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## Review

## Rationale for LDH-targeted cancer immunotherapy



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**Abstract** Immunotherapies have significantly improved the survival of patients in many cancers over the last decade. However, primary and secondary resistances are encountered in most patients. Unravelling resistance mechanisms to cancer immunotherapies is an area of active investigation. Elevated levels of circulating enzyme lactate dehydrogenase (LDH) have been historically considered in oncology as a marker of bad prognosis, usually attributed to elevated tumour burden and cancer metabolism. Recent evidence suggests that elevated LDH levels could be independent from tumour burden and contain a negative predictive value, which could help in guiding treatment strategies in immuno-oncology. In this review, we decipher the rationale supporting the potential of LDH-targeted therapeutic strategies to tackle the direct immunosuppressive effects of LDH on a wide range of immune cells, and enhance the survival of patients treated with cancer immunotherapies.

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## 1. Introduction

Over the past decade, immune checkpoint blockades (ICBs) have significantly improved the survival of patients diagnosed with a wide range of metastatic tumour types. A broad spectrum of ICB agents have been approved, such as antagonistic antibodies, against Programmed Death Receptor 1 (PD1), Programmed Death Ligand 1 (PD-L1) and Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA4).

One of the biggest challenges is to unravel the tumour immune escape mechanisms limiting the ICB efficacy, since the majority of patients will either not benefit from those treatments (primary resistance) [1] or relapse after a period of response (secondary resistance) [2].

There have been many efforts in clinical cancer research to find ways to improve the efficacy of ICB and broaden its clinical applicability. To this end, an area of extensive investigation is identifying tumour-cell intrinsic and extrinsic factors that contribute to immunotherapy resistance. One of the first strategies has been to combine ICB to existing therapies such as cytotoxic chemotherapies and anti-angiogenics, which are believed to also modulate the tumour immunology [3,4].

In this review, we will decipher the rationale for strategies derived from the analysis of circulating lactate dehydrogenase (LDH) to guide personalised ICB therapy. While high LDH levels have been historically considered to be a poor prognostic marker in patients with advanced cancer treated with a broad spectrum of systemic therapies [5], recent studies have demonstrated that it could become a useful biomarker with negative predictive value in patients treated with ICB. Therefore, LDH-targeted therapy seems a promising immune-metabolic anti-cancer therapeutic strategy [6–8]. Briefly, cancer cells preferentially rely on non-oxidative glycolysis pathway (anaerobic metabolism), even under normoxic conditions through the Warburg effect [9]. This shift from an aerobic to anaerobic pathway is one of the hallmarks of cancer [10], and a major step for cancer progression [11] that could be tackled by LDH-targeted therapy.

In this review, we will describe the biology of LDH in the context of cancer, its impact on tumour immune escape and the potential of novel cancer therapeutic strategy blocking LDH activity to enhance the efficacy of ICB.

## 2. The biology of LDH

LDH is an enzyme, which has five tetrameric isoenzymes ensuing from the diverse combination of two subunits A and B (Fig. 1). It is present across multicellular organisms, including human, animals and plants [12].

### 2.1. Subunits A and B

Subunits A (also called M-subunit, for the muscle) and B (also called H-subunit, for the heart), are encoded by two different genes: *Ldh-a* and *Ldh-b* [13]. LDH-A converts pyruvate into lactate, and is mostly present in the skeletal muscle and liver. LDH-B predominates in the heart muscle and converts lactate into pyruvate [14,15]. LDH-A can be found in the cytosol, mitochondria and organelles, and LDH-B appears mostly present in mitochondria [16]. Furthermore, the ratio LDH-A:LDH-B varies according to tissues. Of note, the serum LDH level does not always correlate with the tumour LDH-A expression levels, and these parameters could be considered as two independent but complementary predictors [17].

### 2.2. LDH-1, 2, 3, 4, 5 and X.

LDH-2, composed of three B subunits and one A, is mostly present in red blood cells and in the reticuloendothelial system. LDH-3, made of two of each subunit, is significantly expressed in the lungs. LDH-4, with only one B subunit and three A, is the major enzyme in kidneys [15]. The fifth isoenzyme (LDH-5) is made of only A subunits and has a higher affinity for pyruvate compared to LDH-1 made of only B subunits. Over-expression of the LDH-5 isoenzyme has been identified in tumour cells [18,19]. A sixth isoenzyme, present in

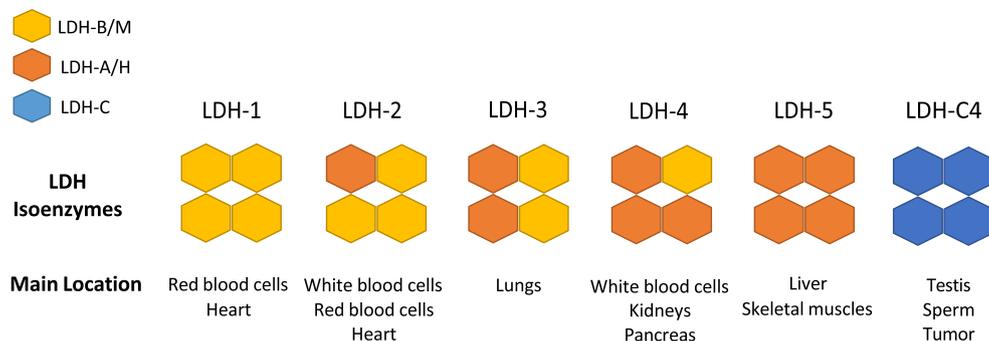


Fig. 1. The different isoenzymes of lactate dehydrogenase (LDH). LDH is a tetramer with five isoenzymes (LDH-1,2,3,4 & 5) depending on the combinations of the two subunits A (H) and B (M). The abundance of each isomer depends on the tissue. A sixth tetramer of the LDH-C subunit is more selectively found in testis, sperm and tumours.

