Warburg and Krebs and related effects in cancer

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Abstract

Warburg and coworkers' observation of altered glucose metabolism in tumours has been neglected for several decades, which, in part, was because of an initial misinterpretation of the basis of their finding. Following the realisation that genetic alterations are often linked to metabolism, and that the tumour micro-environment imposes different demands on cancer cells, has led to a reinvestigation of cancer metabolism in recent years. Increasing our understanding of the drivers and consequences of the Warburg effect in cancer and beyond will help to identify new therapeutic strategies as well as to identify new prognostic and therapeutic biomarkers. Here we discuss the initial findings of Warburg and coworkers regarding cancer cell glucose metabolism, how these studies came into focus again in recent years following the discovery of metabolic oncogenes, and the therapeutic potential that lies within targeting the altered metabolic phenotype in cancer. In addition, another essential nutrient in cancer metabolism, glutamine, will be discussed.

Introduction

The way cancer has been considered and investigated over the last century has been mainly as a genetic disease primarily characterised by dysregulated cell growth. A variety of aberrations in oncogenes leading to gain of function or amplification, and in tumour suppressor genes from inactivating point mutations or epigenetic silencing, have been considered as responsible for the cancer phenotype. Although this understanding has helped to unravel many aspects of this complex disease, it also led to the neglect of early, often biochemical findings, on the role of cancer metabolism, such as the Warburg effect. New evidence is now linking many tumour suppressors and oncogenes to the regulation of metabolism and the need of highly-proliferating cancer cells to juggle nutrient utilisation in a manner that supports continuous and fast cell growth and survival. In over two-third of human cancers, at least one glycolytic gene is constitutively overexpressed (Ref. 1). Thus, current research is actually returning back to classical biochemical studies to further understand their implication in disease initiation and progression with the hope that new applications of those old discoveries will lead to the development of new therapeutics.

Cancer cells, like highly proliferating normal cells, are faced with the task to generate sufficient ATP to maintain energy status as well to produce increasing amounts of biosynthetic metabolites and precursors, whilst balancing the cellular redox status. In particular, for cancer cells, they also need to adapt their metabolic processes to a different environment from normal tissue, where nutrient supplies may be exhausted as well as instability in the redox balance, pH and oxygen levels (Ref. 2). One such adaptive mechanism is aerobic glycolysis or the Warburg effect, in which cancer cells ferment glucose to lactate despite an ample oxygen supply.

This review describes the Warburg effect from a historical perspective, how this fundamental finding on cancer metabolism was forgotten for several decades before re-emerging with the discovery of metabolic oncogenes and how the Warburg effect forms the basis of several therapeutic strategies in cancer currently used or in development.

Historical perspectives on the Warburg effect

Warburg's work on glycolysis in cancer cells came at a time when the scientific community was mainly focusing on lipolysis and/or proteolysis as the energy-providing reactions that drive cancer cell growth rather than on glucose metabolism (Ref. 3). Following on from his observations on the sea urchin egg, for which a significant increase in oxygen uptake and rapid cell division occurred after fertilisation, Warburg expected a similar increase in oxygen uptake in cancer tissues (Refs 3, 4).

Together with Seigo Minami, Warburg observed that Flexner–Jobling rat liver tumours were able to acidify the surrounding isotonic salt solution after the addition of glucose by releasing lactic acid into this solution (Ref. 5). Warburg and coworkers went on to further examine glycolysis in rat liver carcinoma slices by measuring oxygen consumption as well as carbon dioxide production under aerobic and anaerobic settings using his improved manometric technique (Refs 6, 7). In this experimental set up, they made three key observations: (1)
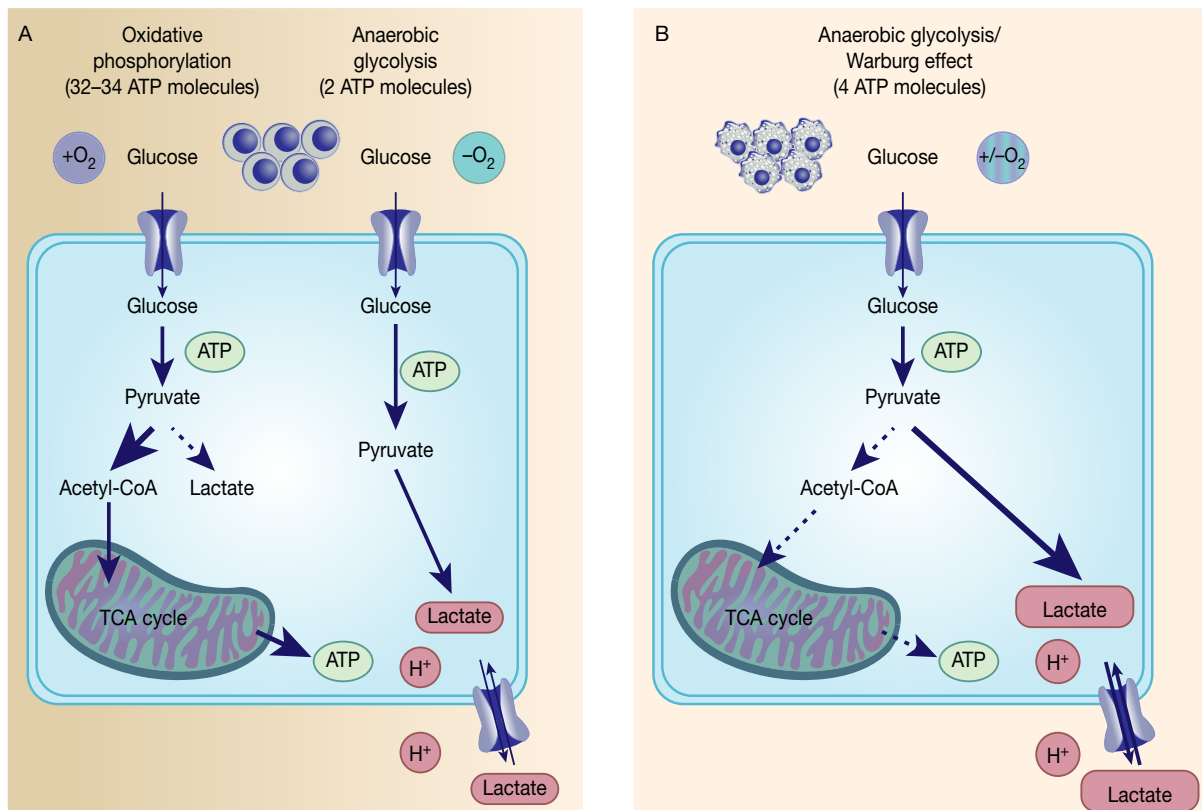
Fig. 1. The Warburg effect in cancer. Differentiated, normal tissues (a) metabolise glucose depending on oxygen levels with normoxic conditions favouring complete glucose metabolism in the mitochondria to maximise ATP production (oxidative phosphorylation). Under low oxygen levels, the majority of glucose will be metabolised to lactate, which is excreted from the cells, but allows sustaining a minimum level of ATP production (anaerobic glycolysis). In contrast, tumour cells (b) mainly metabolise glucose to lactate independent from intracellular oxygen levels and despite of thereby producing less ATP compared with full oxidation of pyruvate within the mitochondria (aerobic glycolysis or Warburg effect).

