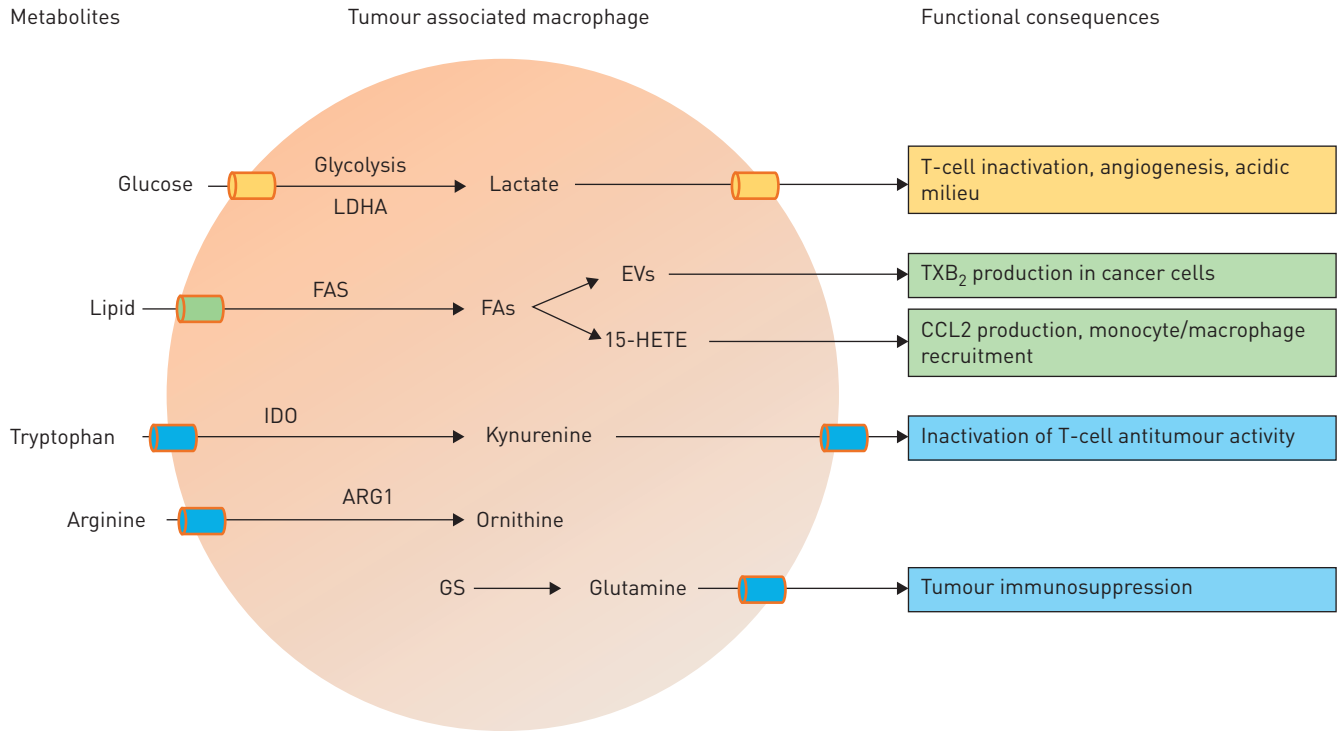
FIGURE 1 Tumour microenvironment (TME) metabolites activate tumour-associated macrophages (TAMs). In TME, glucose, lipid, tryptophan and arginine metabolites activate TAMs. These activated TAMs consume glucose, tryptophan and arginine leading to depletion of these metabolites, which in turn impact TME and its immune status. Moreover, TAM-derived metabolites (*e.g.* lactic acid, kynurenine, glutamine and 15-hydroxyeicosatetraenoic acid (15-HETE)) regulate angiogenesis, T-cell activation and tumour progression. Transporters and/or metabolites of glucose metabolism are indicated as orange, amino acid metabolism as blue and lipid metabolism as green. LDHA: lactate dehydrogenase A; EV: extracellular vesicle; IDO: indolamin-2,3-dioxygenase; ARG: arginase; GS: glutamine synthase; TXB_2: thromboxane B2; FAS: fatty acid synthase; FAs: fatty acids.

TAMs, leading to metabolic cooperation with cancer cells and establishment of a pro-invasive TME. However, further investigations into the contribution of TAM glycolysis in recruitment and function of other immune and stromal cells are required.

