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The oncogenic and clinical implications of lactate induced immunosuppression in the tumour microenvironment

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ABSTRACT

The tumour microenvironment is of critical importance in cancer development and progression and includes the surrounding stromal and immune cells, extracellular matrix, and the milieu of metabolites and signalling molecules in the intercellular space. To support sustained mitotic activity cancer cells must reconfigure their metabolic phenotype. Lactate is the major by-product of such metabolic alterations and consequently, accumulates in the tumour. Lactate actively contributes to immune evasion, a hallmark of cancer, by directly inhibiting immune cell cytotoxicity and proliferation. Furthermore, lactate can recruit and induce immunosuppressive cell types, such as regulatory T cells, tumour-associated macrophages, and myeloid-derived suppressor cells which further suppress anti-tumour immune responses. Given its roles in oncogenesis, measuring intratumoural and systemic lactate levels has shown promise as a both predictive and prognostic biomarker in several cancer types. The efficacies of many anti-cancer therapies are limited by an immunosuppressive TME in which lactate is a major contributor, therefore, targeting lactate metabolism is a priority. Developing inhibitors of key proteins in lactate metabolism such as GLUT1, hexokinase, LDH, MCT and HIF have shown promise in preclinical studies, however there is a corresponding lack of success in human trials so far. This may be explained by a weakness of preclinical models that fail to reproduce the complexities of metabolic interactions *in natura*. The future of these therapies may be as an adjunct to more conventional treatments.

1. Introduction

1.1. Tumour microenvironment

Cancer is a disease, not just of the transformed cells, but also of the surrounding stromal and immune cells, extracellular matrix, blood vessels, and the milieu of metabolites, signalling molecules, and proteins in the intercellular space – collectively known as the tumour microenvironment (TME) [1]. Far from being a passive bystander observing cellular transformation, the TME is an active participant as a major regulator of key hallmarks of cancer including altered energy metabolism, tumour-promoting inflammation, angiogenesis, invasion and metastasis [2].

The immune system's relationship with cancer is Janus-faced: on one hand the immune system is entrusted with detection and elimination of rogue cells that subvert normal proliferative controls; while on the other, the immune system can become corrupted and promote cancer development and progression [3]. Untangling the two is particularly difficult especially as normal immune destruction of malignant cells only leads to selection of the most aggressive and least immunogenic phenotypic cancer clone in the process known as immunoediting [4].

The TME plays a major role in shaping the immune response towards tumours. Tumour growth necessitates adequate oxygen and nutrients; however, the tumour vasculature is chaotic and disorganised meaning it is unable to meet the high metabolic demands of its cells. Moreover, this creates temporally and spatially distinct heterogenous regions within the TME [5]. With cancer expansion, oxygen and nutrients essential for anti-tumour immune cell function are depleted and by-products of tumour metabolism such as lactate and adenosine accumulate in the TME which repress the anti-tumour immune response [6]. Recruitment of immunosuppressive cells including tumour-associated macrophages (TAMs), regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) further dampens effector cell function [7]. The aberrant vasculature in the tumour presents a physical barrier to immune cell

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infiltration due to erratic distribution within the tumour and a repression of the signalling molecules needed for leucocyte extravasation, known as endothelial anergy [8]. Therefore, the TME can adopt an immunosuppressive phenotype to contribute to immune escape.

In this article, we will review the role of lactate – a key oncometabolite that accumulates in the TME – in shaping the immune response to cancer, while also reviewing the clinical implications that lactate levels have for patient prognosis, prediction and treatment.

1.2. Lactate

Altered energy metabolism is a hallmark of cancer. Increased proliferation is predicated on a cell's metabolic state: in order to support a sustained proliferative capacity a cell must have adequate levels of ATP and metabolic intermediates to support the construction of new cells [9]. Lactate is the endpoint of anaerobic glycolysis and for many years its role in cancer was considered as such: an endpoint or waste metabolite. However, this perspective has recently been challenged with evidence suggesting that lactate is a key player in many oncogenic processes, among them metastasis, angiogenesis, metabolism, and immunosuppression [10].

In 1927, Otto Warburg published a landmark paper describing how tumour cells consumed high levels of glucose via glycolysis despite adequate oxygen levels [11]. This phenomenon became known as the Warburg effect or aerobic glycolysis and is now exploited by radiologists as the basis for tumour imaging with FDG-PET (fluorodeoxyglucose-positron emission tomography) [12]. The Warburg effect is thought to provide three key advantages to cancer cells (Fig. 1) [10]. Firstly, while glycolysis is far less efficient in terms of ATP extraction than oxidative phosphorylation, this is offset by the short duration of the former which allows cells dependent on aerobic glycolysis to generate more ATP per unit time than those utilising the conventionally more efficient oxidative metabolism [13]. Secondly, glycolytic intermediates – important sources of carbon – can be siphoned off into alternative pathways to promote macromolecule synthesis needed for cellular construction [9,13]. Finally, upregulation of pathways such as the pentose phosphate pathway (PPP) enables glutathione production via NADPH creation which opposes oxidative stress, facilitating increased cancer cell survival, as well as conferring resistance to chemo- and radiotherapy [14].

An important control mechanism regulating flux between glycolysis and the PPP is the fructose 6-phosphate/fructose 1,6-bisphosphate cycle (Fig. 1) [15]. The conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate is the rate limiting step of glycolysis and is catalysed