the production of lactic acid under anaerobic conditions was much higher in rat carcinoma tissues compared with the reference values from the literature for, for example, human blood or frog muscle, (2) the carcinoma did not take up more oxygen under aerobic conditions than normal tissue and (3) by moving the carcinoma from anaerobic to aerobic conditions, the amount of lactate produced was only marginally reduced (Ref. 6). These findings can be summarised as aerobic glycolysis or later termed the Warburg effect, which is the preference of tumours for fermentation of glucose to lactate even in the presence of sufficient amounts of oxygen. This is in contrast to normal tissue that exhibits the Pasteur effect of completely stopping lactate production in the presence of sufficient amounts of oxygen (Fig. 1) (Ref. 6). Similar results were obtained in different human carcinomas (from larynx, intestine, penis, nose and skin) and comparison with benign tumours showed that malignant tissue produced 3–4 times more lactic acid (Ref. 6). Elegant in vivo experiments by Cori and Cori demonstrated that low lactic acid concentrations within the tumour were maintained by releasing lactic acid into the bloodstream (Refs 8, 9). Later experiments by Warburg and coworkers revealed even higher levels of fermentation in Ehrlich ascites cancer cells from mice compared with the findings in rat sarcomas, a fact probably because of a higher percentage of cancer cells in the mouse ascites cancer (Ref. 10).

Warburg initially reasoned that the observed decrease in oxidative phosphorylation under normoxic conditions was because of defects in the mitochondrial respiration of cancer cells resulting in compromised aerobic respiration and subsequent reliance on glycolytic metabolism (Ref. 11). To him the irreversible injury of respiration was the essential common cause and origin of cancer, a hypothesis that led to major scientific controversies and was

generally not accepted among most biochemists and oncologists because of missing experimental evidence (Refs 12, 13, 14). However, subsequent evidence emerged to suggest that respiration is generally not impaired in cancer cells and today we appreciate that the high rate of glycolysis exhibited by cancer cells under normoxic conditions is not due to damaged respiration, but rather changes in the molecular aspects of glycolysis.

However, the reasons behind the Warburg effect are under continuous investigation. Although oxidative phosphorylation yields between 32 and 34 ATP molecules per molecule of glucose, glycolysis only produces two molecules of ATP per molecule of glucose (Ref. 15) (Fig. 1). Despite being the energetically less favourable pathway, it is likely that there is a selective advantage for cancer cells to adapt their metabolism to glycolysis, such as being a way to balance energy production and generation of necessary building blocks to support proliferation.

Rediscovery of the metabolic role in cancer through the Darwinian evolution of the cancer genome

Over the last two decades, there has been a strongly increasing interest and re-examination of the Warburg effect and cancer metabolism with the reprogramming of metabolism in cancer being added to the original set of hallmarks of cancer from Hanahan and Weinberg in 2011 (Ref. 16).

Prior cancer research had mainly focused on understanding genetic alterations in cancer with the identification of oncogenes and tumour suppressor genes as main types of cancer-causing genes (Refs 17, 18), while oncogenic alterations of cell metabolism were considered as secondary effects. Extensive studies of the genetics of many tumours, facilitated by high-throughput sequencing

techniques, highlight the strong inter- and intratumour heterogeneity, which account for one of the major challenges in the treatment of cancer. Our current understanding of how intratumour genetic complexity arises follows one of the first descriptions of cancer as an evolutionary process from Peter Nowell in the 1970s (Ref. 19). Reminiscent of the Darwinian evolution theory of the 'survival of the fittest', tumourigenesis follows a natural selection process governed by environmental selection forces and cellular adaption strategies in order to confer a selective survival advantage to the cell (Refs 19, 20). This perception had not only brought the importance of the tumour micro-environment back into focus, but also made researchers look more closely at how cancer cells adapt to the particular challenges of a highly proliferative cell, and thus cancer metabolism. Additionally, further elucidation of cancer-related driver mutations, that is, mutations that arise during tumourigenesis and provide a selective advantage for the tumour cells, identified that many have a significant role in core signalling pathways responsible for nutrient uptake and metabolism (Ref. 21). It has been shown that many mutated key signalling pathways come together in order to adapt tumour metabolism and to sustain tumour growth and survival (Ref. 21).

Historically, the first metabolic pathways to be studied biochemically in cancer were the nucleotide de novo and salvage pathways. Enhanced activities of enzymes within these pathways, especially of the salvage pathway, leading to increased nucleotide levels were reported in tumours, such as hepatomas, lung and renal cell carcinoma (Refs 22, 23). Increased nucleotide synthesis in cancer epitomises the adaption of metabolism to cope with the demands of high proliferation and has been a consistent area of interest and target for chemotherapeutics (see 'Antimetabolites'). Although alterations in nucleotide metabolism have been studied continuously, carbohydrate metabolism, the focus of this review, went through a long period of neglect. It was only after the identification of germline mutations in different metabolic enzymes, such as fumarate hydratase (FH), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH), that metabolic enzymes involved in this pathway were considered to play an important role in tumourigenesis (Refs 24–26). Today, not only are mutations in oncogenes or tumour suppressor genes known to impact on metabolic reprogramming, but also the idea of metabolic oncogenes has emerged, such as highlighted in the cases of IDH and 3-phosphoglycerate dehydrogenase (PHGDH).

IDH1/2 as an example in malignant gliomas/AML

Identification of somatic activating mutations in *IDH1* in glioblastoma (GBM) was a key to opening a renewed interest in cancer metabolism and its impact on tumourigenesis (Ref. 26). *IDH1* encodes a cytosolic form of IDH, which catalyses the oxidative carboxylation of isocitrate to α-ketoglutarate (α-KG) within the Krebs cycle. A large sequence analysis of 20 661 protein coding genes in 22 human tumour samples identified point mutations in *IDH1* which all lead to substitutions in the same amino acid (arginine 132) and were mainly prevalent in younger patients (mean age of 33 years) and patients with secondary GBM (Ref. 26). Initial follow-up studies found mutations in *IDH1* or *IDH2* in over 70% of WHO grade II/III astrocytomas, oligodendrogliomas and GBMs (Ref. 27) as well as *IDH1* mutations in about 8% of acute myeloid leukaemia (AML) patient samples (Ref. 28) with later genomic analysis confirming *IDH* mutations in several types of cancer (reviewed in Ref. 29). Both *IDH1* and *IDH2* mutations cause a **gain-of-function** in enzyme activity towards reduction of α-KG to the oncometabolite R-2-hydroxyglutarate (2-HG) while oxidising NADPH/H$^+$ (Refs 30, 31). Although 2-HG is only present at very low concentrations in normal cells with no attributed function (Ref. 29), it

was not clear how 2-HG was potentially driving cancer. The first hint came from a profiling study of DNA methylation patterns in GBM tumours in which a large subset (mainly from the proneural subtype) displayed a hypermethylation pattern of CpG islands (CIMP) together with a frequent *IDH1* mutation (Ref. 32). This correlation between the CIMP phenotype and *IDH1* mutation was further strengthened by showing that the introduction of a mutation in *IDH1* was sufficient to establish the hypermethylator phenotype in gliomas (Ref. 33). Mutational and epigenetic profiling of AML patients revealed *IDH1/2* mutations to be mutually exclusive with loss-of-function mutations in *TET2* (Ref. 34). TET2 is an α-KG-dependent dioxygenase that hydrolyses 5-methylcytosine, and is one of several α-KG-dependent dioxygenase, including histone methyltransferases, histone demethylases and prolyl hydroxylases, that is competitively inhibited by 2-HG (Ref. 35). Thus, a mutation in IDH1/IDH2 leads to the accumulation of the oncometabolite 2-HG which in turn then inhibits various α-KG-dependent dioxygenases resulting in a genome-wide alteration of the histone and DNA methylation pattern (Fig. 2).