The high glycolytic rate of tumour cells results in increased production of lactic acid. Tumour cell-derived lactic acid induces hypoxia inducible factor (HIF)-1α-dependent protumoural polarisation of TAMs and hypoxia is associated with accumulation of protumoral TAMs [119]. When exposed to lactic acid-stimulated macrophages, both murine tumour models using LCC1 and B16-F1 melanoma cell lines resulted in larger tumours compared with co-injection of control medium-stimulated macrophages [40]. To promote neovascularisation, lactic acid induces VEGF production in TAMs by stabilising HIF-1α [40]. Moreover, tumour-derived lactic acid activates mTORC1 to suppress ATP6V0d2-targeted HIF2α degradation in TAMs, leading to M2 polarisation with enhanced HIF2α-mediated VEGF production [41]. Additionally, lactic acid produced by tumours stimulates IL-23 production in TAMs, resulting in tumour growth by inducing the production of IL-17 and IL-22 [42]. Moreover, the acidic TME results from high glycolysis of tumour cells and poor perfusion is characterised as a pivotal factor in tumour progression. Acidity (independent from lactic acid) augments the protumoural polarisation of TAMs in prostate cancer [43]. Tumour cell-derived lactic acid affects glucose metabolism in TAMs. A human macrophage–thyroid carcinoma cell line co-culture model reveal that thyroid cancer-derived lactic acid induces aerobic glycolysis in TAMs through the AKT1/mTOR pathway [33]. Because HIF-1α activation is highly dependent on mTOR activation, this study is in line with the finding that lactic acid produced by tumour cells induces a protumoural phenotype in TAMs by inducing HIF-1α [40]. Apart from the influence of tumour cell-derived lactic acid, enhanced endogenous aerobic glycolysis induced a protumoural phenotype in TAMs. A proteomics analysis demonstrated increased aerobic glycolysis with upregulated HK2, phosphofructokinase and ENO1 in TAMs (figure 2) [31]. Furthermore, increased aerobic glycolysis in TAMs is linked to invasion and metastasis of PDAC [34]. Recently, researchers showed that cancer cell-derived succinate triggers the PI3K–HIF-1α axis in macrophages by activating the succinate receptor, polarising macrophages into protumoural TAMs that induce cancer cell migration and metastasis [44].
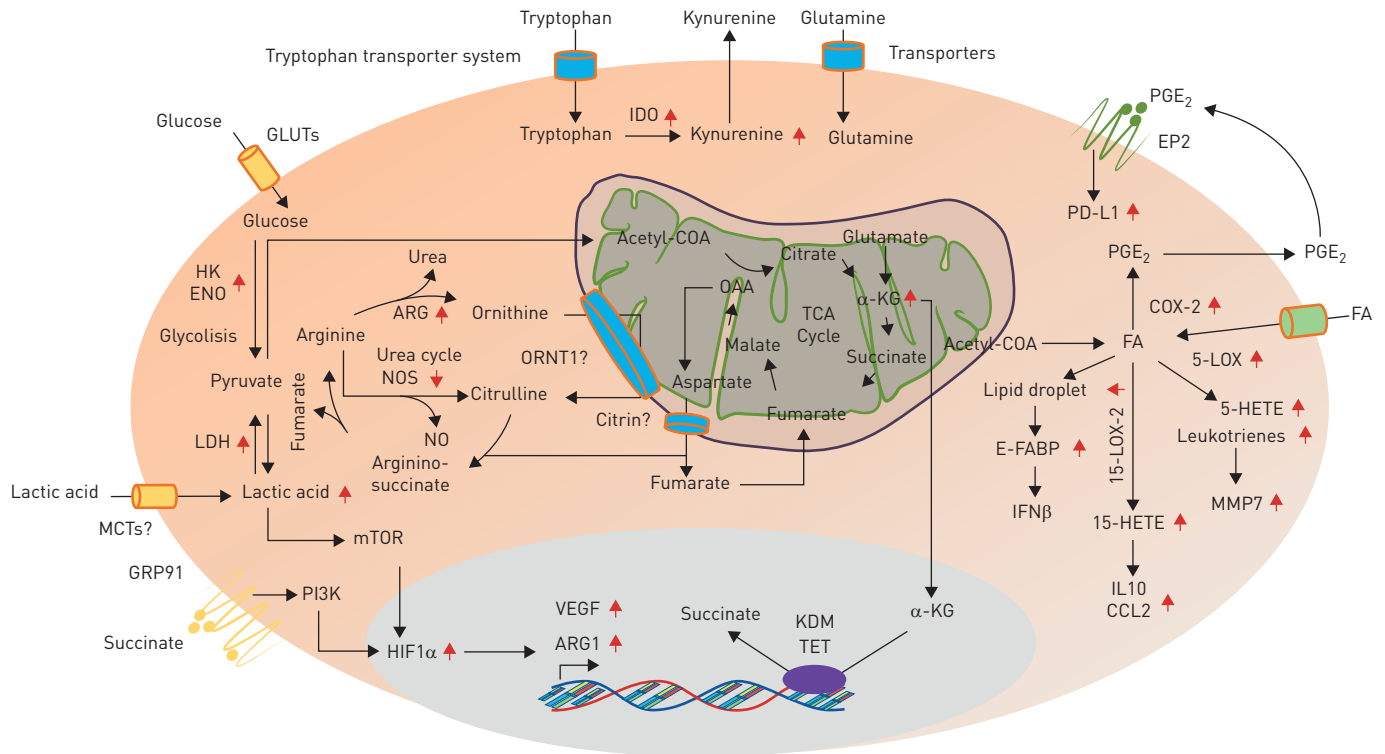
FIGURE 2 Metabolic architecture of tumour associated macrophages. Upward arrows indicate upregulation, downward arrows indicate downregulation and question marks illustrate the potential but inexplicit transporters. Transporters and/or metabolites of glucose metabolism are indicated as orange, amino acid metabolism as blue and lipid metabolism as green. IDO: indolamin-2,3-dioxygenase; GLUT: glucose transporter; HK: hexokinase; ENO: enolase; LDH: lactate dehydrogenase; MCTs: monocarboxylate transporters; ARG: arginase; NOS: nitric oxide synthase; NO: nitric oxide; mTOR: mammalian target of rapamycin; PI3K: phosphoinositide 3-kinases; HIF: hypoxia inducible factor; VEGF: vascular endothelial growth factor; KDM: histone lysine demethylase; TET: ten-eleven translocation; ORNT1: ornithine translocase; OAA: oxaloacetic acid; TCA cycle: tricarboxylic acid cycle; α-KG: α-ketoglutarate; COA: coenzyme A; PGE$_2$: prostaglandin E$_2$; EP2: prostaglandin E2 receptor 2; PD-L1: programmed death-ligand 1; FA: fatty acid; COX-2, cyclooxygenase-2; 5-LOX: 5-lipoxygenase; HETE: hydroxyeicosatetraenoic acid; MMP: matrix metalloproteinase; IL: interleukin; E-FABP: epidermal fatty acid binding proteins; 15-LOX-2: 15-lipoxygenase-2; IFN: interferon; GRP: G-protein-coupled receptor.