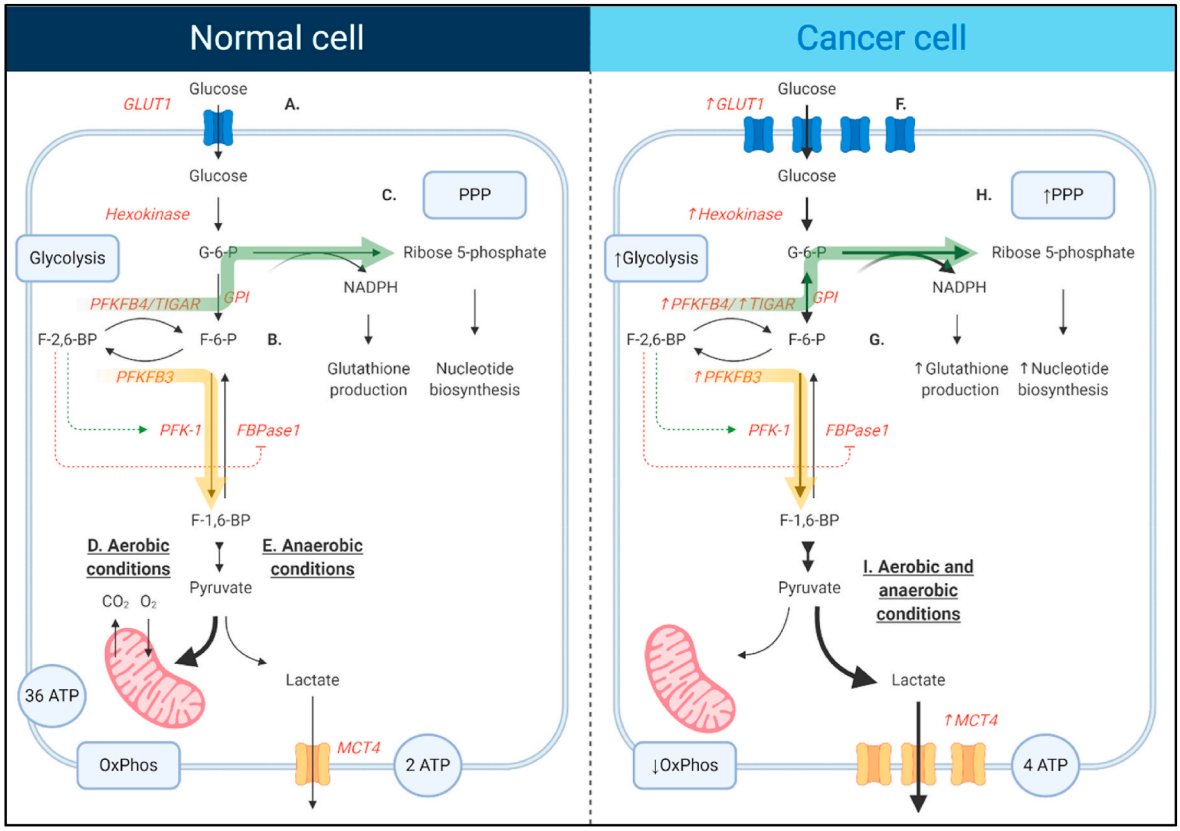

Fig. 1. Accelerated glycolysis regardless of oxygen status confers key advantages on cancer cells. (A) Glucose uptake occurs via glucose transporter 1 (GLUT1) and is metabolised to glucose 6-phosphate (G-6-P) by hexokinase and to fructose 6-phosphate (F-6-P) by glucose-6-phosphate isomerase (GPI). (B) The conversion of F-6-P to fructose 1,6-bisphosphate (F-1,6-BP) by phosphofructokinase 1 (PFK-1) is the rate limiting step that commits glucose to glycolysis. PFK-1 is allosterically regulated by the level of fructose 2,6-bisphosphate (F-2,6-BP); F-2,6-BP is formed by the action of PFKFB3 on F-6-P, therefore, PFKFB3 promotes glycolysis. In contrast PFKFB4 and TIGAR convert F-2,6-BP to F-6-BP removing the allosteric modulation favouring forward flux and in doing so promote accumulation of F-6-P which can be shunted (via conversion to G-P-B by GPI) into (C) the pentose phosphate pathway (PPP) which produces NADPH and precursors for nucleotide biosynthesis. Following conversion of F-1-6-BP to pyruvate (full set of reactions not shown), further metabolism to (D) carbon dioxide in the presence of oxygen via the Krebs cycle and oxidative phosphorylation (OxPhos) in the mitochondria, or to (E) lactate under anaerobic conditions. Lactate is exported extracellularly by monocarboxylate transporter 4 (MCT4). (F) Globally, cancer cells upregulate glycolysis and the PPP (and their associated genes) while down-regulating OxPhos. (H) Activation/overexpression of PFKB4 and TIGAR favours flux through the PPP while (G) PFKFB3 strongly promotes glycolysis and (I) lactate production in aerobic and anaerobic conditions. Oxidative phosphorylation extracts 36 mol ATP/1 mol glucose compared to 2 mol ATP/1 mol of glucose for anaerobic metabolism. Aerobic glycolysis generates 4 mol ATP/1 mol of glucose. FBPase 1, fructose 1,6-bisphosphatase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PFKFB4, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4; TIGAR, TP53 induced glycolysis regulatory phosphatase.

by phosphofructokinase (PFK) 1. PFK 1 is allosterically regulated by fructose 2,6-bisphosphate, a shunt product of PFK 2 action on fructose 6-phosphate. PFK 2 has bifunctional kinase-phosphatase activity (i.e. can produce and degrade fructose 2,6-bisphopate) and has 4 major isoforms (PFKFB1-4), each with different tissue expression and relative kinase-phosphatase activity. PFKFB3 and PFKFB4 are heavily implicated in the regulation of tumour metabolism both being overexpressed in many tumour types including breast and bladder cancers, respectively [16]. PFKFB3 has kinetics which favour net production of fructose 2, 6-bisphopate (namely high kinase and low phosphatase activity) which promotes progression of fructose 6-phosphate through the committed step of fructose 1,6-bisphosphate synthesis. Conversely, PFKFB4 hydrolyses fructose 2,6-bisphosphate into fructose 6-phosphate, thus siphoning glucose into the PPP at the expense of glycolysis by lessening the allosteric agonism for PFK 2. An additional regulator of this junction is TP53-inducible glycolysis and apoptosis regulator (TIGAR) which works in parallel with PFKFB4 as a bisphosphatase, promoting glucose entry into the PPP [16].

Up-regulation of glycolysis occurs in tandem with an up-regulation in glutaminolysis [9,17]. Like glucose, glutamine is a key cellular fuel for numerous processes within cancer cells such as macromolecule synthesis, ATP and anti-oxidant generation [17]. Both glutaminolysis and glycolysis are up-regulated by complex mechanisms including the dysregulation of oncogenes (glutaminolysis: c-Myc, KRas; glycolysis: Akt, PI3K, mTOR, Ras, Raf) and loss of tumour suppressor genes (glutaminolysis: RB; glycolysis: p53, VHL, PTEN) [18,19]. Additionally, hypoxia is central in the acquisition of an altered metabolic phenotype: hypoxia inhibits degradation of hypoxia inducible factor (HIF)-1α (or one of its isoforms: HIF-2α or HIF-3α) allowing it to bind to HIF-1β, translocate to the nucleus, and modulate gene expression by acting as a