However, the clear role of *IDH* mutations in tumour formation and progression is still being investigated. *IDH1* mutations have been proposed as very early events in tumourigenesis in gliomas with *IDH1* mutations occurring prior to the acquisition of *TP53* mutations, loss of chromosome 1p/19q or copy number alterations of phosphatase and tensin homologue (*PTEN*) and *EGFR* (Refs 36, 37). IDH1 R132H mutation was also found to be the only shared mutation between initial and recurrent tumours of 23 GBM patients (Ref. 38) as well as being maintained in 95% of secondary GBMs in another analysed cohort of 106 gliomas (Ref. 39). In addition, studies of haematopoietic disorders found *IDH1/2* mutations in premalignant disease forms, such as myelodysplastic and myeloproliferative neoplasms (Refs 40, 41). However, in vivo mouse models introducing *IDH2* mutations showed that this mutation on its own was insufficient to drive leukemogenesis and rather had the role of a cooperative tumourigenic driver in AML (Refs 42, 43). Although no spontaneous tumour formation was observed after introduction of an *IDH1* mutation in several cell types of the murine central nervous system, mutant IDH1 seems to have a clear role in tumour maintenance as pharmacological inhibition of mutant IDH1 impairs growth in established glioma xenograft models (Refs 44, 45). Although these findings support the role of *IDH* mutations in tumourigenesis and tumour maintenance, further investigations are needed to determine exactly to what extent and in what precise context.

PHGDH: the first enzyme of the de novo serine synthesis pathway

The de novo serine synthesis pathway comprises a three-step reaction cascade from 3-phosphoglycerate to L-serine, consecutively catalysed by PHGDH, phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSPH) (Fig. 3). The first indication of serine synthesis pathway alterations in cancer came from studies by Davis *et al.*, who had observed increased PHGDH activity in rat hepatoma cell lines in comparison to their corresponding normal host liver cells (Ref. 46). Subsequent studies by Snell and coworkers confirmed increased PHGDH activity in rat hepatomas, which was correlated with their growth rate, as well as in human colon carcinoma samples providing yet more evidence of PHGDH's link to tumour initiation and progression (Refs 47, 48).

However, only enzymatic activities had been compared so far and it was not until 25 years later that genomic and metabolomic analysis rediscovered PHGDH in the context of cancer (Refs 49–51). Negative-selection RNAi screening in a human
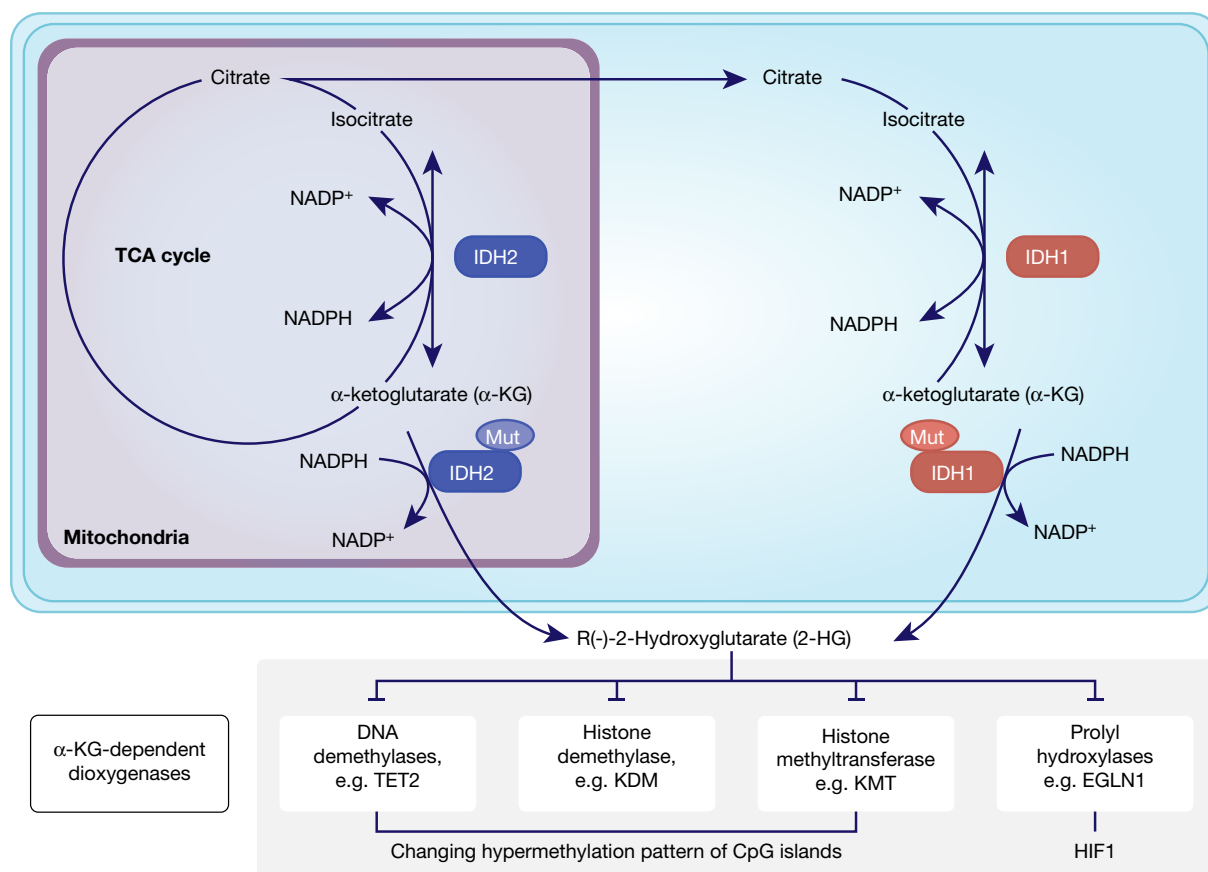
Fig. 2. Somatic IDH1/2 mutations reduce α-KG to the oncometabolite 2-HG. Mutant IDH1/2 (IDH1/2^{mut}) have gained the function to reduce α-ketoglutarate (α-KG) to the oncometabolite R(-)-2-hydroxyglutarate either in the mitochondria by IDH2^{mut} or in the cytosol by IDH1^{mut}, which then inhibits several α-KG-dependent dioxygenases, which leads to genome-wide alteration of the histone and DNA-methylation patterns and HIF1 regulation.