Amino acid metabolism

Amino acid metabolism by TAMs is a coadjutor of cancer cells for suppression of the T-cell immune response. The first evidence of this idea emerged four decades ago when it was shown that macrophages could suppress lymphocyte activity by arginine depletion in culture media [45]. Arginine is utilised by macrophages in either nitric oxide (NO) synthesis or in the arginase pathway to characterise M1- and M2-like polarisation, respectively [46, 47]. More precisely, M1 macrophages are characterised by production of inducible nitric oxide synthase (iNOS) to convert arginine into NO and L-citrulline, while M2 macrophages express arginase 1 (ARG1), which hydrolyses arginine to ornithine and urea. Protumoural TAMs express high levels of ARG1, thereby regulating immune evasion of *Helicobacter pylori*, which is one of the main causes of gastric cancer because it restrains macrophage iNOS expression and NO production and limits the generation of pro-inflammatory cytokines such as interferon (IFN)-γ, IL-17a and IL-12 [48]. Dysregulated metabolism of arginine by TAMs promotes tumour growth and development by impairing the antitumoural immune response. TAMs isolated from murine fibrosarcoma reveal immunosuppressive profiling with a low expression of iNOS [46]. In addition, macrophages overexpressing ARG1 demonstrate increased ARG1 activity with attenuated NO production, leading to a growth-promoting effect on breast cancer cells *in vitro* [47]. More importantly, high levels of ARG in TAMs reduce arginine in the TME, which is associated with loss of the ζ-chain of CD3 in antigen-stimulated T-cells, thereby impairing T-cell antitumoural activity in lung cancer [49]. Interestingly, arginine availability can shift T-cell metabolism towards OXPHOS, rendering a higher survival capacity and antitumoural activity in activated T-cells [50]. Overall, it appears that TAMs, by depletion of arginine in the TME, not only suppress T-cell antitumoural activity, but also force T-cells to adopt glycolysis as their primary metabolic pathway. This is especially pronounced in hypoxic TMEs and in TMEs with high lactic acid levels, as TAMs show higher ARG expression; thus, arginine is reduced in the TME [51].

Tryptophan is another amino acid whose immunoregulatory role was discovered approximately two decades ago when researchers showed that IDO inhibition induced rapid T-cell-associated rejection of the allogeneic conceptus in mice [52]. A year later, the same group revealed that degradation of tryptophan by macrophage IDO suppressed T-cell proliferation *in vitro*, and the inhibition of IDO activity in macrophages reversed the anti-proliferative effect of macrophages on T-cells [53]. IDO is the first and rate-limiting enzyme in the kynurenine pathway, which converts tryptophan into *N*-formylkynurenine [54]. TAMs in different human tumour types, such as lung cancer, express high levels of IDO. The inhibitory effect of TAMs on T-cell proliferation and cytokine production (*e.g.* IFN-γ, TNF-α and IL-2) can be restored by adding exogenous tryptophan or inhibiting IDO [55]. Therefore, consumption of tryptophan in the TME by TAMs can deprive T-cells of tryptophan, which is necessary for biomass generation in activated T-cells, leading to accumulation of kynurenine as an immunosuppressive metabolite in the TME.

Glutamine is a well-known amino acid, which is particularly recognised for its role in the glutaminolysis pathway in cancer cells [56]. Glutamine synthase (GS) is the only enzyme in humans that is known to produce glutamine from glutamate. Interestingly, TAMs from human glioblastoma also exhibit upregulation of genes that are related to the transport and synthesis of glutamine, such as glutamate–ammonia ligase (*GLUL*). Specific knockout of GS in macrophages reduces the metastatic rate in the LLC1 murine model. Although no significant change occurs in the TAM infiltration rate, GS knockout mice-derived TAMs are generally major histocompatibility complex class II^{high} and CD206^{low} compared with their wild-type counterparts [57]. With regard to the TME, TAM-deficient GS enhances the level of intratumoural cytotoxic CD8$^+$ T-cells. Moreover, GS knockout mice show increased vascular integrity, which was concomitant with a reduction in tumour hypoxia [57]. Overall, glutamine production by GS renders a protumoural phenotype in TAMs. However, glutamine and glutamate crosstalk between TAMs and cancer cells should be investigated more precisely because TAM-associated glutamine can be used to replenish the TCA cycle in cancer cells (figure 1) [58]. Furthermore, because glutamine uptake and metabolism is necessary for T-cell activation, competition between cancer cells and T-cells for TAM-associated glutamine indicates another level of cooperation for metabolites [59].