testis and sperm, is composed of another subunit, the C (LDH-X; also termed LDH-C4) [13,20]. This latter one had also been found to be expressed in several types of human tumours, including melanoma, lung and breast cancer [21]. Of note, Chromosome 12p amplification (including the LDH-B gene) is a characteristic feature of testicular germ cell tumours, and those tumours are classically followed for relapse post-surgery by monitoring circulating levels of LDH [22].

### 2.3. Anaerobic glycolysis

LDH is involved in the metabolic pathway of anaerobic glycolysis, which consists of several reactions leading to the catabolism of one molecule of glucose into the production of two molecules of pyruvate and  $\text{NADH}^+$ . Pyruvate can then either be converted into lactate by LDH or enter into the tricarboxylic acid cycle (TCA) after its conversion into Acetyl-CoA. Once released into the bloodstream, the produced lactate is metabolised into pyruvate by the liver through the Cori cycle via LDH (Fig. 2) [23].

### 2.4. Warburg effect

As cancer cells increase their glucose uptake, the glycolysis rate increases about 200-fold compared to non-cancerous tissues [7], where glycolytic tumours have up to 40-fold elevated lactate levels compared to

peripheral tissues [24]. Whereas lactates were considered as waste from the Warburg effect (aerobic glycolysis), Faubert *et al.* and Hui *et al.* showed that they were used, over glucose, as fuel of the mitochondrial TCA cycle [25,26], where lactic acidosis can alter the metabolic pathways used for generating ATP [27].

### 2.5. Cancer metabolism

In the first study, Faubert *et al.*, infusions of  $^{13}\text{C}$ -lactate led to the presence of  $^{13}\text{C}$  TCA cycle metabolites in the tumour in both humans and mice suffering from lung cancer. Higher expression of both LDH-A, LDH-B and both monocarboxylate transporter 1 (MCT1), MCT4 lactate transporters were detected in tumours exhibiting the highest rates of lactate metabolism. Moreover, distant metastasis or tumour recurrence in the lung of patients was associated with increased lactate metabolism markers. In the second study, Hui *et al.*, flux assessment of  $^{13}\text{C}$ -labelled nutrients following intravenous infusions of healthy fed or fasting mice showed that in all tissues, but the brain, lactate exceeded glucose to fuel the TCA cycle. Similar observations were made in normal and tumoural lung tissues from genetically engineered fasting mice. Additionally, mass spectrometry studies tracking  $^{13}\text{C}$  TCA cycle metabolites in lung and cervical cancer cells have shown that  $^{13}\text{C}$  lactate can localise to the mitochondria, where LDH-B in the inner mitochondrial membrane can oxidise lactate to pyruvate [28]. These studies highlight that tumours are