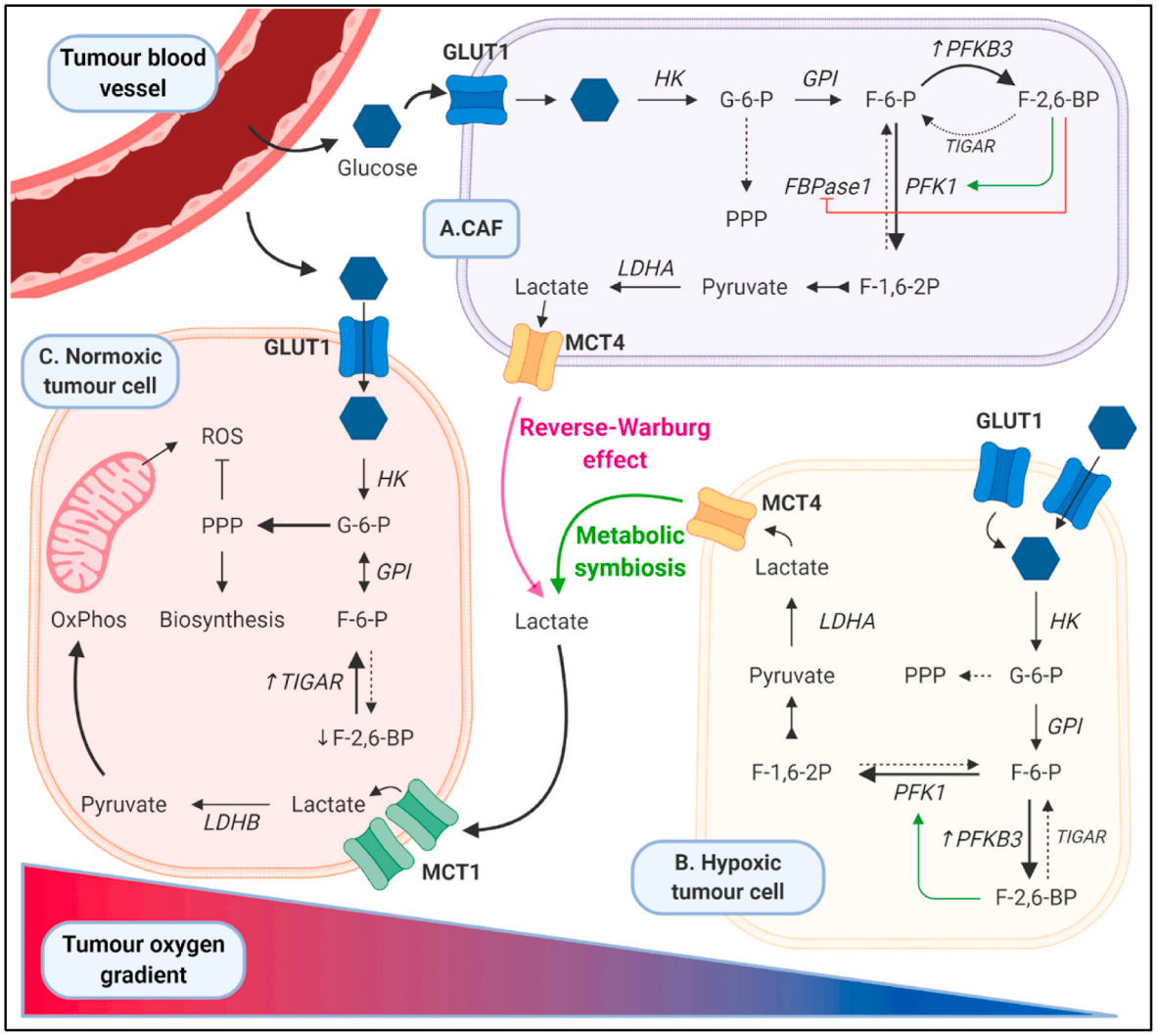

Fig. 2. Diverse metabolic programs among different cancer cell types can support tumour growth via lactate shuttling. Different regions of tumours are reliant on different metabolic pathways. (A) Cancer-associated fibroblasts (CAFs) and (B) hypoxic cells display up-regulated glycolysis. Tumour cells can also display up-regulated glycolysis in aerobic conditions i.e. the Warburg effect (not shown). Glycolytic metabolism is favoured by expression of GLUT1 and MCT4 which allow uptake and extrusion of glucose and lactate, respectively. Up-regulation of PFKB3 is also important in committing glucose molecules to glycolysis; PFKB3 increases F-2,6-BP levels – this allosterically activates PFK1, the rate-limiting step of glycolysis, while negatively regulating FBPase 1 (not shown in B). By committing glucose to glycolysis, flux through the pentose phosphate pathway (PPP) is reduced; however, TP53-inducible glycolysis and apoptosis regulator (TIGAR) reduces F-2,6-BP levels which promotes flux through the PPP generating anti-oxidants, to neutralise reactive oxygen species (ROS), as well as precursors for nucleotide biosynthesis. The endpoint of glycolytic metabolism is lactate which is produced from lactate dehydrogenase A (LDHA) action on pyruvate. Lactate can be exported to the extracellular environment by MCT4. Extracellular lactate can be taken up by (C) aerobic tumour cells via MCT1 and used to generate ATP via the Krebs cycle and oxidative phosphorylation (OxPhos). The metabolic symbiosis between (B) hypoxic/glycolytic and (A) normoxic/oxidative cancer cells spares glucose for hypoxic tumour cells. The cooperativity between (A) the stroma (CAF) and (C) malignant cells is termed Reverse-Warburg effect and contributes to the establishment of lactate and glucose gradients within tumours.

transcription factor [20]. These factors induce the expression of glycolytic genes including; glucose transporters to drive glucose uptake; glycolytic enzymes such as hexokinase, phosphofructokinases, pyruvate kinase, and lactate dehydrogenase (LDH); monocarboxylate transporters (MCT) to shuttle lactate out of the cell into the TME [21,22]. Increased expression of these glycolytic genes are well-described segregators of normal, precancerous and malignant cells in several tumour types including oesophageal adenocarcinoma and hepatocellular carcinoma, highlighting their importance in the tumourigenic process [23,24]. The high flux through both the glycolytic and glutaminolytic pathways in cancer cells are major contributors to lactate accumulation in the TME.

The TME itself is also another important source of lactate. Malignant epithelial cells can corrupt elements of the TME, such as cancer-associated fibroblasts, to adopt a Warburg-like phenotype involving up-regulation of glycolysis and lactate production. The exported lactate generated by this 'reverse-Warburg effect' can be taken up by aerobic tumour cells for oxidative metabolism (Fig. 2) [25]. Moreover, lactate shuttling can create a metabolic symbiosis between cells in different tumour regions and aids hypoxic cells which can use the spared glucose [26,27]. This phenomenon may explain how lactate accumulates in tumours that are not totally dependent on aerobic glycolysis [28–30]. As a consequence of these metabolic derangements lactate accumulates in the extracellular environment and may reach levels of up to 30–40 mM – over 10 times greater than physiological lactate concentrations [13,22].

2. Immunosuppressive roles of lactate within the TME

The last decade has seen a revolution in oncology: the rise of immunotherapies has rewritten the prognosis for a range of cancers such as melanoma and leukaemia and is now termed the 5th pillar of cancer care, joining the traditional pillars of surgery, chemotherapy, radiation, and targeted therapy [31]. Despite the fanfare surrounding immunotherapy – particularly immune checkpoint inhibitors – a 2019 study found that only 12.46% of patients responded to such drugs [32]. Additionally, more conventional treatment options such as chemotherapy and radiation therapy are also dependent on the immune response to eliminate cancerous cells [33]. However, the efficacies of immunotherapy, and other treatment modalities, are limited by an immunosuppressive TME in which lactate accumulation plays a major role.

Several groups have investigated the role that lactate might play in shaping the response to immune checkpoint inhibition including *Kelderman* et al. who concluded that in patients with advanced cutaneous melanoma, long-term benefit of ipilimumab treatment was unlikely for patients with baseline serum LDH greater than twice the upper limit of normal; moreover, baseline LDH was shown to be the strongest predictive factor for overall survival (OS) [34]. Also in patients with advanced melanoma, *Nosrati* et al. incorporated elevated LDH into a validated 5-factor prediction scale for the clinical activity of PD-1 antibodies [35], while *Schouwenburg* et al. identified LDH as a predictive biomarker of anti-PD-1 and anti-CTLA-4 therapeutic response in melanoma patients previously treated with BRAF inhibitors [36]. In non-small cell lung cancer, a 2019 meta-analysis found that serum LDH levels can predict response to treatment with immune checkpoint inhibitors – those with high pre-treatment LDH level had significantly shorter progression-free and OS [37]. Similarly, in oesophageal squamous cell carcinoma patients, serum LDH can be used as an independent biomarker for predicting response to immune checkpoint blockade [38]. However, a 2020 meta-analysis of immune checkpoint inhibitors in metastatic breast cancer did not find a utility for LDH in predicting response to these treatments, illuminating the complexity in interpreting and forecasting tumour-immune interactions [39].

2.1. Adaptive immunity

Lymphocytes, especially T cells, are classically associated with anti-tumour immunity and are one of the major executors of malignant cells. Suppression of this lineage is vital if tumours are to progress and spread. A diverse array of T cell subtypes exists; simplistically, the major classifications include cytotoxic lymphocytes (CTL) – originating from $CD8^+$ T cells – and various $CD4^+$ subsets including T_H1, T_H2 and Tregs. CTLs are the major effectors of tumour cell lysis and are supported in this capacity by T_H1 cells which recruit, prime and enhance CTL function. T_H2 cells are chiefly regarded as pro-tumour as they do not contribute to CTL responses and may even suppress beneficial anti-tumour T_H1 cells [40]. Tregs suppress anti-tumour immune responses and in most tumours, including melanoma, cervical, renal, and breast cancers, high infiltrates correlate with worse prognosis [41].

Just as transformed cells rely on accelerated glycolysis to support the biosynthetic programs needed for sustained mitosis, activation of naïve $CD4^+$ and $CD8^+$ lymphoid cells causes a shift in metabolic activity away from oxidative phosphorylation and towards increased glycolysis as well as up-regulating glutaminolysis [42]. In addition to supporting proliferation, glycolysis is also involved in cytokine production: glycolysis regulates interferon-gamma (IFNγ) production in $CD4^+$ T cells via post-transcriptional and epigenetic mechanisms [43]. However, these processes are disrupted in the nutrient-deficient TME as infiltrating CTLs and $CD4^+$ cells must compete with tumour cells (and other immune cells) for scarce and exhausted resources which impedes their function [44].