Amino acid-restricted TAMs exhibit an antitumoural phenotype with reduced TAM infiltration, tumour growth, and an increased response to immunotherapies in syngeneic tumour models of prostate and renal cell carcinoma (RCC) [34]. Hence, amino acid metabolism in TAMs exhibits a fundamental influence on phenotypic polarisation. Kynurenine is produced by tumour cells as a result of the enzymatic activity of IDO-1 and tryptophan 2,3-dioxygenase. Glioblastoma cell-derived kynurenine activates the aryl hydrocarbon receptor (AHR) in TAMs. By enhancing CCR2 expression, the AHR promotes TAM recruitment. Additionally, AHR drives protumoural polarisation of TAMs by upregulating Krüppel-like factor 4 and suppressing nuclear factor (NF)-κB activation [60]. Furthermore, glioblastoma cells secrete high levels of glutamate into the extracellular TME. TAMs isolated from human glioblastomas and co-cultured with glioblastoma cells display an immunosuppressive profile with upregulation of genes related to glutamate transport and metabolism, indicating that glioblastoma cell-derived glutamate might be utilised by TAMs to generate a protumoural phenotype [61]. The production of α-ketoglutarate *via* glutaminolysis is important for M2 macrophage activation; however, whether intrinsic glutamate deprivation enhances the antitumoural phenotype of TAMs requires further investigation [62]. Moreover, hypoxia-induced semaphorin 3A acts as an attractant for TAMs by triggering VEGF receptor 1 phosphorylation through neuropilin-1 (Nrp1) and plexin A1/plexin A4. Depletion of Nrp1 in TAMs is related to upregulation of iNOS and downregulation of ARG1 (figure 2) [63]. Therefore, hypoxia in the TME is one factor that enhances activation of the arginase pathway in TAMs. Researchers reported that TAM-derived IL-1β and TNF-α regulate arginine metabolism in neuroblastoma cells [64]; however, further studies are required to elucidate the cancer cell-derived triggers that affect arginine metabolism in TAMs.

Lipid metabolism

The lipogenic phenotype is one of the metabolic hallmarks of cancer. Fatty acid metabolism plays a pivotal role in regulating cancer cell survival and therapeutic resistance [65]. In addition to secreting leptin and transforming growth factor-β, which enhances cancer cell malignancy, adipocytes in the TME are a major lipid source [66]. Fatty acids are important substrates that are used in cellular membrane architecture, energy production and generation of signalling molecules. Additionally, lipid mediator products of fatty acids were reported to stimulate resolution in cancer by enhancing macrophage phagocytosis of tumour cell debris and counter-regulating the secretion of pro-inflammatory cytokines by macrophages [67].

Although glucose and amino acid metabolism mostly renders the protumoural phenotype of TAMs, metabolism of fatty acids in TAMs can act as a double-edged sword that can confer a protumoural function to TAMs and can lead to an antitumoural phenotype. Antitumoural TAMs express the epidermal

fatty acid-binding protein (E-FABP), which is a lipid chaperone that can coordinate the distribution and metabolism of intracellular lipids. Upregulation of the E-FABP modulates the inflammatory response of TAMs, especially by increasing the activity of the IFN-β signalling cascade. Given that the E-FABP is involved in lipid metabolism, inhibition of lipid droplet formation impairs IFN-β production in macrophages, which illustrates an E-FABP–IFN-β lipid droplet axis in protumoural TAM activation [68]. Furthermore, tumours from E-FABP$^{-/-}$ mice contained a significantly higher percentage of CD4$^+$, CD8$^+$ T-cells, and natural killer cells in the early stages (*e.g.*, 1 week after tumour implantation) [68]. Therefore, it seems that E-FABP-associated lipid metabolism of TAMs is involved in the antitumoural immune response, especially in the initial stages of tumour formation by arming TAMs with IFN-β production and enhancement of immunoprotective cell recruitment to the tumour site.