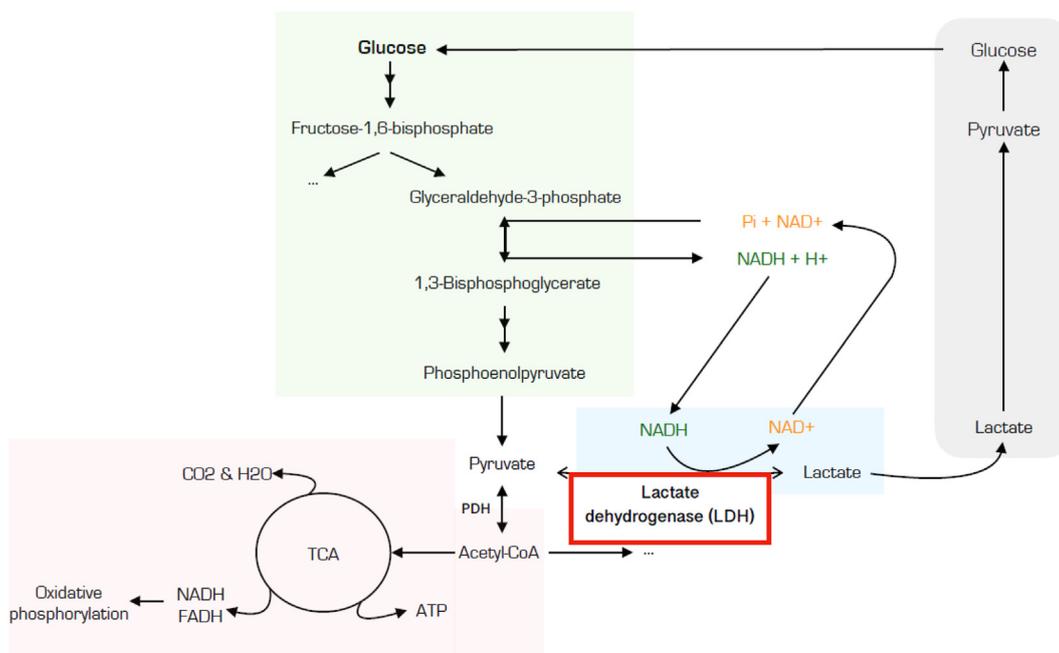


Fig. 2. **The glucose catabolism pathway.** When glucose enters into the cell, it is transformed into pyruvate through the glycolysis pathway (in green). Pyruvate can then follow the anaerobic (in blue) or the aerobic pathway (in red). The aerobic pathway is an oxygen-dependent pathway. Lactate dehydrogenase metabolises reversibly the conversion of pyruvate and lactate.  $\text{NADH}$  from the previous reaction is needed at this point, which is transformed into  $\text{NAD}^+$ . This way,  $\text{NAD}^+$  can be reused for the glycolysis. Lactate goes into the bloodstream and will be finally metabolised into glucose through the cori cycle in the liver (in grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

able to use lactate as a source of energy, especially under low glucose conditions [26], suggesting lactate metabolic pathways are a potential target to limit tumour progression.

### 3. Negative prognostic impact of elevated LDH in oncology

A meta-analysis of 76 studies confirmed the negative impact of LDH by an association with poor outcomes (HR for (overall survival) OS of 1.7 (95% confidence interval (CI): 1.62–1.79;  $p < 0.00001$ )) in solid tumours, notably renal cell, melanoma, gastric, prostate, nasopharyngeal and lung cancers (all  $p < 0.00001$ ) [29]. Another study on 5,799 participants confirmed the association of high LDH-levels in the serum and dismal survival [30].

These studies, among others [31–34], show that high LDH levels are associated with poor outcomes in many tumour types. Besides this negative prognostic impact of elevated LDH, the LDH-A expression is associated with the tumour growth, maintenance and tumourigenesis [35,36]. It is an important factor as a later study showed that tumour volume determined by computed tomography (CT)-scans could be used as a prognostic marker in immunotherapies [37]. Of note, in that study, CT-scan tumour burden per Response Evaluation Criteria in Solid Tumours did not correlate with circulating LDH levels in melanoma and non-melanoma patients, challenging the usual correlation that is widely accepted in the oncology community.

Using artificial intelligence techniques, Dercle *et al.* have recently demonstrated that elevated serum LDH was the dominant predictor of primary cancer resistance to anti-PD(L)1 immunotherapy [38]. In this study, artificial intelligence-driven prediction of OS showed that OS probability started to decrease linearly when LDH was above normal levels to reach a minimum value when LDH levels were above twice the lab normal limits. Among 33 baseline routine medical variables derived from CT images, clinical and biological metadata in 695 patients, elevated LDH and liver metastatic disease were dominant and independent predictors of short OS in patients treated with anti-PD(L)1 immunotherapies; whereas patients with normal LDH levels and no metastatic liver disease had significantly more prolonged overall survival when treated with immunotherapy than other systemic therapies ( $p = 0.00002$ ) [38]. This result was in accordance with another recent study in non-small cell lung cancer (NSCLC) [39].

### 4. Immunosuppressive effects of LDH-derived lactate and LDH in the tumour microenvironment

Increasing evidence suggests a role for the metabolite lactate in the modulation of several biological processes,

including the immune response. Lactate induces tumour cell proliferation, and may also modulate immune cell infiltration or function within the tumour microenvironment. Specifically, as presented below, lactate may alter both myeloid (monocytes, macrophages neutrophils, dendritic cells and mast cells) and lymphoid cell populations (natural killer cells, T cells and, in particular, the differentiation of Th1, Th17 and Tregs), thus affecting tumour immunosuppression (Fig. 3).

Exogenous lactate and protons (from lactic acid) in the tumour microenvironment can mediate their effects through activation of pH-sensing G-protein-coupled receptor (Gpr) 81 or 132 expressed on antigen-presenting cells and through the ubiquitously expressed MCT 1, responsible for the cellular uptake of lactate [40,41]. Once inside the cell, lactate is primarily thought to negatively regulate glycolysis, diverting cellular metabolism and modifying ATP production. Moreover, the expression in CD4+ and CD8+ T-cells of the sodium lactate transporter Slc5a12 and the lactic acid transporter Slc16a1, respectively, is also induced and impacts the function and motility of those immune cells [42].

Recent appreciation for the inherent ties between metabolic processes and immune cell fate and function puts forward the notion that cellular metabolic changes ultimately impact the type of immune response [43]. Put simply, cells with mainly inflammatory roles preferentially metabolise glucose, whereas cells with regulatory functions will prefer oxidative phosphorylation [44]. Thus, the immune suppressive role of lactate can be partially attributed to metabolic modulation. Other modes of action of lactate on the metabolic rewiring of immune cells can be attributed to changes in cell signalling pathways and epigenetic reprogramming, termed lactylation [45]. In this section, we will briefly summarise the impact of lactate, its preferential metabolism by cells of regulatory function, as well as its direct immune suppressive effects on lymphocytic cell populations.