breast cancer xenograft model identified *PHGDH* within a predefined set of 133 metabolic enzymes as an essential gene for tumourigenesis and within a region of frequent copy number alterations (Ref. 50). Further analysis of a cohort of human breast tumours revealed 70% of ER-negative tumours having increased levels of PHGDH mRNA and protein (Ref. 50). This supported a previous study that had already shown a correlation between high PHGDH mRNA levels and poor 5-year survival in ER-negative, basal breast cancers (Ref. 51). Next to genomic amplification, PHGDH expression can also be upregulated by the transcription factors ATF4 (Ref. 52) and c-Myc (Ref. 53) or through epigenetic activation of *PHGDH* by histone H3 lysine 9 methyltransferase (Ref. 54). In addition, reinvestigation of a pooled analysis of somatic copy number alterations revealed *PHGDH* as a frequently amplified gene across 3131 cancer samples (Ref. 55). *PHGDH* is located on chromosome 1p12 in a peak region that displays repeated copy number gain in 16% of the cancers assessed with no known oncogenes contained in that particular region (Refs 49, 55). The most common tumour type with *PHGDH* gain was melanoma, with 40% frequency in *PHGDH* amplification (Ref. 55). Consistent with the genetic data, reduced proliferation was seen in melanoma and breast cancer cell lines containing *PHGDH* copy number gain or elevated PHGDH expression following knockdown of PHGDH (Refs 49, 50). However, translation of these findings to xenograft models had different outcomes with studies in breast cancer and non-small cell lung cancer xenografts showing the suppression of tumour growth following PHGDH knockdown (Refs 50, 56), whereas in another study no effect of PHGDH knockdown was observed in established breast cancer xenograft models (Ref. 57).

To date, many tumour types have been reported to exhibit increased levels of PHGDH expression, such as cervical, glioma, colon, melanoma, pancreatic, liver as well as kidney tumours (Ref. 58). However, how PHGDH drives tumourigenesis and progression still needs to be further validated. Overexpression of catalytic dead PHGDH fails to rescue the PHGDH knockdown phenotype (Ref. 59), similarly, the supply of exogenous amounts of serine or methyl-serine-ester to the cell culture media does not restore cell proliferation following PHGDH knockdown (Ref. 50). Exploring a role for PHGDH beyond serine synthesis identified human PHGDH as being able to catalyse the NADH-dependent reduction of α-KG to the oncometabolite 2-HG, a function previously only known for PHGDH from *Escherichia coli*, but at the same time the amounts of 2-HG generated were 100–1000 times lower than those in *IDH* mutant cancers (Ref. 60). Another study showed that hypoxia-induced HIF-1 and HIF-2 expression increased the expression of PHGDH and other enzymes of the de novo serine synthesis pathway, in breast cancer cell lines and breast cancer stem cells-enriched cell populations (Ref. 61). Under hypoxic conditions, PHGDH knockdown resulted in decreased NADPH levels and subsequent increase of mitochondrial ROS levels and sensitised breast cancer cell lines to carboplatin (Ref. 61). Additionally, PHGDH has been reported to stabilise the transcription factor FOXM1 in glioma, which in turn leads to increased expression of genes involved in tumour invasion, angiogenesis and cell cycle regulation (Ref. 62). PHGDH has also been shown to be linked to the NAD$^+$ salvage pathway with breast cancer cell lines overexpressing PHGDH being highly sensitive to inhibition of the NAD$^+$ salvage pathway through NAMPT inhibitors (Ref. 63).
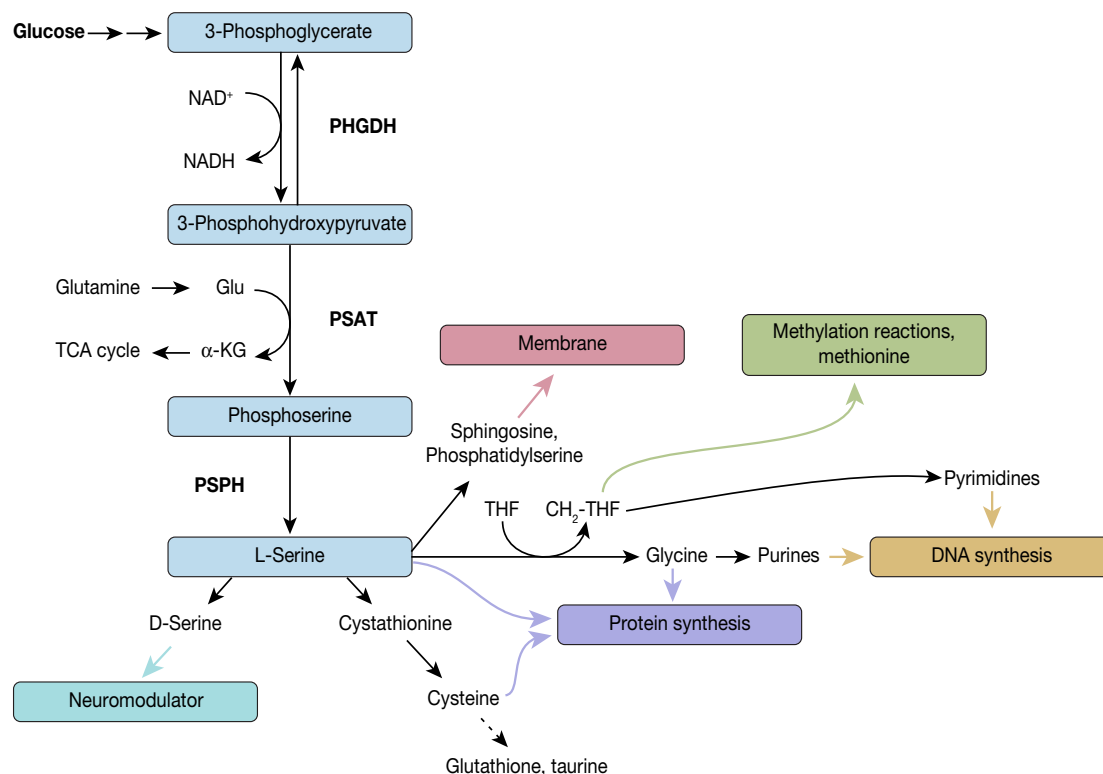
Fig. 3. Diversion into the synthesis of biomolecules through PHGDH. PHGDH diverts the glycolytic flux by oxidising 3-phosphoglycerate (3-PG) to 3-phosphohydrox-ypyruvate (3-PHP) and thereby reducing NAD$^+$ to NADH. 3-PHP is further converted to phosphoserine by phosphoserine aminotransferase (PSAT), which is combined with the formation of α-ketoglutarate from glutamate, followed by dephosphorylation by phosphoserine phosphatase (PSPH) leading to L-serine. L-serine itself is a precursor for various biomolecules and by these means implicated in several biological processes, such as neuromodulation, protein and membrane synthesis, methylation reactions and DNA synthesis.

Oncogenes and tumour suppressors change the metabolic gene expression underlying aerobic glycolysis

In addition to the genetic mutations of metabolic enzymes, there are also other changes in molecular mechanisms that have been shown to contribute to the Warburg phenotype in cancer, such as activation of transcription factors (e.g. HIF-1 or Myc) or signalling pathways (e.g. PI3K/Akt) as well as the inactivation of tumour suppressors (e.g. p53). These genetic alterations have an impact on several aspects of cancer metabolism, but within the scope of this review, we will be focusing on mechanisms related to aerobic glycolysis.