Supporting the antitumoural function of lipid metabolism in TAMs, researchers recently showed that TAM extracellular vesicles (TAM-EVs) composed an immunostimulatory molecular pattern similar to antitumoural macrophages [69]. Lipid metabolism, especially the arachidonic acid pathway, is enriched in TAM-EVs. Notably, cyclooxygenase (COX)-1, thromboxane A synthase-1, and certain cytochrome P450 epoxygenases are upregulated in the proteome signature of TAM-EVs [69]. Interestingly, TAMs exert antitumoural functions by rewiring the arachidonic acid catabolic pathway in cancer cells through TAM-EVs, to induce TXB$_2$ production and reduce prostaglandins [69]. This finding shows that lipid metabolism in the TME is fine-tuned by cellular crosstalk in which lipid metabolites exhibit intrinsic immune properties and can regulate the metabolic profile of other cell types. However, it is unknown how TAMs communicate with other immune cells through extracellular vesicles. Lipid metabolism also confers a protumoural phenotype to TAMs. RCC-derived TAMs produce a high amount of eicosanoid 15-hydroxyeicosatetraenoic acid (15-HETE), which is synthesised by 15-lipoxygenase (15-LOX). Conversely, the level of prostaglandin E$_2$ (PGE$_2$) was the same in RCC-derived TAMs and a normal kidney. RCC-derived TAMs show a higher expression of the 15-LOX2 isoform, concomitant with lower expression of COX-2, which is responsible for PGE$_2$ production. Interestingly, inhibition of 15-LOX2 rather than COX-2 reduces IL-10 and CCL2 production by TAMs and consequently impairs the TAM protumoural phenotype [70]. The 5-LOX pathway has also been shown to be involved in lung cancer, with 5-LOX-expressing alveolar macrophages increased in the lungs of human hepatocellular carcinoma patients with lung metastasis by producing leukotriene B$_4$, a potent tumour growth promoting mediator [71]. In a mouse model, deactivating myelocytomatosis (MYC) lowered 5-LOX mRNA levels and inhibiting 5-LOX *in vivo* reduced leukotriene B$_4$ levels as well as lung tumour burden [72]. The extracellular vesicles from lung cancer pleural exudates were shown to transform cysteinyl-leukotriene C$_4$ (LTC$_4$) to LTD$_4$, which stimulated cancer cell migration and survival [73]. Accordingly, the pro-metastatic effect of exosomes can be mediated by the leukotriene machinery, further supporting the use of CysLT1 receptor antagonists for lung cancer therapy (*e.g.* montelukast). Additionally, inhibition of lipid droplet formation *in vitro* can reduce the antitumoural capability of TAMs [68]. Furthermore, targeting lipid droplet formation in TAMs through reduction of fatty acid transportation into lipid droplets impairs tumour growth in murine models by decreasing the proportion of protumoural TAMs in tumours [74]. Recently, it has been shown that TAMs increase the lipid accumulation *via* upregulation of scavenger receptor CD36. TAMs use the accumulated lipids as a source of energy by fatty acid oxidation. Interestingly, inhibition of lipid uptake by blocking CD36 or suppression of fatty acid oxidation in macrophages inhibit the generation of TAMs, thereby reduce the protumour functions of TAMs [75]. Therefore, depending on the tumour stage, TAMs can use lipid metabolism as either a protumoural or an antitumoural tool. Moreover, the fate of lipid precursors can differ based on the phenotype and function of TAMs.

Increased intracellular enrichment of lipids is associated with infiltration and phenotypic switching in TAMs. The effects depend on multiple factors, including lipid content and cancer types and stages. For example, TAMs enriched with the polyunsaturated fatty acid (PUFA) and linoleic acid (18:2) demonstrate antitumoural effects on early stage breast tumours and protumoural effects on human ovarian carcinoma, while TAMs accumulated with the saturated fatty acid, stearic acid (18:0), or the PUFA arachidonic acid (20:4) primarily display protumoural functions [68, 70, 76–80]. In response to linoleic acid treatment, the E-FABP, an intracellular lipid chaperone that is highly expressed in M1 macrophages, was upregulated in TAMs to enhance IFN-β responses by increasing lipid droplet formation in the early stages (*e.g.*, 1 week after tumour implantation) in a murine breast tumour model [68]. Stearic acid did not exert a similar M1 polarisation effect, which is in agreement with the finding that macrophages enriched with stearic acid are not tumouricidal [68, 80]. In contrast, TAMs accumulated in the later stages (on average 3 weeks after tumour implantation) of breast cancer exhibited high adipocyte/macrophage FABP expression, which promoted tumour growth *via* IL-6/STAT3 signalling [81]. Lipid droplet formation in the late stages of murine breast tumour models correlates with TAM protumoural polarisation; caspase-1 inactivates medium-chain acyl-CoA dehydrogenase by cleaving peroxisome proliferator-activated receptor (PPAR)-γ

at Asp64, thereby inhibiting fatty acid oxidation and inducing lipid droplet formation in TAMs [82]. However, it is unclear if adipocyte FABP expression in advanced tumour stages occurs in a lipid droplet formation-dependent manner. Moreover, high concentrations of linoleic acid and arachidonic acid in the TME are agonists of PPARβ/δ and facilitate protumoural polarisation of TAMs [82, 83]. Lipid droplet formation serves as a pool to enrich TAMs with PPARβ/δ ligands, leading to upregulation of PPARβ/δ target genes and polarisation of TAMs to a protumoural phenotype in ovarian carcinoma [76]. In addition to cancer cells, immune cells can also shape the metabolic profile of TAMs. Recently, it has been shown that Tregs can promote protumoural TAMs by modulating lipid metabolism. Mechanistically, Tregs block the inhibitory effect of $CD8^+$ T-cell-associated IFN-γ on sterol regulatory element-binding protein-1 of TAMs, inducing fatty acid synthesis in M2-like TAMs [84].