#### 4.1. Myeloid cells

As activated immune cells can also produce lactate, macrophages with up-regulated toll-like receptors (e.g. TLR2 and TLR4) can use a MCT4, lactate transporter, to maintain a high glycolytic rate and secrete lactate; thereby, likely regulating the anti-tumour response [46]. Beyond lactate secretion, the lactate-rich tumour microenvironments have been shown to enhance the polarisation of M2-like macrophages in several cancer subtypes. Ohashi *et al.* observed higher head and neck squamous cell carcinomas tissue expression of CD163 and CSF1R in patients with higher lactic acid concentrations, suggesting a modified differentiation of macrophages toward an M2 phenotype [47]. In mouse models of lung carcinoma and melanoma, tumour-derived lactate induced the polarisation of bone

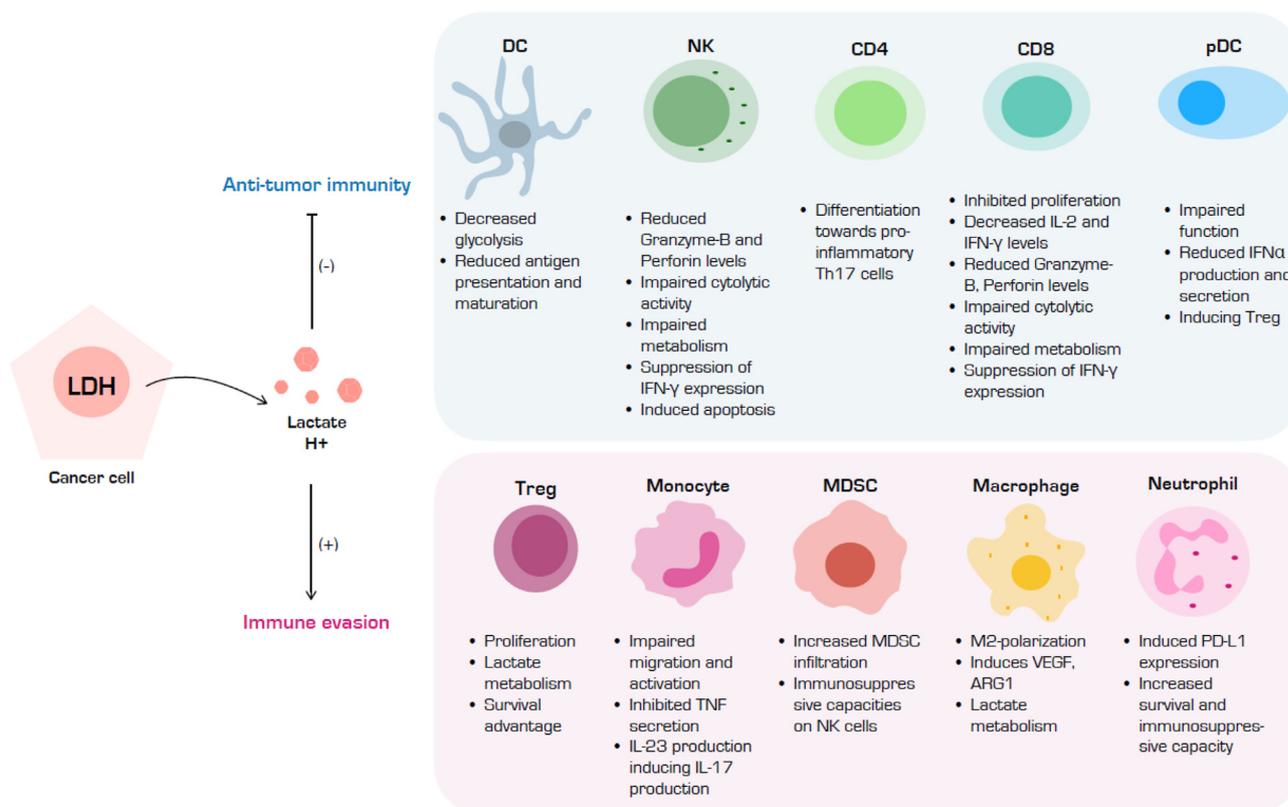


Fig. 3. **The immune suppressive effect of lactate dehydrogenase.** Via the lactate production, the LDH enzyme has an immune suppressive effect on various immune cells that are becoming ineffective, tolerogenic and promoting metastasis, angiogenesis and inflammation.

marrow-derived macrophages toward an M2-like phenotype, inducing proangiogenic vascular endothelial growth factor (*VEGF*) and arginase 1 (*ARG1*) expression through stabilisation of HIF-1 $\alpha$  [48]. In a breast cancer model, lactate-mediated ERK/STAT3 signalling was responsible for the increased expression of *VEGFA* and *ARG-1* in monocytes [49]. *In vitro*, the migration and activation (characterised by IL6 and tumour necrosis factor cytokine release) of human blood monocytes were impaired in the presence of lactate [50]. More specifically, a separate study identified the secretion of tumour necrosis factor by monocytes to be dependent on glycolysis, which was inhibited in the presence of exogenous lactic acid and lactate influx into the cells [51]. Recent work on the study of specific metabolic characteristics of heterogeneous tumour-associated macrophages (TAMs) subsets showed that MHC-II<sup>lo</sup> ‘pro-tumoural’ TAMs in NSCLC metabolically adapt to use lactate as metabolic fuel compared to MHC-II<sup>hi</sup> TAMs. This metabolic plasticity provides the MHC-II<sup>lo</sup> subset a clear survival advantage within a lactate-enriched tumour microenvironments potentiating their T-cell suppressive capacities [52].