Activation of transcription factors

Amplified or overexpressed *MYC* has been identified in a plethora of human cancers and it was shown to transcriptionally regulate a variety of processes, such as proliferation, cell size, apoptosis and metabolism (Ref. 64). Following activation, c-Myc and its cofactor Max bind to the 'E-boxes' consensus sequences present in a majority of target genes involved in metabolism (Ref. 65). Using ^{13}C-labelled glucose, Myc has been shown to upregulate glucose consumption in Burkitt lymphoma cells (Ref. 66). Myc expression promotes glucose uptake through increasing the expression of glucose transporter GLUT1 as well as upregulating glucose catabolism through increased expression of glycolytic enzymes, such as hexokinase 2, phosphofructokinase-M 1 and enolase 1 (Refs 65, 67). In addition, Myc was shown to block the transcription factor MondoA in triple-negative breast cancer, which results in the inhibition of thioredoxin-interacting protein to then stimulate aerobic glycolysis (Ref. 68). Myc also upregulates the transcription of heterogeneous nuclear ribonucleoproteins and thereby indirectly promotes the expression of isoform M2 of pyruvate kinase

(PKM2), which is the predominant isoform in cancer, supporting the diversion of glycolytic intermediates towards anabolic metabolism (Ref. 69). The metabolic switch in cancer is further favoured by c-Myc-mediated activation of the enzymes within the de novo serine synthesis pathway, with PSPH being particularly important for c-Myc-driven hepatocellular cancer progression in vitro and in vivo (Ref. 70). Another glycolytic gene upregulated by Myc is Lactate dehydrogenase A (*LDHA*), with lactate dehydrogenase A being responsible for the final step in aerobic glycolysis: the conversion of pyruvate to L-lactate (Ref. 71). In addition, Myc also induces overexpression of the bidirectional monocarboxylate transporter MCT1, which facilitates lactate excretion from the cells (Ref. 72). Lactate has been proposed to support cancer aggressiveness by triggering angiogenesis and was shown to stimulate the accumulation of hypoxia-inducible factor 1 α (HIF-1α) activation in GBM cells (Refs 73, 74).

HIF-1 and HIF-2 are major transcription factors made of the heterodimeric complex of α and β subunits and responsible for the adaptive response to hypoxic conditions (Ref. 75). HIF1α is better characterised with regards to cancer metabolism. Under normoxic conditions, HIF1α levels are tightly regulated, but become aberrantly activated by oncogenic signalling pathways, such as PI3K/AKT/mTOR (Ref. 76), or mutations in tumour suppressor proteins, such as the von Hippel–Lindau protein (VHL) (Ref. 77). Independent of oxygen level mutations in the Krebs cycle, enzymes FH and SDH lead to overexpression and stabilisation of HIF1α by fumarate and succinate suggesting a positive feedback mechanism as HIF1α in turn promotes glycolysis (Refs 78, 79). Similar to Myc, glucose transporters as well as glycolytic enzymes are also under the control of HIF-1 and promote glucose import and glycolysis under normoxic conditions (Refs 80, 81). In addition, HIF-1 also prevents the flux of pyruvate into the mitochondrial TCA cycle by activating pyruvate
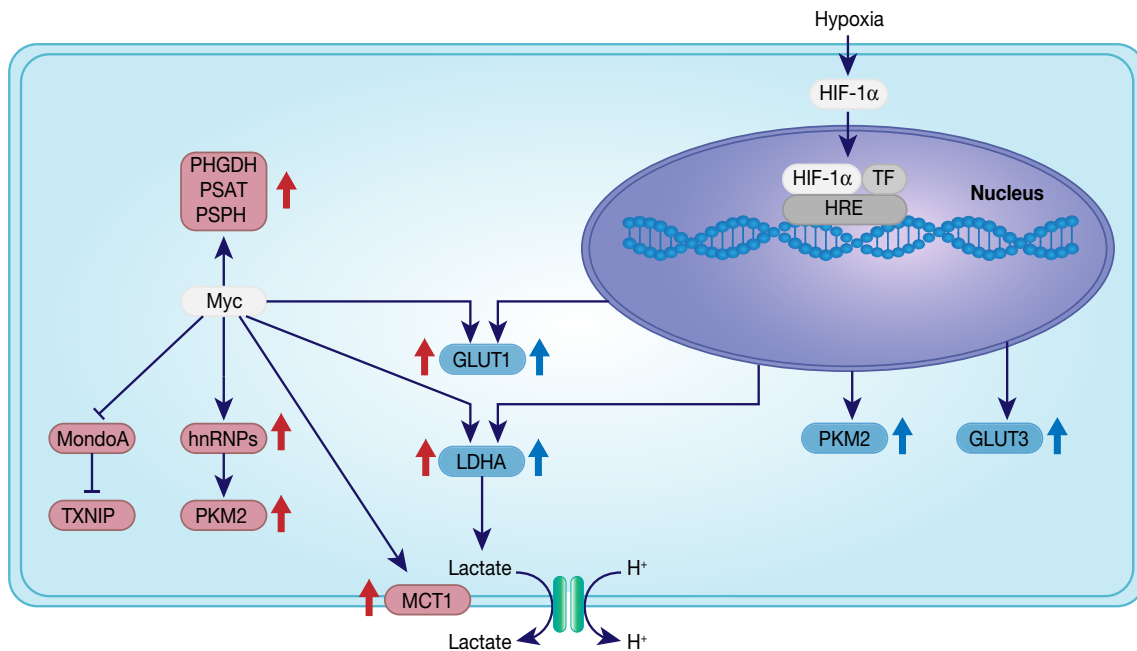
Fig. 4. Myc- and HIF1α-activated pathways. Myc and HIF1α promote the Warburg effect in cancer cells by upregulating critical pathways: increased glucose uptake through high expression of glucose transporters GLUT1 and GLUT3, increased lactate synthesis and excretion through overexpression of LDH-A and MCT1, diversion of the glycolytic metabolites to anabolic metabolism through upregulated PKM2, stimulation of aerobic glycolysis through inhibition of MondoA.

dehydrogenase kinase 1 (PDK1) as well as LDHA (Refs 81, 82). PDK1 phosphorylates and thereby inactivates pyruvate dehydrogenase (PDH) and thus prevents the conversion of pyruvate to acetyl coenzyme A, the metabolite to enter mitochondrial TCA, while increased LDHA expression results in more pyruvate being metabolised to lactate.

Overall these activated transcription factors contribute to the reprogramming of the glucose metabolism according to Warburg's observations by: (1) promoting glucose uptake through upregulation of glucose transporters, (2) supporting aerobic glycolysis through increased expression of glycolytic enzymes under normoxic conditions and (3) supporting lactate production and excretion via monocarboxylate transporters (MCT) to divert pyruvate from the TCA cycle in the mitochondria (Fig. 4).