Increased arachidonic acid metabolism is associated with protumoural polarisation of TAMs. Arachidonic acid can be utilised to synthesise PGE_2 through the activated phospholipase A_2/COX-2/microsomal PE_2 synthase 1 (mPGES1) pathway. PDAC-derived arachidonic acid is delivered to TAMs *via* exosomes, leading to an immunosuppressive protumoural phenotype with increased secretion of protumoural molecules such as PGE_2, VEGF, monocyte chemoattractant protein-1, IL-6 and matrix metallopeptidase (MMP)-9 [77]. In addition, tumour cells can induce expression of programmed cell death protein ligand 1 (PD-L1) in TAMs *via* the COX-2/mPGES1/PGE_2 pathway, which deactivates cytotoxic T-cells and facilitates tumour escape from immune surveillance in murine bladder tumours [78]. Aside from the effect on polarisation of TAMs, arachidonic acid metabolism influences TAM infiltration in the TME. The enzyme 5-LOX converts arachidonic acid into 5-HETE and leukotrienes. Under hypoxic conditions, ovarian cancer cells produce high levels of 5-LOX to promote TAM infiltration, through upregulation of MMP-7 [79]. Similarly, the 15-LOX-2 pathway in TAMs is activated in human RCC, resulting in an increase in the secretion of the arachidonic acid metabolite 15-HETE, the chemokine CCL2, and the immunosuppressive cytokine IL-10 [71]. Monocytes are recruited to the site by binding to CCR2, while IL-10 mediates the development of immune tolerance [23, 70]. This suggests that arachidonic acid directly influences TAM infiltration and polarisation in the TME (figure 2).

Metabolic reprogramming of TAMs to favour a tumouricidal phenotype as a potential antitumoural therapeutic strategy

Specialised tumour metabolism supports cancer cell energetics and plays a critical role in establishing an immunosuppressive TME. Blocking TAM recruitment is one of the approaches that can be used to inhibit tumour progression [23]. 5-LOX metabolites facilitate TAM infiltration, but blocking recruitment of TAMs by manipulating 5-LOX should be further investigated [79]. Nevertheless, repolarising TAMs towards an antitumoural phenotype is more effective than blocking recruitment to prevent tumour growth [85]. β-catenin plays a pivotal role as a regulatory hub in several cellular processes including metabolism [86]. A recent study from our group provides strong evidence that β-catenin-mediated transcription plays a central role in the transition of tumour-inhibiting M1-like TAMs to tumour-promoting M2-like TAMs. Therefore, targeting β-catenin in TAMs may provide a new immunotherapeutic option to reactivate antitumour immunity in the TME of the lung [87].

Activated caspase-1, which causes lipid accumulation and differentiation of TAMs towards a protumoural phenotype, is exclusively detectable in TAMs but not in tumour cells or normal tissues. Hence, caspase-1 inhibition is a potential strategy for reprograming the TME. Caspase-1 inhibitors, including NCX-4016, YVAD and VAD, repolarise TAMs towards an antitumoural phenotype and suppress tumour growth *in vivo* [82]. Given that the COX-2/mPGES1/PGE_2 pathway is involved in the regulation of PD-L1 expression in TAMs, selective inhibition of COX-2, mPGES1 or genetic overexpression of the PGE_2-degrading enzyme, 15-hydroxyprostaglandin dehydrogenase, could alleviate immune suppression and re-establish the antitumour immune response in the TME [78, 88]. Cancer cells promote TAM membrane cholesterol efflux, which is regulated by the ATP-binding cassette transporter (ABC transporter), leading to protumoural programming with inhibition of IFN-γ-induced gene expression in TAMs. Genetic deletion of ABC transporters reverts the tumour-promoting functions of TAMs to an antitumoural phenotype in murine bladder carcinoma and melanoma, as well as in ovarian carcinoma models [89, 90]. Overexpression of ABC transporters confers multidrug resistance through increasing efflux of drugs from cancer cells. However, clinical trials demonstrated that application of ABC transporter inhibitors as chemotherapeutic sensitisers exhibits limited or no benefit for patients with cancer [91]. Additionally, studies suggest that preventing cholesterol efflux in TAMs by targeting the ABC transporter could block the protumoural functions of TAMs to restore antitumoural immunity [89, 90]. Therefore, the development of TAM-specific ABC transporter inhibition strategies might be a potential novel antitumoural therapeutic approach.

Aerobic glycolysis is a hallmark of cancer cells. Tumour cell-derived lactic acid drives TAMs to favour a protumoural state [40, 92]. Nonspecific inhibition of the glycolytic pathway, which involves