#### 4.2. Neutrophils and myeloid-derived suppressor cells

Other regulatory cell subsets infiltration is enhanced by tumour lactate production, including myeloid-derived

suppressor cells. Investigating the effect of tumour-derived lactate on innate immune cell subsets, mice harbouring *LDH-A*-deficient pancreatic cancer had a significant decrease in myeloid-derived suppressor cells cell infiltration. In addition, these cells had reduced immunosuppressive capacities on NK cell function *ex vivo* [53]. In hepatocellular carcinoma, tumour-derived lactate can induce PD-L1 surface expression on tumour-infiltrating neutrophils via the NF $\kappa$ B/COX-2 pathway, increasing both their survival and immunosuppressive capacity [54].

#### 4.3. Dendritic cells

Proper activation of dendritic cells requires glycolysis [55,56], suggesting that the presence of lactate would negatively impact their maturation and antigen-presenting capacity. Specifically, while pro-inflammatory dendritic cells (DCs) express higher levels of LDH-A and produce higher lactate levels, modulated by sterol regulatory binding protein and HIF-1 $\alpha$ , lactate rich environments can condition DCs to express anti-inflammatory cytokines, such as IL-10, and lower pro-inflammatory cytokine production, such as IL-12, in response to toll like receptor activation [57–59]. Further, lactic acid inhibits the differentiation of monocyte-derived DCs to CD1a+IL-12+ subset *in vitro*; in mixed lymphocyte reactions, the addition of

lactic acid with peptide-pulsed dendritic cells showed a concentration-dependent blockade of antigen-specific CD8<sup>+</sup> T cell proliferation [58]. Plasmacytoid DCs (pDCs), major producers of type I IFNs and critical in anti-tumour immunity, are also impacted by tumour-derived lactate exposure [60]. In the breast cancer tissue, pDCs express higher levels of pH sensing Gpr81 and MCT1 transporter on the cell surface. *In vitro*, extracellular lactate exposure through Gpr81 and MCT1 on pDC-reduced IFN $\alpha$  production and secretion. In addition, lactate-exposed pDC in breast cancer induced greater Treg differentiation *ex vivo*, mediated by elevated tryptophan metabolism [60].

#### 4.4. Tregs

Treg cells highly contribute to an immunosuppressive tumour microenvironment. Compared to effector T cells, they present a different metabolic profile. By suppressing MYC, the transcription factor FOXP3 endows Tregs with a metabolic and survival advantage in low-glucose and high-lactate environments [61]. Specifically, unlike effector and cytotoxic T-cells, Foxp3 suppresses glycolysis and induces oxidative phosphorylation, where high lactate concentrations suppress T-cell effector potential and sustain Treg suppressor functions [52]. The function of Tregs and their immunosuppressive capacity are highly dependent on the use of lactate as a carbon source to fuel the TCA cycle [62]. Because glucose is detrimental to the suppressive function of Treg cells as well as their stability, they proliferate and thrive within an otherwise hostile lactic acid-rich environment [62].

#### 4.5. T-cells and NK cells

In the presence of elevated lactate levels, cytotoxic T-cells and NK cells are also directly impacted by lactate uptake. Upon lactate exposure, the intracellular levels of IL-2 and IFN $\gamma$  are decreased and the cytolytic activity of T-cells is impaired due to the reduced amount of Granzyme-B and Perforin in their intracytoplasmic granules [63]. In NK cells, exposure to lactate inhibits their production of Perforin and Granzyme-B and decreases their cytotoxicity [53]. The accumulation of lactate intracellularly alongside with decreased pH levels leads to an impaired metabolism measured by a drop in ATP production in both CD8<sup>+</sup> T and NK cells [65]. Moreover, the upregulation of the transcription factor NFAT is inhibited, resulting in the suppression of IFN $\gamma$  expression in CD8<sup>+</sup> T and NK cells. *In vivo*, IFN $\gamma$ -mediated cytotoxicity of T and NK cells is strongly inhibited in the mouse B16.SIY model, rescued by *LDH-A* inhibition of the tumour cells and reduced glycolytic activity [65].

In colorectal cancer liver metastasis, human NK cells are significantly depleted and undergo apoptosis *in vitro*

in the presence of tumour conditioned medium, associated with elevated lactate levels [66]. Increased apoptosis of NK cells is directly linked to a decreased intracellular pH and elevated mitochondrial ROS expression.

Among CD4<sup>+</sup> T-cells, lactate can promote their differentiation towards pro-inflammatory Th17 cells [67,68]. Lactate uptake by naïve CD4<sup>+</sup> T-cells diverts their metabolism towards fatty acid synthesis, a signalling pathway essential to Th17 cell function in chronic inflammatory diseases [69]. In line with these findings, tumour cell line-secreted lactic acid stimulates IL-23 production by monocyte/macrophages, which in turn induces IL-17 secretion by antigen-specific T-cells, potentiating tumour growth [70].

Overall, a lactate-rich tumour microenvironment can metabolically support regulatory cell populations while strongly inhibiting cytotoxic and effector T-cell immunity. Targeting tumour metabolism may provide a promising approach in restoring anti-tumour immunity and enhancing response to immunotherapy checkpoint blockade in multiple cancer types.