Inactivation of tumour suppressors: exemplified by p53

Several tumour suppressors have been reported to regulate cancer metabolism, such as PTEN and retinoblastoma protein as well as VHL, mentioned above (Ref. 83). However, the most prominent representative of this group is p53 with missense mutations in its encoding gene *TP53* occurring in up to 50% of all cancers (Ref. 84). Loss of p53 function has been shown to contribute to the Warburg effect through several mechanisms. Glucose import into the cell is partly regulated by p53 through direct repression of the transcription of GLUT1 and GLUT4 as well as through inhibition of NF-κB, which represses GLUT3 expression (Refs 85, 86). In addition, p53 induces the transcription of *TIGAR* (TP53-induced glycolysis and apoptosis regulator), which results in lowering the intracellular fructose-2,6-bisphosphate (F26BP) levels (Ref. 87). As F26BP is the allosteric activator of phosphofructose kinase 1 (PFK1), its depletion decreases PFK1 activity and thus promotes diversion of glycolysis into the pentose phosphate pathway. P53 also intervenes with the conversion of pyruvate to lactate or acetyl-CoA by downregulating the monocarboxylate transporter MCT1 and thus lactate excretion from the cells on one hand whilst promoting conversion of pyruvate to acetyl-CoA by PDH by downregulating pyruvate dehydrogenase kinase-2, which in turn results

in PDH activation on the other hand (Refs 88, 89). In addition, p53 upregulates the expression of the E3 ubiquitin ligase Parkin, which ubiquitylates PKM2 and decreases its activity and thereby the glycolytic flux (Ref. 90). In line with p53 negatively regulating glycolysis, p53 also promotes oxidative phosphorylation in the mitochondria. Synthesis of cytochrome c oxidase 2 (*SCO2*) is one of the genes that is induced in a p53-dependent manner and SCO2 protein is one of the cytochrome c oxidase assembly factors and thereby necessary for the proper function of the mitochondrial respiratory chain (Ref. 91). Apoptosis inducing factor (*AIF*) is another gene upregulated by p53 and the translated mitochondrial flavoprotein AIF is required for proper assembly of mitochondrial respiratory complex I (Ref. 92).

This shows that a growing body of evidence supports the role of p53 in regulating cellular metabolism and in particular promotes the reversion of the Warburg effect (Fig. 5). However, p53 is mutated in about 50% of cancers in which it leads to compromised oxidative phosphorylation and support of glycolysis.

Beyond Warburg: Krebs and glutamine metabolism

Cancer metabolism is a complex and flexible system involving the orchestration of energy metabolism, cell growth and proliferation with nutrients besides glucose, such as glutamine, also playing important roles in tumours. Glutamine is the most abundant amino acid accounting for more than 20% and 40% of the free amino acid pool in the plasma and muscle, respectively (Ref. 93).

Hans Krebs, who is probably more well-known for his characterisation of the tricarboxylic acid cycle (or Krebs' cycle), played a major role in elucidating glutamine metabolism (Ref. 94). In 1935, he published his findings on glutamine synthesis as an endothermic reaction that is catalysed by an enzyme, glutaminase, which exists in two distinct forms (Ref. 94). Glutamine is a conditionally essential amino acid, becoming essential under conditions that exceed the body's synthetic capabilities, especially in hypercatabolic stress states, such as injury, sepsis and inflammation resulting in increased glutamine consumption by the gastrointestinal tract, immune cells, inflammatory tissue and kidney
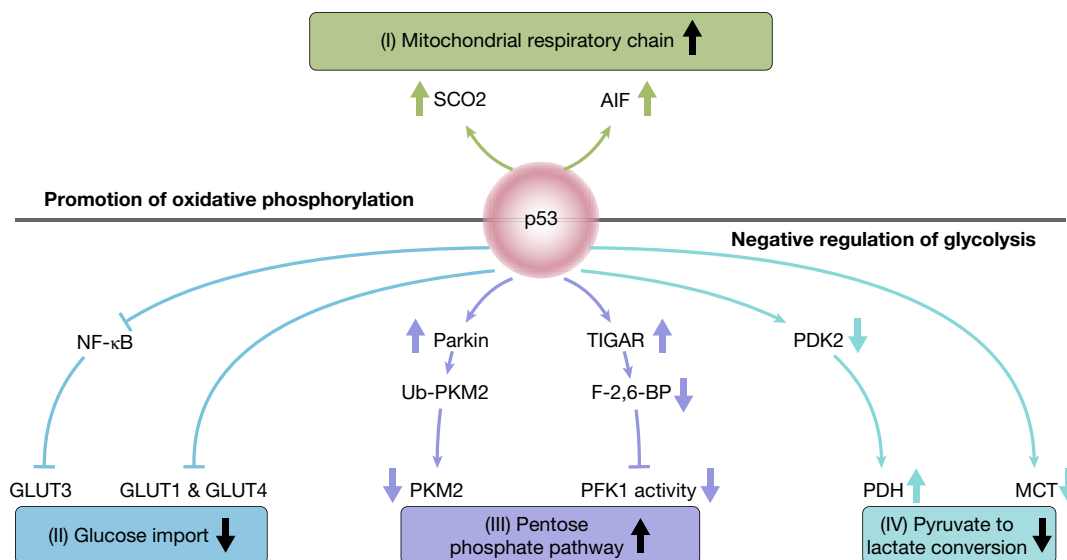
Fig. 5. Impact of p53 on glucose metabolism. Scheme of the pathways by which p53 promotes oxidative phosphorylation directly or through negative regulation of glycolysis. (I) P53 induces the expression of proteins implicated in the proper function of the mitochondrial respiratory chain: SCO2 and AIF. (II) P53 reduces glucose import through direct repression of GLUT1 and GLUT4 transcription and indirect repression of GLUT3 through inhibition of NF-κB. (III) P53 promotes glycolytic flux through the pentose phosphate pathway by downregulating PKM2 through the promotion of its ubiquitination and reducing PFK1 activity through F-2,6-BP depletion. (IV) P53 reduces the conversion of pyruvate to lactate by minimising lactate efflux through MCT downregulation and promoting the conversion of pyruvate to acetyl-CoA through the decrease of PDK2 activity and consequently increase in PDH activity.

(Ref. 95). Glutamine is not only a precursor for a variety of biomolecules, such as proteins, nucleotides and amino sugars, but also plays a role in the acid–base balance because of being a precursor of urinary ammonia, functions as a nitrogen carrier between tissues, is involved in the regulation of glycogen synthesis in the liver and can work as respiratory fuel (Ref. 96) (reviewed in Ref. 97). Several cancer cells have been shown to be addicted to glutamine, making it a prerequisite for culture conditions (Ref. 98) and this glutamine-dependency finding was also extended to the growth of certain tumours in vivo (exemplified in Refs 99, 100, 101).

Glutamine metabolism in cancer

Glutamine is actively transported across the cell membrane by one of 14 membrane transporters belonging to four distinct gene families of the solute carrier (SLC) group transporters: SLC1, SLC6, SLC7 and SLC38 (Ref. 102). Among those, SLC1A5 has been heavily studied and shown to be upregulated in many solid cancer types, exemplified by but not limited to triple-negative breast cancer (Ref. 103), melanoma (Ref. 104), neuroblastoma (Ref. 105) or colorectal cancer (Ref. 106). Another route of amino acid uptake in general is micropinocytosis. This internalisation process through vesicles has been shown to provide a route to contribute to glutamine-dependence by micropinocytic uptake of proteins in Ras-transformed pancreatic and urinary bladder cancers (Ref. 107).

After entering the cell, glutamine is metabolised to glutamate and an ammonium ion by glutaminase, an enzyme that exists in several tissue-specific isoforms and is encoded in humans by the genes *GLS* and *GLS2* (Ref. 108). Through alternative splicing, *GLS* transcribes for two transcripts, translated into the mitochondrial kidney-type glutaminase (KGA) and glutaminase C (GAC), while *GLS2* encodes the two proteins GAB and liver-type glutaminase through a surrogate promoter mechanism (Refs 108, 109). GAC is upregulated in several solid cancers, such as breast (Ref. 110), lung (Ref. 111), colon (Ref. 112) and prostate (Ref. 113). Following the conversion from glutamine to glutamate by glutaminases, glutamate is further metabolised to α-KG by either glutamate dehydrogenase (GLUD) or aminotransferases.