Lactate accumulation in the TME is the net result of the increase in glycolysis from immune and neoplastic cells. However, the metabolic changes immune cells need for activation are somewhat of an Achilles heel as lactate efflux is dependent on the concentration gradient across the plasma membrane. The altered ratio of extracellular to intracellular lactate, secondary to the Warburg effect, 'asphyxiates' immune cells by their inability to rid themselves of this waste [10]. In the case of CTLs, this disturbs their metabolic capacity resulting in impaired cytotoxicity [45].

Increased levels of lactate are a major contributor to acidosis in the TME – although other factors such as CO_2 also play a role [46]. Observations across a range of malignancies have characterised tumour pH values as low as 5.6 although most fall between 6.0 and 7.0 [47,48]. Decreased extracellular pH has been shown to impair almost all aspects of $CD8^+$ and $CD4^+$ lymphocyte function: activation, cytotoxicity, chemotaxis, motility, and proliferation [47,49]. These effects appear, at least in part, to be reversible: *Calcinotto* et al. showed restoring normal pH using esomeprazole, a proton pump inhibitor, reverted an acidotic-induced anergic state in human and mouse tumour-specific $CD8^+$ T lymphocytes [50]. These findings were supported by work published by *Pilon-Thomas* et al. who showed abolition of CTL IFNγ secretion under acidic conditions and used bicarbonate to reverse this effect; moreover, restoration of neutral pH improved CTL infiltration and response to anti-CTLA-4, anti-PD-1 and adoptive cell transfer immunotherapies in murine melanoma and pancreatic cancer models [51].

A mechanism underlying this lactate-mediated immunosuppression was proposed by *Brand* et al. who showed that lactic acid and tumour acidosis inhibited nuclear factor of activated T cells (NFAT), the key activating transcription factor in tumour-infiltrating $CD8^+$ T cells and NK cells, resulting in reduced IFNγ production [52]. They also found that tumour LDHA expression negatively correlated not only with T activation markers, but also with survival in patients with melanoma. Additionally, lactate concentrations above 20 mM induced $CD8^+$ T and NK cell apoptosis; such levels, as alluded to previously, can be found intra-tumourally and represents another mechanism of immune evasion. *Bosticardo and colleagues* found that *in vitro* activation of CTLs at low extracellular pH resulted in impeded cytokine secretion and proliferative capacity. Affected CTLs exhibited up-regulation of IFNγ-R2 chain and CTLA-4 expression, which rendered them sensitive to negative regulatory signals [53]. It has been postulated that TME acidity can interfere with mammalian target of rapamycin complex 1 (mTORC1) signalling which coordinates key events in the T cell lifecycle including

immune receptor signalling, metabolic regulation and migratory activity culminating in the promotion of T_H1 and T_H2 effector cell differentiation, while suppressing Treg induction and T cell anergy [47,54].

Following antigen presentation, multiple secondary co-stimulatory or co-inhibitory signals are delivered to naïve T cells. A dynamic balance between such signals at the immunological synapse influences the fate of the T cell response: if co-inhibitory signals outweigh the co-stimulatory signals, T cell anergy is induced. Conversely, when the co-stimulatory signals outweigh the co-inhibitory signals, a T cell proliferates and differentiates into an effector cell [55]. There is emerging evidence that lactate can induce co-inhibitory T cell ligands such as PD-L1 on tumour cells which can contribute to anergic T cell development [56]. *Seth* et al. showed that deletion of LDHA in macrophages increased T-cell numbers in a murine model of lung carcinoma by suppressing PD-L1 expression [57]. Similarly, in a murine model of melanoma mice with LDHA-deficient tumours had improved response to anti-PD-1 therapy compared to mice implanted with wild-type LDHA tumours; this adds credence to the emerging importance of lactate in the regulation of the PD-1 axis [58].

As well as suppressing effector T cell function, lactate accumulation in the TME favours immunosuppressive Treg development. Tregs are far more reliant on oxidative metabolism than effector T cells [59]. FoxP3, a key Treg transcription factor, interacts with LDH allowing Tregs to convert lactate into pyruvate; this confers a metabolic edge on Tregs enabling them to thrive in low-glucose, lactate-rich environments which hinders conventional T cell function [60]. Lactate has a role in shaping the polarisation of infiltrating T cells: *Comito* et al. showed using an *in vitro* prostate cancer model that cancer-associated fibroblast-derived lactate reduced anti-tumoral T_H1 cells and increased Treg cells via a lactate-based modulation of their respective transcription factors, T-bet and FoxP3 [61].

While the role of T lymphocytes is firmly established in mediating the immunosurveillance hypothesis, the role of B cell-mediated immunity in cancer is an emerging area of interest [62]. Recent reports highlighting the importance of tumour-associated B cells for immune-checkpoint inhibitor therapy success hint at their broader significance in anti-tumour immunity [63,64]. However, this perspective is highly controversial as reports of B cell involvement in tumourigenesis exist [65]; it is probable therefore that the role B cells in cancer mirrors that of their T cell counterpart, with various subtypes (including conventional and regulatory B cells) exhibiting differing properties which are likely highly context-dependent [66]. How lactate affects the anti-tumour aspect of B cell function including antigen processing and presentation, and plasma cell formation is not currently known. However, as will be discussed, lactate impacts the antigen presentation and processing of other professional antigen presenting cells, notably dendritic cells (DCs) and TAMs: thus, we hypothesis that lactate may skew B cells towards a more tolerogenic phenotype. Additionally, it has transpired that metabolism plays a crucial role in B cell activities: *Caro-Maldonado* et al. showed inhibition of glycolysis or deletion of GLUT1 in human B cells suppresses *in vivo* antibody production [67]. Other authors have found that glutamine restriction is the major controller of B cell function: *Waters* et al. demonstrated that glutamine restriction markedly impaired murine B cell growth and differentiation; however, glucose restriction did not affect B cell functions [68]. Interestingly, *Garcia-Manteiga* et al. showed that following antigen presentation, naïve B to plasma cell differentiation was accompanied by increases in glucose oxidation and lactate accumulation; however, as antibody secretion ensued, this diminished in favour of a greater reliance on glutamine metabolism [69]. Thus, it may be plausible that in the nutrient-depleted TME B cell function may suffer accordingly, yet the clinical significance of this as well as any direct effect of lactate on B cells has yet to be elucidated.

2.2. Innate immunity

Although adaptive immunity garners much of the attention of the world of immuno-oncology, cells of the innate immune system play a major role – especially in the arena of immunosuppression and promoting tolerogenic responses. See Fig. 3 for a summary.

Lactate has been shown to favour TAM polarisation towards a protumour M2 phenotype [70]. *Colegio* et al. attributed this to lactate-mediated stabilisation of HIF-1α leading to up-regulation of M2-associated genes such as TGFβ, VEGF, and arginase (ARG1) [71]. *Mu* et al. recently provided evidence that in a murine breast cancer model that lactate induces M2 macrophage polarisation via the activation of the ERK/STAT3 signalling pathway which up-regulates similar genes to HIF-1α including ARG1 [72]. According to *Chen* et al., lactate in the TME interacts with Gpr132, a pH-sensing membrane receptor on macrophages; activation of this receptor can increase the expression of M2 polarisation-associated genes [73]. Furthermore, they demonstrated that loss of Gpr132 in breast cancer-bearing mice inhibited metastasis, while decreased Gpr132 expression in patients with breast cancer correlated with improved metastasis-free survival. Recently, *Zhang* et al. demonstrated in murine models of lung cancer and melanoma, that lactate has a role in epigenetic regulation in macrophages: extracellular and intracellular lactate levels induced lysine lactylation (addition of lactyl groups to histone proteins) leading to increased expression of M2-like genes such as ARG1 in M1 macrophages [74].