### 5. Negative impact of LDH on immune checkpoint targeted therapies

Although the precise role of LDH biology in cancer remains only partially understood, circulating levels of LDH have been independently identified as a negative predictor of patient response to ICB in multiple studies.

First, a randomised controlled open-label phase III trial for patients with advanced melanoma treated with nivolumab monotherapy or nivolumab in combination with ipilimumab showed improved five-year OS in patients with physiologically normal levels of LDH. The median OS in patients with normal levels of LDH in the combination group was >60 months compared with 17.4 months in patients with high LDH levels [71].

Second, in a phase III clinical trial for patients with small cell lung cancer in the second-line setting, normal physiological levels of LDH ( $\leq$ ULN) and the absence of liver metastasis were both independently associated with improved OS with nivolumab monotherapy compared to chemotherapy (HR, 0.76 [95% CI, 0.59–0.98], and 0.74 [95% CI, 0.59–0.94] respectively) [72].

Third, a retrospective meta-analysis including 1136 patients on the impact of high serum LDH in patients with NSCLC treated with immune checkpoint immunotherapies found significant association between elevated LDH and poor progression free survival (PFS) and overall survival (OS) (HR, 1.62 [95% CI, 1.26–2.08], and HR, 2.38 [95% CI, 1.37–4.12]) [73]. In addition, a retrospective meta-analysis including 16,159 patients with malignant melanoma treated with combination targeted therapies and immunotherapy observed strong association between LDH and poor PFS and OS

(HR, 1.83 [95% CI, 1.53–2.2], and HR, 1.72 [95% CI, 1.6–1.85]) [74].

Finally, a recent prospective study on multiple solid tumour types, including patients in phase I clinical trials testing single or combination immunotherapy, observed elevated baseline LDH as independently associated with worse PFS and OS (HR, 1.97 [95% CI, 1.30–2.99], and HR, 2.06 [95% CI, 1.26–3.28]) [75].

Considering the differential affinity and thus metabolic activity of the various LDH isoenzymes, as mentioned previously, Wilpe *et al.* [67] assessed the impact of LDH-isotyping in the blood of melanoma patients as a better predictor of response to combination PD1 and CTLA4 blockade, especially the measurement of LDH-5, known to be significantly increased in tumours. They observed that baseline levels of LDH-1 were decreased in patients showing no clinical benefit (PFS < 6 months), though not significant. Surprisingly, no patients had high levels of LDH-5. They did not find differences in other LDH isoenzymes frequency and clinical benefit, suggesting that this approach may not wholly reflect the glycolytic activity within tumours [76].

The use of more robust biomarkers associated with tumour glycolysis in addition to LDH levels may help predict the immunosuppressive capacity of malignant cells. Comparing the expression of glycolysis-related genes (including *LDH-A*) and T-cell infiltration in melanoma and NSCLC samples from TCGA and PROSPECT cohort, Cascone *et al.* [68] observed reduced T-cell signatures in patients with high glycolysis gene expression. This was confirmed by immunohistochemistry staining of T-cell infiltration on paired FFPE samples [77]. An additional study on the measurement of glycolytic-related gene index based on the log<sub>2</sub> weighted co-expression of 14 genes (including *LDH-A* and *MCT1*) found that a ‘high glycolytic score’ was negatively correlated with PFS in melanoma patients treated with anti-PD1 [78]. Gene expression profiling from four breast cancer datasets showed patients with poor survival had high LDH-A/HIF-1 $\alpha$  levels and low CD3/CD4/CD8 expression levels [79]. Overall, these data suggest that LDH is associated with local and systemic tumour immunosuppression, warranting further research on its role and potential as a novel targeted therapy in combination with checkpoint blockade.

## 6. Therapeutic targeting of LDH metabolism for cancer immunotherapy

Based on the tumour cell preferential usage of lactate as a fuel source and the deleterious effects of elevated serum LDH on patient outcomes with immune checkpoint blockade in different tumour types, a promising

therapeutic strategy would be to target tumour cell metabolism through direct or indirect blockade of LDH.

### 6.1. Pre-clinical models

Recent studies on the effects of LDH-A deletion in pre-clinical models provide supportive evidence for the link between tumour cell metabolism and the immune response. Serganova *et al.* [79] demonstrated that LDH-A deletion in the 4T1 murine breast cancer significantly reduced primary tumour growth and metastatic potential in immunocompetent mice. Phenotypic changes in the tumour microenvironment included reduced HIF-1 expression, vessel normalisation, increased infiltration by CD3+ and CD4+ T-cells and decreased TAMs infiltration [79]. This study demonstrated the regulation of the tumour microenvironment by LDH-A via HIF-1 mediated production of VEGF. Interestingly, conditional deletion of LDH using a tamoxifen-inducible Cre K-Ras-mutant lung cancer and LDH-A inhibition in B16F10 melanoma model reduces PD-L1 expression on tumour cells, enhancing T-cell mediated anti-tumour immunity [80]. Seth *et al.* also studied the separate effect of LDH-A deletion in tumour-associated macrophages, which increased their activation (CD86 expression/M1 polarisation) and modified their cytokine secretion profile, influencing T-cell PD-1 expression and Tc17 (IL17-producing CD8+ T cell) polarisation. The Tc17 is a cell subtype with known anti-tumour properties [81]. A more recent study on mouse splenic CD8+ T-cell activation demonstrated raised LDH-A expression in IL-2-treated versus IL-21-treated cells as a consequence of amplified glycolysis and reduced incorporation of pyruvate into the TCA cycle [82]. As opposed to IL-2, IL-21 is known to induce stem cell memory-like phenotype and anti-tumour immunity. Use of a small LDH-A inhibitor on CD8+ T cells promoted the oxidation of pyruvate via the TCA cycle in IL-2-treated cells. This study confirmed that transient inhibition of LDH-A during CD8+T cell differentiation *in vitro* significantly improved anti-tumour immunity in an adoptive transfer mouse model of melanoma [82]. In a mouse model of B16F10 melanoma, the deletion of LDH-A in tumour cells in combination with anti-PD1 improved the efficacy of immune checkpoint blockade compared to anti-PD1 alone [83]. This was mediated by increased NKT and T-cell infiltration, metabolic activation through facilitated oxidative phosphorylation and cytotoxic degranulation of the cells.