downregulation of pyruvate kinase isozyme 2 as a promoter of aerobic glycolysis, in a CT26 colon cancer cell–M2 macrophage co-culture system reverses macrophage M2 polarisation by reducing CD206 and legumain, while inducing STAT1 and TNF-α [93]. Additionally, administration of the pyruvate dehydrogenase kinase inhibitor, dichloroacetate, enhances the tumouricidal activity of TAMs in a murine T-cell lymphoma model. TAMs are one of the major producers of IL-1β, which promotes tumour progression. Production of IL-1β by TAM activation of both the NF-κB and mTOR pathways occurs in a glucose-dependent manner [94]. A prototypical inhibitor of mTOR, rapamycin, repolarises protumoural macrophages towards an antitumoural phenotype by suppressing mitochondrial ROS and NLRP3 inflammasomes, suggesting that targeting upper stream factor of glucose would be beneficial for antitumoural responses in TAMs [95, 96]. Expectedly, the glucose-lowering drug, metformin, reduces M2 polarisation in TAMs in murine pancreatic tumours and osteosarcoma tumour models and reduces IL-1β production [97, 98]. Similarly, inhibition of glucose metabolism in the TME with 2-deoxyglucose, a competitive inhibitor of HK2, impairs IL-1β production [94]. Moreover, instead of inhibiting the glycolytic pathway in the entire TME, specific inhibition of aerobic glycolysis with 2-deoxyglucose in protumoural TAMs is already sufficient to disrupt the prometastatic phenotype of TAMs [99].

Protumoural TAMs are characterised by upregulation of ARG1, which is promoted by the Ron-receptor tyrosine kinase (RTK), which is specifically expressed by protumoural TAMs [34]. This suggests that targeting RTK activity using RTK inhibitors such as sunitinib malate in protumoural TAMs could be therapeutically beneficial. Additionally, specific targeting of ARG in TAMs could exhibit significant therapeutic implications. The ARG1 inhibitor, L-norvaline, reverses the proliferative effect of protumoural TAMs overexpressed with ARG1 *in vitro* [47]. Moreover, anti-PD-1 therapy decreases the expression of ARG1 in TAMs, which is in line with the principle of PD-1 targeting immunotherapy [100]. Given that the rate-limiting enzyme for arginine biosynthesis, arginine-succinate synthetase (ASS1), may be elevated in TAMs, further research should be carried out to investigate the role of ASS1 in channelling arginine to ARG and iNOS (table 1) [101].

Future perspectives

One of the main challenges in targeted cell therapy is finding the molecular pathways, which are specific and unique to the interested cell. This challenge has a higher level of complexity, especially for TAMs by considering their plasticity. Studies on immunometabolism, in particular the metabolism of TAMs, provide novel therapeutic opportunities to TAMs-targeted therapy. In this line, finding metabolism-associated functions in TAMs can open new avenues for TAM-based therapy. Recruitment and migration of TAMs are two energy-consuming pathways. Although it has been shown that glycolysis impacts macrophage migration [22], the involvement of other metabolic pathways especially, OXPHOS and fatty acid oxidation in actin and cytoskeleton rearrangement, as two main migration compartments, need to be investigated during monocyte recruitment into the tumour site [22]. Furthermore, based on significant roles of metabolism in trained immunity, deciphering the metabolism contribution to trained immunity of TAMs can reveal novel specific targets involving TAMs plasticity and epigenetic programming [111]. In regard to the tumour acidosis that is dependent upon metabolic status of TME cellular components [112], it has been shown that TME acidification can induce pro-tumour TAMs whereas the contribution of TAMs in TME acidosis is completely unknown [113]. As modulation of TME acidosis can improve the immunotherapy [114], deciphering the TAMs-associated acidosis can reveal some novel opportunities to use not only for specific targeting of TAMs, but also for combination therapy especially in the immune checkpoint field. TAMs display a high level of functional plasticity that is symbolised by high sensitivity to the surrounding TME for phenotypic alteration. Therefore, aside from interference with TAM survival and inhibition of TAM infiltration, repolarisation of protumoural TAMs towards an antitumoural phenotype is a potential therapeutic approach for cancer.

Several strategies for reprogramming protumoural TAMs exist, such as manipulation of TME stimuli and influencing the NF-κB, mitogen-activated protein kinase/extracellular signal-regulated kinase, and Wnt/ß-catenin pathways [23, 87]. Although evidence shows that metabolic regulation is crucial to phenotypic switching in TAMs, such as metabolic regulation by metformin [97, 98], better characterisation of the mechanisms underlying the intrinsic metabolic signals that drive TAM activation *via* cross-talk with cancer cell-derived metabolites is required.

As Treg cells can regulate TAMs metabolism, our surmise is other TME cellular components also can modulate TAMs metabolism [84]. However, further studies are needed to identify the immune/stromal cells metabolism cross-talk with TAMs. In addition, epigenetic regulation, which is strongly affected by metabolic changes, plays a pivotal role in macrophage polarisation. For instance, α-ketoglutarate is important for regulating Jmjd3-dependent polarisation in M2 macrophages [62]. A need exists for more preclinical studies to identify the epigenetic and metabolic networks that reprogram protumoural TAMs as a means to enhance cancer immunotherapy.