TAMs have numerous mechanisms of subverting anti-tumour immune responses and their presence constitutes a negative prognostic marker [75]. They limit M1 macrophage-mediated innate immune responses and restrict T cell activation. TAMs produce IL-10, an immunosuppressive cytokine, favouring T_H2 polarisation [76]. TAMs can also express co-inhibitory molecules such as PD-L1 which drives apoptosis following ligation with its cognate receptor, PD-1, on activated T lymphocytes [77]. They express CCL22 attracting Treg cells and can sabotage T cell metabolism by expressing ARG1 which deprives T cells of L-arginine, a key nutrient for their growth [78,79]. Consequently, lactate-driven TAM polarisation is a key mechanism of immune escape for malignant cells. Furthermore, TAMs are implicated in many other domains of cancer progression such as promoting angiogenesis, invasion and metastasis and chemotherapy resistance [80].

Lactate has also been implicated in release of high-motility group box 1 (HMGB1), a danger-associated molecular pattern (DAMP), from activated macrophages [81]. Emerging evidence is suggestive of an important role for DAMPs, including HMGB1, in initiating and perpetuating chronic inflammation that exhausts and impairs anti-tumour immunity as well as contributing to cancer progression [82,83]. However, HMGB1 is also known to have anti-tumour properties and can interestingly suppress glycolysis in tumour cells resulting in metabolic cell death [84]. Clarification is thus warranted on the interplay of tumour metabolism and the dual role that DAMPs such as HMGB1 can play in advancing or impeding cancer progression.

MDSCs are a population of immature myeloid cells which similarly to TAMs promote an immunosuppressive phenotype in the TME, also contributing to cancer growth and metastasis [85]. Cytokines and growth factors, such as TGFβ, IL-10, and VEGF, in the TME are well-established in MDSC differentiation and regulation [86]. However, lactate is emerging as a key regulator of MDSCs. *Husain* et al. showed that exogenous lactate acted as an adjuvant in MDSC development, increasing the *in vitro* frequency generated from mouse bone marrow cells with GM-CSF and IL-6 [87]. Additionally, knockout of LDHA led to decreased numbers of MDSCs in the spleens of tumour-bearing mice further supporting the role lactate may play in MDSC development. Similarly to Tregs, MDSCs are more dependent on oxidative metabolism (specifically fatty acid oxidation), thus they obtain a selective growth advantage over anti-tumour leucocytes who must compete with the tumour and each other for scarce glucose [88].

Lactate is also implicated in repression of innate lymphocyte

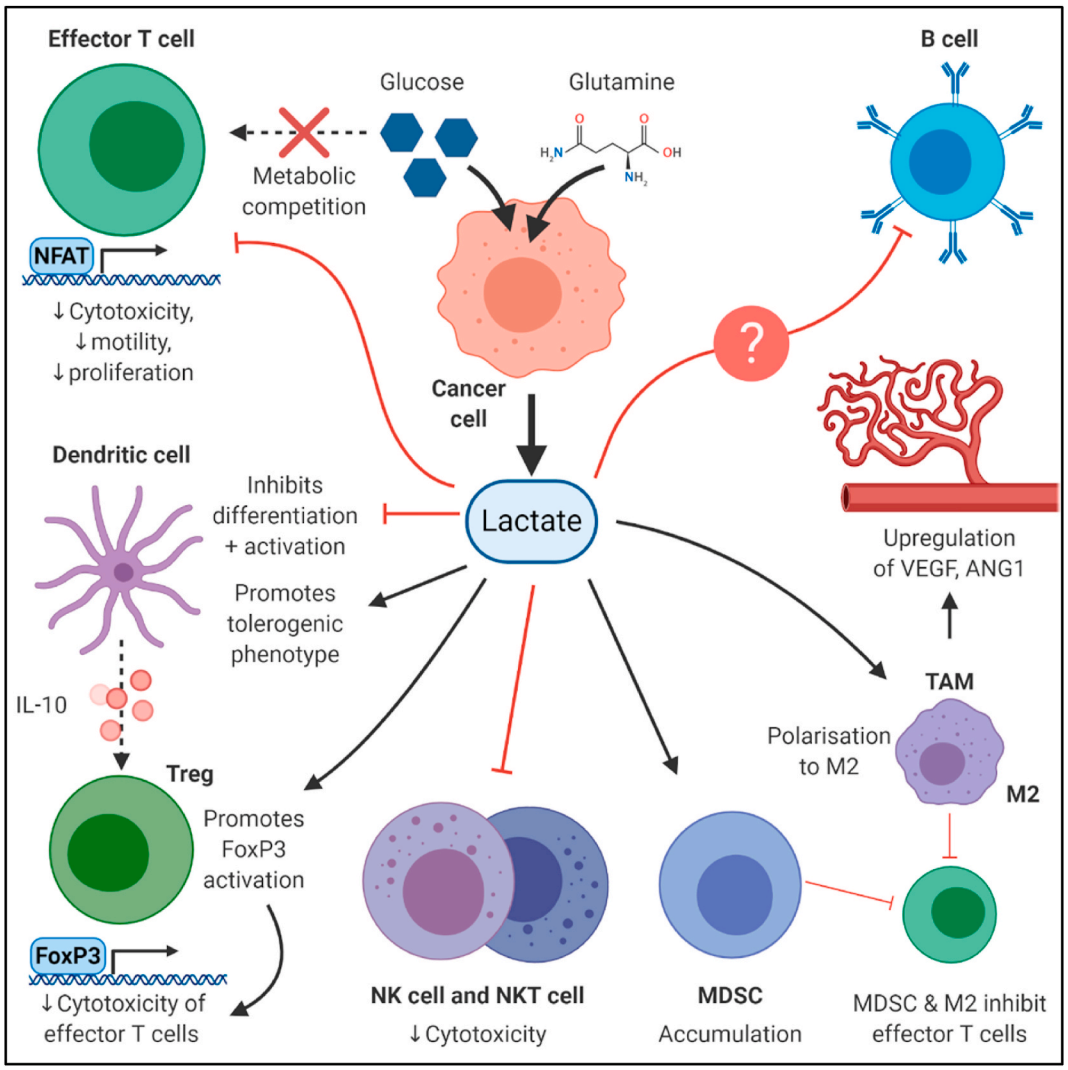

Fig. 3. Lactate hinders anti-tumour immunity by direct effects on effector cells and by promoting immunosuppressive cells. Lactate accumulates in the TME due to up-regulation of glycolysis and glutaminolysis in cancer cells. Key nutrients such as glucose and glutamine are diverted to tumour cells as fuel which consequently starves tumour-resident immune cells of energy substrates. Lactate can directly inhibit T cell function by interfering with the transcription factor NFAT. Dendritic cells are indispensable for T cell activation, however, their activation and maturation which is a prerequisite for this is impaired by lactate. Moreover, lactate induces a tolerogenic DC phenotype promoting regulatory T cell (Treg) polarisation. Tregs suppress anti-tumour immunity. Additionally, lactate can up-regulate expression of FoxP3, an important transcription factor in Treg development and function. Lactate can directly inhibit the cytotoxic functions of innate lymphocytes such as natural killer (NK) and natural killer T (NKT) cells. Lactate causes accumulation and polarisation of myeloid derived suppressor cells (MDSCs) and M2-tumour-associated macrophages (TAMs), respectively, which inhibit anti-tumour immune responses. M2 macrophages are involved in angiogenesis via up-regulation of vascular endothelial growth factor (VEGF) and angiopoietin 1 (ANG1). The effect of lactate on B cell-mediated immunity is unclear at present, but is thought to involve some element of inhibition possibly mediated by metabolic disruption.

function. As early as 1991 reports of lactate-induced NK cell cytolytic dysfunction surfaced [89]. More recently, *Husain* et al. showed in a murine pancreatic cancer model that treating NK cells with lactate (15 mM) decreased production of perforin and granzyme B resulting in weakened cytotoxic capabilities when tested *in vitro* [87]. In parallel with this discovery, they found that NK cells from LDHA-depleted murine pancreatic tumours had improved cytolytic function. Natural Killer T (NKT) cells are an innate subset of lymphocytes with important roles in anti-tumour immunity and as cellular immunotherapeutics [90]. Lactate has been shown to inhibit NKT cell IFNγ and IL-4 secretion via a blockade on mTOR signalling in cell culture experiments [91].