Overall, these pre-clinical studies demonstrate the potential to improve immune checkpoint immunotherapies efficacy through the targeted deletion of LDH-A, through its role not only on tumour cell immune evasion, but also macrophage differentiation and CD8+ T cell fate.

## 6.2. Safety profile

The inhibition of LDH is generally believed to be safe, as individuals harbouring lactate dehydrogenase-A deficiency develop mainly symptoms of exercise intolerance (muscle fatigue and rigidity) [84]. An overview of the principal therapeutic inhibition methods is presented in Table 1. Galloflavin inhibits both LDH-A and LDH-B isoforms, competitively inhibiting NADH interaction with LDH [85]. It shows anti-tumour effects through induction of the apoptotic pathway *in vitro* on breast cancer and endometrial cancer cell lines [86,87].

## 6.3. N-hydroxyindole-based pathway modulation

The upregulation of glucose transporter I by cancer cells can be hijacked by the use of glycoconjugates, such as the LDH inhibitor N-hydroxy-indole-based conjugated with D-glucose [88]. In an orthotopic mouse model of pancreatic cancer, glycoconjugate N-hydroxy-indole-Glc-2 was highly potent at inhibiting tumour growth, compared to chemotherapy gemcitabine [89].

## 6.4. Pyruvate pathway modulation

Other inhibitors of LDH-A can act indirectly, including the pyruvate analogue oxamate. *In vitro*, studies show that oxamate exhibits known inhibitory effects on LDH, decreasing both proliferation and lactate production [90] in gastric cancer cell lines and increasing radiosensitivity through increased intracellular ROS and ATP depletion in NSCLC cell lines [91]. Interestingly, oxamate inhibition of medulloblastoma cell line metabolism was more effective than siRNA of LDH-A, leading to the hypothesis that the direct silencing of LDH-A does not fully inhibit the function of other LDH subunit LDH-B as compared to the wider range of inhibition mediated by pyruvate analogues [92].

## 6.5. NADH-pathway inhibitor

The NADH-competitive inhibitor, gossypol, has shown anti-tumour effects *in vitro* as well as *in vivo* in patients with

advanced or metastatic cancer in early phase I/II clinical trials [93,94]. A recent clinical trial of gossypol/AT-101 in patients showed encouraging results, with 11/13 patients enrolled presenting a clinical CR to combination AT-101 treatment and chemo-radiation, improving OS. Immunohistochemistry staining in surgically resected specimens from two patients confirmed pre-clinical results, showing targeted downregulation of cancer-stem cell protein expression YAP1 and SOX9 [95].

A derivative of gossypol, FX11, in combination with NAD<sup>+</sup> inhibitor FK866, induced tumour cell death in human cancer cell lines dependent on glycolysis for survival (RCC4, MCF-7 and P493 B). In this study, Le *et al.* noted that the efficacy of FX11 *in vivo* using lymphoma P493B and pancreatic cancer was dependent on tumour hypoxia, noting hypoxic regions of tumours responded better than non-hypoxic regions; demonstrating the dynamic and spatial complexity in the response to LDH-A inhibition [96]. In addition, the NADH<sup>+</sup> competitive inhibitor for LDH-A quinoline-3-sulfonamide was also effective only in human and breast cancer and hepatocellular carcinoma cell lines deficient in LDH-B expression in hypoxic conditions [97].

## 6.6. Co-inhibition of LDH-A/B

Due to the metabolic plasticity of cancer cells, the inhibition of either LDH-A/B may promote amplification of alternative metabolic pathways through enhanced mitochondrial oxidative phosphorylation [98]. To this end, a recent study on the co-inhibition of LDH-A/B, using a specific inhibitor NCI-006 in combination with Mitochondrial complex I blockade (IACS-010759) in mouse xenograft models of human pancreatic and colorectal cancer, showed enhanced anti-tumour effects [99].

## 6.7. Disruption of LDH subunit association

More recently, the development of small peptides disrupting the subunit association of LDH-A subunits (e.g. the tetramerization of LDH-5) showed promise through their high specificity and are in the stages of pre-clinical testing [100,101].