Although GLUD catalyse the reversible deamination of glutamate by releasing ammonia, aminotransferases, according to their name, transfer the nitrogen onto other biomolecules, such as pyruvate or 3-phosphohydroxypyruvate, as within the second step of de novo serine synthesis (Ref. 114). In tumours, glutamate is mainly metabolised by aminotransferases, whose expression is upregulated while GLUD expression is low (Ref. 115). Generated α-KG can be further metabolised to the oncometabolite 2-HG, a reaction catalysed by IDH as described earlier.

Similar to the Warburg effect, there are several oncogenes, such as KRAS, Myc and p53, that contribute to reprogramming glutamine metabolism in cancer and a few examples will be given herein. KRAS enhances the diversion of glutamine into the TCA cycle or transamination in order to sustain the synthesis of biomolecules (Ref. 116). In pancreatic ductal adenocarcinoma (PDAC), oncogenic KRAS has been shown to promote aspartate transaminase (GOT1) expression, while reducing GLUD1 expression, thereby supporting the irreversible deamination of glutamate (Ref. 100). On similar lines, induced Myc expression in mouse embryonic fibroblasts leads to rewiring of their metabolism and an addiction to glutamine and stimulation of the mitochondrial glutamine catabolism (Ref. 117). Further studies on Myc-regulated glutamine metabolism reveal that Myc transcriptionally represses miR-23a and miR-23b, which leads to increased expression of mitochondrial glutaminases in lymphoma and prostate cancer cell lines (Ref. 118). Recently Myc has also been reported to support glutamine synthesis through increasing glutamine synthase expression initiated by promoter demethylation, which then leads to an increased usage of glutamine in nucleotide and protein synthesis (Ref. 119). P53 has been shown to induce the expression of glutaminase 2 isoforms, which results in shifting glucose metabolism towards oxidative phosphorylation and reduction of tumour cell growth (Ref. 120).

Collaboration of glucose and glutamine metabolism in cancer

With the shift of cancer cell metabolism towards the Warburg effect, that is, reduced glycolytic flux through the mitochondria
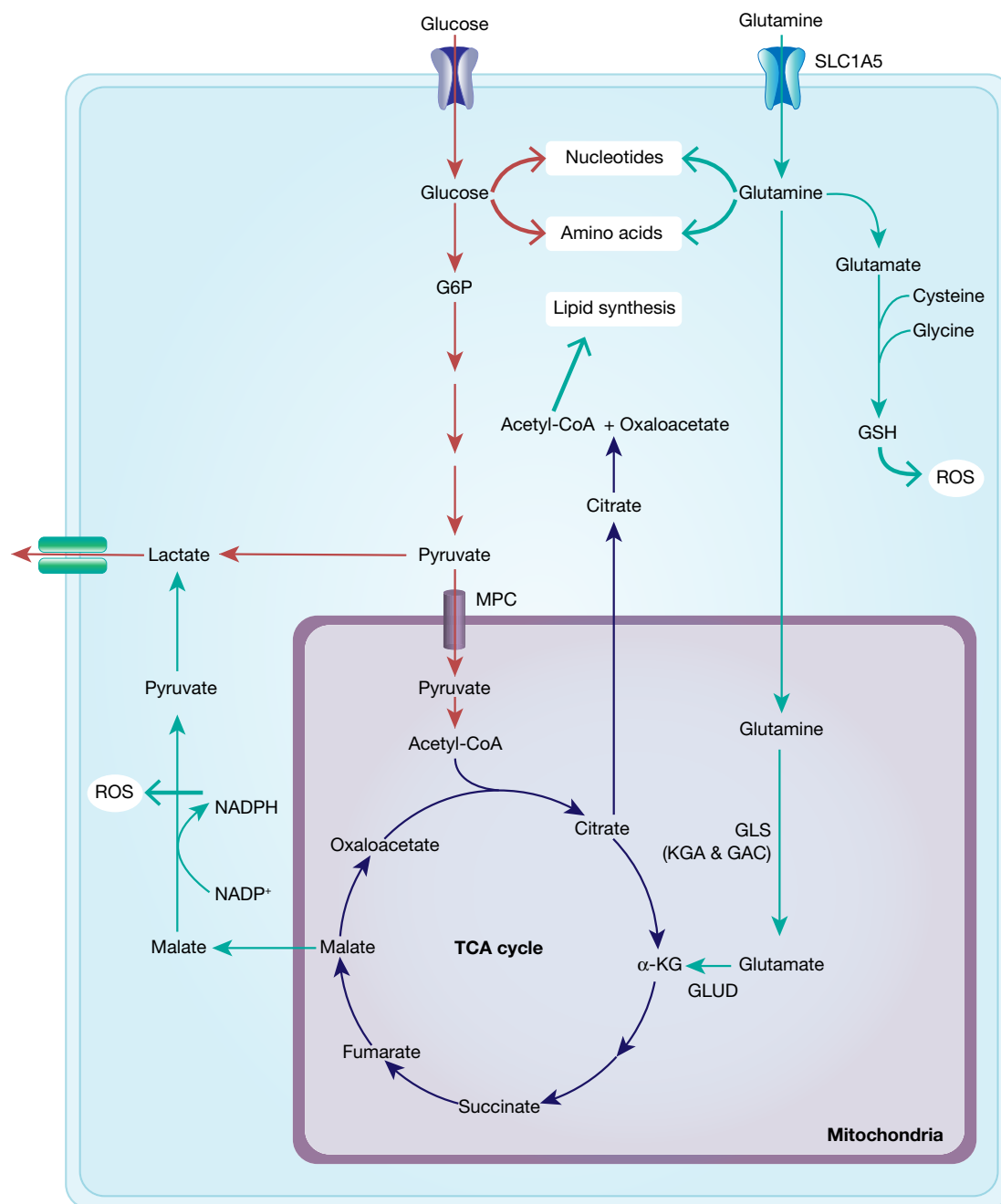
Fig. 6. Interplay between glucose and glutamine metabolism to drive cancer growth. Glucose is transported into the cells and mainly metabolised into pyruvate and lactate and thereby contributes to nucleotide and amino acid synthesis. Glutamine is equally transported into the cells and metabolised to glutamate and α-KG, fuelling into the TCA cycle and thus into ATP generation and lipid synthesis. TCA-cycle-derived malate is further metabolised to pyruvate, thereby generating NADPH and thus contributing to maintaining redox balance. In addition, glutamate fuels into GSH synthesis, which is an important ROS scavenger.

and lower ATP production, glutamine is able to step in as an alternative carbon source in order to guarantee sufficient energy production and synthesis of biomolecules (nucleic acids and proteins) necessary for proliferation (Fig. 6).

Metabolic studies with ^{13}C-labelled glutamine and glucose in the human GBM cell line SF188 have shown that a large part of glutamine is replenishing the TCA cycle with glutamine-derived α-KG (anaplerosis) (Ref. 121). In line with this, inhibition of pyruvate transport into the mitochondria via the mitochondrial pyruvate carrier in glioma cells activates GLUD, which catalyses the reaction of glutamate to α-KG in the mitochondria, fuelling into the TCA cycle (Ref. 122). In addition, glutamine-derived carbon can also support lipid synthesis as the TCA cycle intermediate, citrate, is exported into the cytosol where it is cleaved into acetyl-CoA and oxaloacetate, a necessary precursor for lipid

synthesis. While this pathway has been reported to be predominant in cancer cells under hypoxia (Ref. 123), glucose-derived citrate is considered to be the main source for acetyl-CoA under normoxic conditions (Ref. 124).