DCs sit at the junction of innate and adaptive immunity and are critical for tumour-specific immune responses. The cornerstone of their utility is antigen processing and presentation to T cells, which is the first signal enabling the conversion of naïve T cells to fully functional effector T cells. As discussed, appropriate co-stimulation – also known as signal two – is indispensable to avoid anergy. While signal three takes the form of cytokine secretion which determines the functional outcome or polarisation – T_H1, T_H2, Treg etc. – of the recipient cell [92]. Lactate can inhibit differentiation and activation of DCs which is a prerequisite for initiating adaptive immunity [93]. Moreover, *in vitro* studies have shown lactate induces a tolerogenic phenotype in DCs whereby IL-10 and IL-12 production is enhanced and attenuated, respectively, skewing the immune profile of the TME towards Treg dominated environment and away from anti-tumour T_H1 responses [93–95]. Additionally, lactate inhibited the ability of DCs to induce the proliferation of allogeneic T cells *in vitro*. Restoration of homeostatic conditions using diclofenac in a murine glioma model to eliminate lactate reversed the tolerogenic effect on tumour-infiltrating DCs [96]. However, some controversy exists in the literature surrounding these findings as two studies showed that a low extracellular pH augmented DC function as measured by increased endocytosis, antigen presentation, costimulatory molecule expression

and ability to induce T cell replication [97,98]. Furthermore, *Yu* et al. described how lactic acid could potentiate the immunogenicity of an irradiated whole-tumour cell vaccine via enhanced DC function [99]. Mechanistically, *Tong* et al. described acid-sensing ion channels which mediated these effects and showed that neutralisation of pH using non-steroidal anti-inflammatories such as diclofenac abrogated acidosis-induced increases in DC function [97]. *Gottfried* [93] and *Nasi* [94] were only able to modestly repress DC function using acidity alone in their *in vitro* models, therefore, questions remain about the true effect of lactate and acidity on DC function. Further clarification is needed on this point. The metabolic reconfiguration driven by DC activation is similar to that of effector T cells: resting DCs are primarily dependent on fatty acid oxidation and oxidative phosphorylation, while maturation induces a switch to aerobic glycolysis with lactate generation [100]. However, the high concentrations of lactate in the TME can block endogenous lactate extrusion leading to impeded DC metabolism [101].

2.3. Stromal cells

Lactate has a profound influence on the stromal component of the TME such as cancer-associated fibroblasts, endothelial cells and pericytes by promoting secretion of immunosuppressive and tumour-promoting factors [25,102]. Extruded lactate from cancer cells promotes hepatocyte growth factor release from cancer-associated fibroblasts [103]: hepatocyte growth factor attenuates $CD8^+$ CTL activity and induces tolerogenic DC and Treg cell populations [104,105]. In endothelial cells lactate influx through MCT1 drives tumour angiogenesis by an NF-κB/IL-8–dependent mechanism [106]; such nascent vasculature constitutes a barrier to immune infiltration and effective immunosurveillance of tumours [8]. Pericytes are an often-overlooked component of the TME, yet have important contributions to many hallmarks of cancer including angiogenesis, metastasis, and immune evasion [107]; knowledge regarding the impact of lactate on pericyte function is currently lacking.

3. Clinical implications

3.1. Prognostic and predictive utility

As might be expected given its immunosuppressive effects, in addition to its other roles in cancer – promoting angiogenesis, invasion, and metastasis – lactate is generally associated with more aggressive tumours and higher intertumoral levels correlate with worse outcomes in several cancer types including cervical and head and neck cancers.

In lung cancer, elevated systemic lactic acid is a negative prognostic factor in the metastatic setting: stage IV for non-small cell lung cancer and extensive stage for the small cell subtype [108]. *Vlachostergios* et al. found that lactic acid level $\geq$1.4 mmol/L (normal range: 0.5–1 mmol/L) was associated with shorter OS in this cohort (n = 85) of patients. While historically many cases of raised systemic lactate levels in patients with solid tumours were complemented with extensive liver metastasis – as the liver is the major site of lactate metabolism – only 21.2% of patients in cohort had liver involvement. The authors also excluded patients with conditions such as sepsis and acute kidney injury as these may present with hyperlactatemia. Additionally, hypoxic causes of raised lactate levels did not play a major factor in the results. Together this study lends credence to the theory of overproduction i.e. the raised lactate was due to dysregulated tumour metabolism. This is consistent with lung cancer, especially the small cell subtype, being noted for its rapid mitotic capacity and being thus reliant on glycolysis to support the high rate of proliferation [109]. Blood lactate levels were found by *Wei* et al. to be higher in metastatic colorectal cancer patients compared to those without systemic disease. They also found that elevated serum lactate combined with serum LDH levels were independent prognostic factors for OS in this cohort of patients [110].

Intratumoural lactate is also well-established as a biomarker. *Ping* et al. found that intracellular lactate was significantly increased in tumour-infiltrating lymphocytes in gastric carcinomas [111]. Increased lactate level was correlated negatively with percentages of T_H1 cells and CTLs in the tumour reflecting an altered and impaired immune capacity within the TME. *Walenta* et al. established that lactate concentrations were significantly higher in primary cervical tumours with metastatic spread compared to primary malignancies in patients without metastases using the Mann-Whitney test. This was reflected in the log-rank survival analysis: patients with high tumour lactate concentrations had lower overall and disease-free survival, in addition to higher recurrence rates, compared to those with low tumour lactate concentrations [112]. In head and neck cancers, raised tumour lactate concentration was predictive of subsequent nodal or distant metastatic spread [113].

New technologies such as nuclear magnetic resonance spectroscopy (MRS) and hyperpolarised (HP) ^{13}C magnetic resonance imaging (MRI) are changing the landscape of intratumoural lactate measurement increasing its feasibility as a tumour biomarker [114–116]. These techniques have the advantage of allowing real-time visualisation of LDH activity and lactate production across the entire tumour without the need for invasive, costly and time-consuming biopsies that may fall foul of sampling bias in heterogeneous tumours. The former tool has been used to show that raised intratumoural lactate levels is an adverse prognostic index in breast cancer [117]. Furthermore, raised intratumoural lactate levels have been correlated with HER2 addiction status and trastuzumab (an anti-HER2 drug) susceptibility in HER2-positive breast cancer; thus, lactate may have utility as a predictive biomarker allowing optimal anti-HER2 drug prescription to such patients [118]. In patients with diffuse intrinsic pontine glioma, MRS detection of lactate is predictive of a poor prognosis [119].