Table 1

Overview of the principal therapeutic methods targeting directly or indirectly the lactate dehydrogenase.

| Target                                       | Mechanism   | Drug  | References |
|--|---|---|------------|
| Direct lactate dehydrogenase (LDH)-targeting | Genetic deletion  | shRNA   | [70,80–83] |
|  | Binding the free enzyme                                 | Gallic acid (galloflavin)                         | [85–87]    |
|  | LDH subunit dissociation                                | Drug – peptides                                   | [100,101]  |
| Indirect LDH-targeting                       | Acting by pyruvate competition                          | Oxamic acid and derivatives (e.g. sodium oxamate) | [90–92]    |
|  | Acting by enzyme cofactor NADH and pyruvate competition | Gossypol and derivatives (e.g. FX11)              | [93–97]    |
|  |   | quinolines (e.g. quinoline 3-sulfonamide)         | [88,89]    |
|  |   | N-hydroxy-indole (NIH-1,2)                        | [105–108]  |
| Monocarboxylate transporter 1/4 inhibition   |   | AZD3965   | [105–108]  |
|  |   | NSAID (e.g. diclofenac)                           |            |
| Glucose availability                         | Depleting glucose availability                          | Ketogenic diet, vitamin C, anti-diabetics         | [102,103]  |
| Co-inhibition LDH and OXPHOS pathway         | Mitochondrial complex I blockade                        | Anti-diabetics metformin and phenformin           | [104]      |
|  |   | NCI-006/IACS-010759                               | [99]       |

### 6.8. Depleting glucose availability

Lastly, there are other interventions to reduce the LDH levels by depleting glucose availability through a ketogenic diet, high vitamin C consumption, or anti-diabetic drugs [102,103]. Interestingly, anti-diabetic drug metformin targets Mitochondrial complex I and has been tested in combination with LDH blockade *in vitro* in human pancreatic cancer. The combination treatment was highly effective at inhibiting tumour proliferation and inducing cell death under both normoxic and hypoxic conditions [104].

### 6.9. Modulating lactate efflux/influx

A different mode of action to target the immunomodulatory effects of lactate is through the inhibition of MCT1/MCT4, which shuttle lactate across the cell membrane, enabling lactate efflux by tumour cells and influx by cells within the tumour microenvironment. In mouse models of B16 melanoma, MCT1-deficient Tregs display reduced immunosuppressive effects in primary tumours, improving T-cell cytotoxicity and response to anti-PD1 treatment [62]. The MCT1-inhibitor, AZD3965, has shown high efficacy inducing tumour cell death in a diffuse large B-cell lymphoma (DLBCL) SCID mouse model, resulting in increased dendritic cell maturation and NK cell infiltration [105]. Furthermore, a recent dose-escalation trial for AZD3965 in 11 patients with diffuse large B-cell lymphoma and Burkitt Lymphoma demonstrated proof-of-mechanism with complete response in one patient with a reduction in tumour FDG uptake [106]. Tumour-targeted delivery of AZD3965 by intravenous injection of pH-sensitive nanoparticles (AZD-UPS NP) increases the efficacy, and significantly reduces adverse toxic effects compared to orally administered drug in a mouse model of TC-1 [107]. Combination of AZD-UPS NP with anti-PD1 significantly reduced tumour growth and improved survival in two mouse models of TC-1 and B16F10 compared to either treatment alone. The authors concluded that lactate export by tumour cells was effectively inhibited by AZD-UPS NP, which primed the tumour microenvironment to improve immunotherapy response. Indeed, combination treatment enhanced infiltration by antigen specific CD8+ T cells in this model.

The non-steroidal anti-inflammatory drug diclofenac inhibits MCT1 and MCT4, inhibiting lactate efflux from B16F10 tumour cells. *In vivo*, the combined treatment of diclofenac, anti-PD1 and anti-CTLA4 significantly improved survival, compared to immunotherapy or diclofenac treatment alone [108].

Early phase I and II clinical trials testing inhibition of LDH show promising results in certain cancer types, warranting confirmation in randomised controlled

clinical trials. Furthermore, research on systemic and intratumoural biomarkers of immunological response following these novel treatment strategies can aid in the development and application of combination therapeutic strategies in cancer immunology. Ultimately, improved understanding of the temporal complexity of cancer metabolism and progression in association with immune escape is crucial to the correct timing and combination of treatments.

## 7. Conclusion

One of the hallmarks of cancer cells is their metabolic switch to anaerobic glycolysis, with LDH representing a key enzyme in this process. Circulating LDH levels are high in many advanced cancer patients. While increasing levels of LDH can be considered as a consequence of cancer cell metabolism, it is also a cause of many inherently tied hallmarks of cancer, including angiogenesis, invasion, metastasis and immune evasion. LDH-inhibition has been deemed relatively safe and has demonstrated promising results, experimentally, in the context of immunotherapy combinations in pre-clinical models. Because there is a clear mechanistic association between LDH elevation and poor survival in cancer, inhibiting LDH could improve patient outcome and make immunotherapies more effective in various types of cancer. However, there is a need for more investigations to determine, apart from the safety and efficacy, which LDH isotype should be targeted and how and which patients should be treated with an LDH inhibitor. Understanding the specificity of LDH changes in the context of the anti-tumour response can guide the design of selective therapeutic strategies. Importantly, the level of evidence accumulated over the recent years on the negative impact of high circulating levels of LDH across cancers should make mandatory the stratification of cancer immunotherapy clinical trials on this easily implementable biomarker.

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## Author statement

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## Conflict of interest statement

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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