TABLE 1 Selected agents targeting metabolism for treatment of various metabolic or inflammatory diseases and cancer

Drug	Target	Metabolic pathway/consequence	Disease model	Clinical trials/status	Effect on TAMs
Glucose metabolism					
2-deoxyglucose (2-DG)[#]	Hexokinase 2 (glucose uptake)	Inhibition of glucose uptake and therefore aerobic glycolysis	Cancer in general, rheumatoid arthritis	Stopped due to toxicity (hypoglycaemia) [102]	Repolarisation [34]
Enasidenib (AG-221), AG-120 (Ivosidenib), AGI-5198, AG-881,	Mutant IDH1/2	Inhibition of α-KG reduction to 2-HG by mutant IDH leading to impaired demethylation	Acute myeloid leukaemia, bile duct cancer, glioma, haematological malignancies, solid tumours [103, 104]	Enasidenib and ivosidenib approved for acute myeloid leukaemia	
CB-839	Glutaminase 1	Inhibition of glutamin metabolism (increased dependence of glutamine in cancer cells) [105, 106]	Colorectal cancer, NSCLC, renal cell carcinoma, melanoma	NCT03263429, NCT03831932, NCT02771626	
Metformin[#]	AMPK	Reduction in glycolytic pathway, reduced glucose blood levels, increased FAO, inhibition of respiration, inhibition of mTOR	Type II diabetes, cancer in general, rheumatoid arthritis	Approved for type II diabetes, NCT02019979, NCT02640534, NCT01310231, NCT02312661	Repolarisation [97, 98]
Nucleotide biosynthesis					
Methotrexate, Pemetrexed	DHFR, GARFT	Impaired nucleotide biosynthesis	Breast cancer	Phase II trial (methodextrate)	
Amino acid metabolism					
L-asparaginase (Elspar, Kidrolase), PEG-BCT-100 (ADI-PEG20), AEB-1102	Circulating arginine	Breakdown of arginin, targeting cancer cells without ability for arginase *de novo* synthesis (ASS1 silenced cancer types)	Melanoma, hepatocellular carcinoma, acute lymphocytic leukaemia	L-asparaginase approved for acute lymphocytic leukaemia	
Rapamycin, RAD001[#]	mTOR	Deregulation of proliferation and protein/lipid/nucleotide production	ALS, glioma, NSCLC	NCT03359538, NCT01158651, NCT01063478	Repolarisation [95, 96]
L-norvaline, CB-1158[#]	Arginase 1	Disruption of *de novo* arginine synthesis	Advanced solid tumours, Alzheimer's disease models [107]		Repolarisation [47]
PHGDH inhibitors	Phosphoglycerate dehydrogenase	*De novo* serine synthesis	Breast cancer, lung adenocarcinoma, melanoma [108–110]		
Lipid metabolism					
ND-646	ACC	Impaired *de novo* fatty acid synthesis	Lung tumour models [82]		
Pralnacasan, NCX-4016, YVAD, VAD[#]	Caspase-1	Inhibition of inflammasome/lipid accumulation in inflammatory cells	Rheumatoid arthritis, osteoarthritis, inflammatory bowel disorders, cancer, autoimmune diseases	Pralnacasan studies stopped after phase II	Specifically targets and repolarises TAMs [82]
Paclitaxel, Methodextrate, Doxorubicin[#]	ABC transporter	Impaired efflux leading to accumulation of *e.g.* xenobiotics or cholesterol in the cell	Multidrug resistant cancer [91]	Studies ongoing *e.g.* phase III trial for breast cancer (NCT02488967)	Repolarisation [90]
Meclofenamate sodium, Zileuton[#]	5-LOX	Conversion of arachidonic acid to 5-HETE and leukotrienes	Pain relief, rheumatoid arthritis, osteoarthritis, asthma		TME infiltration [79]

TAM: tumour-associated macrophages; IDH: isocitrate dehydrogenase; α-KG: α-ketoglutarate; 2-HG: 2-hydroxyglutaric acid; NSCLC: nonsmall cell lung cancer; AMPK: AMP-activated protein kinase; FAO: fatty acid oxidation; mTOR: mammalian target of rapamycin; DHFR: dihydrofolate reductase; GARFT: glycinamide ribonucleotide formyltransferase; ALS: amyotrophic lateral sclerosis; ACC: acetyl-CoA carboxylase; TME: tumour microenvironment; 5-LOX: 5-lipoxygenase; 5-HETE: 5-hydroxyeicosatetraenoic acid. [#]: potential specific TAM metabolic targets.

Selective targeting of TAM metabolism *in vivo* is an ongoing challenge. As mentioned previously, cichloroacetate exhibits potential in TAM M2-to-M1 reprogramming. However, dichloroacetate also inhibits aerobic glycolysis and induces differentiation of Tregs, which might result in decreased immunosurveillance in cancer therapy [115]. Hence, the therapeutic potential of dichloroacetate might be hampered in the absence of a specific TAM-targeting strategy. Although nanoparticle and liposome-based systems aid in efficient drug delivery to TAMs, the dynamic ability of TAMs to adapt to a specific microenvironment increases the difficulty for *in vivo* metabolic targeting. Therefore, investigations into the metabolic features of TAMs at a spatial and temporal resolution using specialised experimental technologies, such as *in vivo* tracer analysis and single-cell technologies in combination with high-resolution mass spectrometry for accurate metabolite identification, would offer more precise guidance for metabolic regulation [116, 117]. Furthermore, depletion of TAMs generates a less hypoxic TME and reduces tumour glycolysis, leading to increased PD-L1 expression in tumours. As some patients with lung cancer acquire resistance to immune checkpoint therapies and some groups do not respond [118], a combination of immunotherapeutic agents such as PD-L1 inhibitors and TAM metabolic interventions could be beneficial for cancer therapy.

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