HP ^{13}C MRI using [1–^{13}C]pyruvate as a substrate has been shown to be safe and feasible for metabolic imaging in a variety of cancers including breast and prostate [120–123] and has been validated to probe pyruvate-to-lactate conversion in cancer and the TME driven by the Warburg effect [122]. This modality has been used to correlate intratumoural lactate levels with increased Gleason grade, a marker of tumour differentiation or aggressiveness, in prostate cancer [124]. In patient-derived tumour xenografts models of pancreatic cancer, both MRS and HP ^{13}C MRI of pyruvate-to-lactate conversion were used to show that the most aggressive tumour models (defined by time-to-harvest) had a significant increase in lactate production from HP pyruvate [125].

In glioblastoma (where the utility of conventional FDG-PET is hindered by the high background glucose uptake in the metabolically-active brain), several metabolic imaging modalities have been established including H^1 MRS, C^{13} MRS and P^{31} MRS [126]; H^1 MRS can segregate *in vitro* IDH1 mutant and wild-type gliomas (an important prognostic distinction) on the basis of a decreased drop in lactate, glutamate and phosphocholine [127]. Additionally, HP ^{13}C MRI with [1–^{13}C]pyruvate as a substrate was shown to be feasible for detecting early response to temozolomide treatment using an orthotopic human xenograft models; suggesting potential for its use as a predictive biomarker of response [128]. However, this modality is not suitable for low-grade IDH1 mutant gliomas which produced significantly less HP [1–^{13}C]lactate than glioblastoma and had no post-temozolomide associated changes in lactate production, findings consistent with the comparably lower growth and hypoxia levels in this tumour type compared to glioblastoma [129]. Moreover, in breast cancer a pilot study by *Xu* et al. failed to conclude that tumour metastatic risk is associated with the high levels of glycolysis and lactate production [130]. Such results shed light on a key concept in tumour metabolism: different cancer types institute a variety of metabolic programs and heterogeneity in this respect exists both within and between tumours [131]. Therefore, future development of metabolic imaging must account for the different metabolic phenotypes that tumours can exhibit.

There are several ongoing clinical trials that are investigating the

utility of lactate imaging as companion biomarkers of response in patients undergoing cancer treatment: NCT02913131 (patients with advanced solid tumour malignancies receiving PI3K/mTOR pathway inhibitors), NCT04346225 and NCT03581500 (systemic abiraterone or apalutamide for advanced prostate cancer). Other studies, such as NCT04258462 (renal cancer) are using HP ^{13}C pyruvate MRI to predict tumour aggressiveness.

3.2. Therapeutic implications

Given the wealth of evidence implicating lactate and altered cellular metabolism in a range of cancer hallmarks, targeting these features is now a major focus of pharmaceutical drug development (Fig. 4). Here we discuss inhibitors of GLUT1, hexokinase, LDH, MCT1 and HIF.

GLUT1 is overexpressed in a variety of cancers and correlates with markers of invasive and metastatic behaviour [132]. Inhibitors of this transporter, such as STF-31 and WZB117, have been tested preclinically with some promising results: *Chan* et al. showed that STF-31 could selectively kill renal carcinoma cells *in vitro* while *Liu* et al. demonstrated both *in vitro* and *in vivo* growth inhibition with WZB117 in a murine lung cancer model [133,134]. Additionally, STF-31 induced apoptosis and sensitised myeloma cells to chemotherapy (melphalan, doxorubicin, and bortezomib) *in vitro* [135]. WZB117 exerted a synergistic effect on apoptosis induction and growth inhibition in *in vitro* breast cancer cells when combined with MK-2206, an Akt inhibitor, and when used as monotherapy inhibited tumour growth in an intrahepatic cholangiocarcinoma patient-derived xenograft model [136,137]. To our knowledge, no clinical trial has ever investigated the safety or efficacy of these two agents in humans yet the above results suggest further exploration is warranted.

2-deoxy-D-glucose (2-DG) is a competitive inhibitor of hexokinase. Being structurally similar to glucose allows 2-DG to be metabolised by hexokinase, however, 2-DG is unable to be processed any further by the glycolytic pathway and thus accumulates in the cytoplasm. Hexokinase inhibition results in glucose and ATP depletion. 2-DG has undergone phase I clinical trials to estimate dosing in several solid tumours including prostate cancer and glioblastoma on the basis of preclinical efficacy [138,139]. Recently, 2-DG has been used as an adjuvant alongside sorafenib (multi-kinase inhibitor) and metformin in hepatocellular carcinoma and breast cancer cell lines, respectively, showing synergistic growth retardation *in vitro* measured by cell cycle arrest [140,141]. It also showed synergistic effects when combined with GnRH-II antagonists in endometrial and ovarian cancer cells *in vitro* by decreasing cell viability in addition to increasing apoptosis [142]. 2-DG has also been shown to enhance $CD8^+$ memory cell formation and anti-tumour function – encompassing augmented lymphocyte homing to lymph nodes (connoting greater antigen presentation), increased production of IFN-γ and TNF-α, and improved tumour regression in mice with melanoma tumours [143]. Such results suggest that the place of metabolic modulators may be as companions to traditional drugs to increase their efficacy by curbing the immunosuppressive effects of TME.

The desire to inhibit LDH stems from knockout experiments demonstrating the crucial role for carcinogenesis in many cancer types [144–146]: inactivation of LDH precipitated impeded *in vitro* breast cancer and oesophageal squamous cell cancer cell growth as well as decreased tumorigenesis and disease regression in murine models of lung, oesophageal and breast cancer. Several types of LDH inhibitors exist, however, none are yet clinically viable. N-hydroxyindoles have been shown to decrease the *in vitro* growth of pancreatic and cervical cancer cell lines [147] and can synergistically increase apoptosis when combined with gemcitabine in pancreatic cancer cell lines [148].

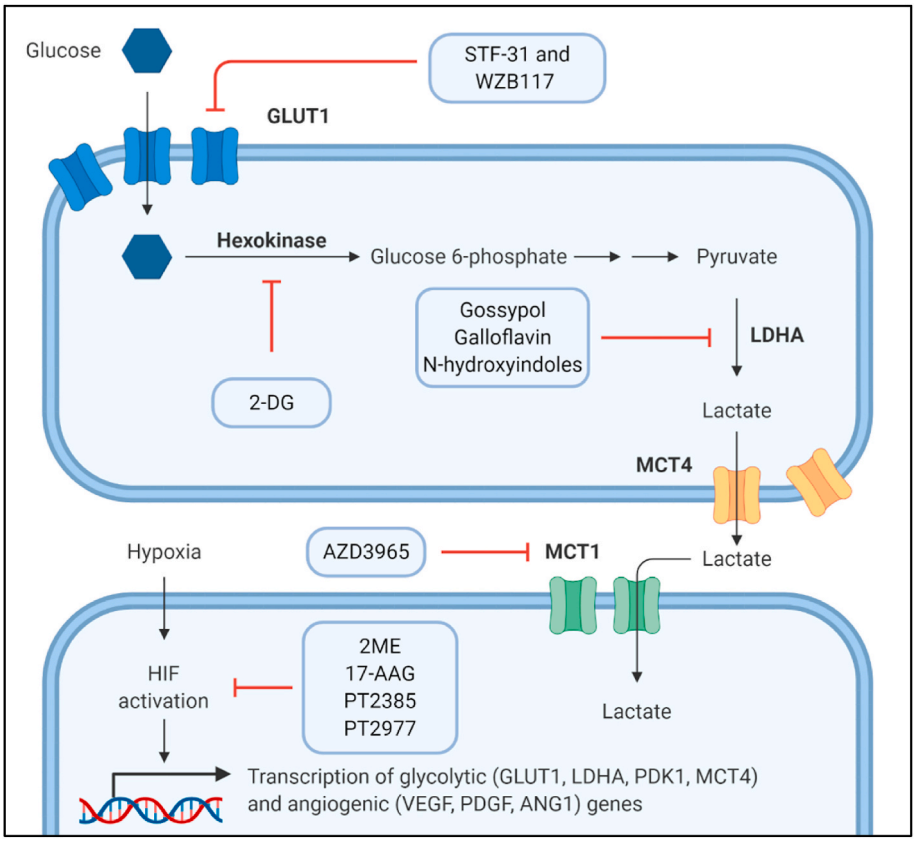

Fig. 4. Pharmacological inhibitors of lactate metabolism under pre-clinical and clinical development. GLUT1, glucose transporter 1; 2-DG, 2-deoxy-D-glucose; HIF-1α, hypoxia inducible factor 1α; LDHA, lactate dehydrogenase A; MCT1, monocarboxylate transporter 1; MCT4, monocarboxylate transporter 4; PDK1, pyruvate dehydrogenase kinase 1; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor; ANG1, angiopoietin 1.

Galloflavin can induce apoptosis in breast cancer and hepatocellular carcinoma cell lines: both effects are attributable to blockade of aerobic glycolysis [149,150]. More recently, galloflavin in conjunction with metformin was shown to inhibit proliferation of and induce pancreatic ductal adenocarcinoma cell death [151]. However, to our best knowledge neither of these agents have been tested in humans. *Hermans* et al. developed an LDH inhibitor that re-invigorated IL-2-induced effects in memory $CD8^+$ T cells most significantly promoting effector-like metabolism (aerobic glycolysis) and reducing the expression of inhibitory immune checkpoints including LAG-3, PD-1, 2B4, and TIM-3; the authors also showed that LDH inhibition prior to adoptive T cell transfer into murine melanoma models led to improved tumour clearance [152]. Yet perhaps the most successful candidate at present is gossypol which reached phase I/II trials in humans, however, lack of efficacy has hindered its progress towards approval: *Poznak* et al. found no partial or complete responses among 20 women receiving gossypol for metastatic breast cancer refractory to doxorubicin and paclitaxel [153]. Similarly, *Baggstrom* et al. halted a phase II study of gossypol in extensive-stage small cell lung cancer (n = 14) during interim analysis as all patients failed to meet the primary end point of the trial (objective response) response to therapy [154]. Recently, *Xie* et al. investigated the efficacy of the negative enantiomer of gossypol in adrenal cortical carcinoma, but after none of the first 21 patients achieved even a partial response the trial was halted [155]. Given these disappointing results conflict with the strong preclinical evidence, perhaps cancer cells *in situ* are less dependent on LDH than animal models would suggest.

MCTs play a major role in lactate shuttling between oxygenated and hypoxic regions of tumours; oxidative cancer cells displaying MCT1 are able to absorb lactate excreted by MCT4-expressing glycolytic cancer cells [156]. Disrupting this process using MCT1 inhibitors has shown exciting potential retarding tumour growth and increasing cell death in breast cancer and myeloma cell lines *in vitro* [157–159]. Murine studies in lymphoma, breast, lung and colorectal cancer confirmed the anti-proliferative effects of this strategy as well as demonstrating augmented immune function via increased intra-tumoural DC and NK cell infiltration, in addition to a radio-sensitisation effect [159–161]. NCT01791595 is a first-in-man phase I trial of AZD3965, an MCT1 inhibitor, in patients with advanced solid tumours, diffuse large B cell lymphoma and Burkitt's Lymphoma to define the maximum tolerated dose. The estimated completion date is May 2021.

Induction of glycolytic genes by hypoxia and HIF-1α is a key mechanism by which tumours adopt an altered metabolic signature [20]. Moreover, the HIF-1-hypoxia axis is implicated in a range of tumour-promoting domains, classically angiogenesis, but also metastasis and therapeutic resistance. Therefore, there exists a convincing rationale for targeting this pathway: early efforts included 2-methoxyestradiol (2 ME) and tanespimycin (17-AAG), however, initial preclinical results showing efficacy *in vitro* and *in vivo* animal models were not replicated in human phase II trials [162]. In more contemporary times, several clinical trials have investigated the efficacy of HIF inhibitors some of which are ongoing, among them the HIF-2α inhibitors PT2385 and PT2977 (also known as MK-6482). NCT03216499 investigated PT2382 in patients with recurrent glioblastoma with 17 of 24 progressing; NCT03108066 and NCT02293980 are both ongoing trials investigating PT2385 in patients with advanced clear cell renal carcinoma. NCT03634540 is recruiting patients with clear cell renal carcinoma for treatment with PT2977 in combination with cabozantinib; NCT04195750 is also recruiting patients with advanced renal cell carcinoma for treatment with PT2977.

4. Conclusion and future directions

Lactate is a major saboteur of immune function in the TME. It can mediate its effects directly on cells such as by blocking cytotoxicity, motility or transcription factor function etc., as well as indirectly by inducing immunosuppressive cell types such as Tregs, TAMs and MDSCs. Immune escape driven by lactate within the TME is a major contributor to cancer growth, progression and metastasis, therefore, it is not surprising that lactate is a prognostic biomarker in many cancer types. The immune system is also critical in eliminating tumours in response to radio-, chemo- and immunotherapy, thus, lactate is emerging as a predictive marker of treatment response. In the age of personalised medicine, biomarker development is paramount to optimising patient treatment yet financial constraints of development, validation and use hinder the progress in this area. Lactate can be measured routinely in peripheral circulation and non-invasively with MRS and HP-MRI which exempts it from many of the factors which shackle biomarker discovery and validation.

Despite preclinical promise, clinical success with drugs targeting lactate metabolism is lacking. This is likely explained by the fallacies of preclinical models which cannot capture the spatial and temporal complexity of the TME are not representative of the harsh, nutrient-deprived and oxygen-poor conditions that tumours develop in Refs. [131]. Moreover, emerging evidence suggests that cancer cell metabolism is dynamic, rather than static, and evolves over time as tumours progress from premalignant lesions to disseminated metastatic entities; thus, targeting a single aspect or pathway is undermined by the ease in which cancer cells can recalibrate their metabolic phenotype [163]. Armed with this information, we should be cognisant of the fact that oncometabolites may have varying effects at different timepoints within the lifetime of a cancer. Future research should expand on our currently limited understanding in this area. Moreover, this knowledge would likely have important implications in deciding the optimal time to intervene with anti-metabolic drugs and the best combinations to choose. Key to achieving these goals is the development of better tumour models that more accurately capture the *in vivo* conditions transformed cells develop in and are exposed to.

A major unanswered question, thus far, is the impact lactate has on humoral immunity, DC function and ramifications of such for immune evasion and treatment with immunotherapies. Additionally, the literature is unclear regarding the role lactate may play in perpetuating chronic tumour-driving inflammation via release of molecules such as HMBG1 and if modulation of these pathways could be harnessed for therapeutic benefit. Further illumination of the role these factors play in creating and conserving an immunosuppressive TME will help the development and improvement of novel and existing therapeutics targeting same, respectively. Additionally, employing novel combination strategies of metabolic inhibitors with conventional drugs, may help overcome the hurdles that immune cells face in eliminating cancerous cells. In light of the evidence suggesting ameliorated immune function in conjunction with 2-DG, LDH and MCT1 inhibitors, it may be pertinent to investigate the efficacy of such drugs as adjuvants for immunotherapies. Lactate imaging has the potential to identify patients with high-lactate tumours who might benefit from this approach. Moreover, successful therapeutic targeting of lactate metabolism would likely lead to gains in other areas beyond augmented anti-tumour immunity by impacting the other functions of lactate within the TME such as acting as an alternative metabolic fuel, and promoting angiogenesis, invasiveness and metastatic potential of tumour cells, which were beyond the scope of this review.

Author contributions

Conceptualization: MD and ND; writing - original draft: CH; writing - review & editing: CD, MD and ND; supervision: MD and ND.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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