Glutamine has also an important role in the regulation of oxidative stress. Malate, a biomolecule generated within the TCA cycle, can be exported from the mitochondria and converted to pyruvate by malic enzyme, a process that generates NADPH and thus contributes to the cellular redox balance. In human PDAC cells, glutamine-derived aspartate is converted to oxaloacetate, which is subsequently converted into malate then pyruvate and with the last catalytic step generating NADPH (Ref. 100). Furthermore, glutamate is used in the de novo synthesis of reduced glutathione (GSH), an essential scavenger of reactive oxygen species (ROS) and depletion of glutamine has been shown to reduce

Table 1. Labelled glucose and glutamine derivatives used for imaging for cancer diagnosis and progression

Imaging drug	Phase of development	NCT number ('https://www.clinicaltrials.gov')
[18]FDG PET	Approved and in clinical use	
[18]FG-PET	PET imaging (cancer, metastatic cancer, metastatic brain cancer)	NCT03721055
[18]FG-PET	PET imaging (lung cancer)	NCT03568799
[18]F-Gln-BF$_3$	PET imaging in xenograft model	

FDG, fluorodeoxyglucose; FG, fluoroglutamine; Gln, glutamine; PET, positron emission tomography.

the cellular GSH concentration in Myc-driven cells (Ref. 125). In anchorage-independent growth conditions, IDH1-dependent reductive carboxylation of glutamine was shown to generate NADPH in the mitochondria and thereby suppress mitochondrial ROS (Ref. 126).

Despite the interplay of glutamine and glucose metabolism, the different pathways are not necessarily co-regulated. Although the PI3K/AKT signalling pathway stimulates glucose uptake and metabolism, glutamine uptake and metabolism is independent of this signalling cascade in Myc-dependent glioma cells (Ref. 117).

Therapeutic strategies arising from the Warburg effect and altered glutamine metabolism

The altered metabolic pathways, reflecting the demands of highly proliferating cancer cells and their tumour environment, may be exploited diagnostically and therapeutically. It should be noted that there are more metabolic pathways, besides glucose and glutamine addiction, that are altered in cancer, which represent additional directions for the development of cancer therapeutics by targeting cancer metabolism, but are beyond the scope of this review.

Imaging: diagnosis and monitoring of tumours

The first medical procedure really based on Warburg's findings is ^{18}F-deoxyglucose positron emission tomography ([18]FDG-PET), which makes use of increased glucose and also [18]FDG uptake in most cancers compared with normal tissue and was implemented in the clinic in the 1980s (Ref. 127).

With the emerging evidence for the role of glutamine and increased glutamine uptake in tumours, several studies have investigated glutamine-based tracers for PET scans. 5-[11]C-(2S)-glutamine and [18]F-(2S,4R)-4-fluoroglutamine ([18]FG) have been proposed as complementary tools to guide glutamine-targeted therapies with [18]FG showing a different tumour-to-background contrast compared with [18]FDG in glioma patients (Ref. 128). ^{18}F-Gln-BF$_3$ is another tracer that has shown significant uptake in xenograft studies (Ref. 129) (Table 1, Fig. 7).

Antimetabolites

Chemotherapeutic agents targeting metabolic enzymes have an even longer history in the treatment of neoplastic disease. In the late 1940s, successful targeting of cancer cell metabolism was already achieved using the antifolate drug aminopterin to induce remission in children with acute lymphoblastic leukaemia (ALL) (Ref. 130). More antifolate drugs, such as methotrexate, were developed and were shown to exhibit their antitumour effect through inhibition of dihydrofolate reductase, which subsequently leads to the loss of reduced folates and in turn inhibits thymidylate and purine synthesis causing cytostasis or cell death (Ref. 131). Antifolate drugs have a broad application in the treatment of different solid tumours and haematologic malignancies and there has been a continuous effort in the development of the next generation of antifolates, such as raltitrexed and pemetrexed (Ref. 132).

Analogues of purines (6-mercaptopurine, azathioprine, 6-thioguanine) and pyrimidine (5-fluorouracil, cytarabine, gemcitabine) are also used extensively in cancer therapy, often in combination with DNA repair chemotherapeutics. They exert their antiproliferative activity by blocking nucleotide metabolism and becoming misincorporated into DNA following intracellular conversion to their corresponding nucleotides (Refs 133, 134, 135) (Table 2, Fig. 7).

Glucose metabolism

Based on an increasing understanding and interest in the Warburg effect and the importance of glucose metabolism in cancer cells, the pharmacological intervention of these pathways has come into more focus (Table 3, Fig. 7). Many cancers show upregulation of different glucose transporters, such as GLUT1, 3 and 4 and this fact is exploited in [18]FDG-PET imaging. Several GLUT1 inhibitors from different chemical classes have been developed, such as WZB117, STF31, Silybin and Resveratrol showing promising results in in vitro experiments in different cancer cell lines (Ref. 136) with Silybin also having been investigated in phase I clinical studies in prostate cancer (Ref. 137). 2-Deoxyglucose, in contrast, was established as a molecule that is able to enter cells via the glucose transporters but cannot be metabolised beyond 2-deoxyglucose-6-phosphate (2-DG-P) and therefore accumulates (Ref. 138). 2-DG-P competitively inhibits phosphoglucose isomerase as well as hexokinase in a non-competitive manner and thereby slows further glucose uptake and metabolism leading to cancer cell death in vitro (Refs 139, 140). Although several preclinical studies demonstrated anti-proliferative effects in different cancers, these results could not be translated into clinical settings for 2-DG monotherapy (Ref. 141). One major limitation for the usage of 2-DG is its toxicity associated with hypoglycaemia. This toxicity is potentially due to more than one mechanism as 2-DG has been shown to interfere with several biological processes beyond glycolysis, such as induction of oxidative stress, interference with N-linked glycosylation in the endoplasmic reticulum or induction of autophagy (Refs 141, 142).

Another route currently explored to target aerobic glycolysis in caner is through interference with lactate production or excretion. LDHA catalyses the conversion of pyruvate to lactate and as outlined before, LDHA expression is upregulated by HIF-1 and c-Myc. Many cancers were found to have higher levels of LDHA compared with normal tissue (Ref. 143) and LDHA has been shown to be required for initiation, maintenance and progression of various tumours making it an interesting cancer target (Refs 144, 145). To date, several inhibitors of LDHA have been reported and mainly validated in biochemical or cell-based models as pharmacokinetic properties or selectivity were not good enough for in vivo studies (Ref. 146). Among the known LDHA inhibitors is the natural product gossypol, which was investigated in phase I/II clinical studies for different cancers, but is also a reported pan-inhibitor of anti-apoptotic Bcl-2 family protein members ('https://www.clinicaltrials.gov', Refs 146, 147). Following lactate formation, MCT, especially 1 and 4, are responsible for transporting lactate out of the cells, thereby being part of metabolic reprogramming in cancer and thus attractive targets for antitumour therapy (Refs 73, 148). Hence, MCT1 inhibition has
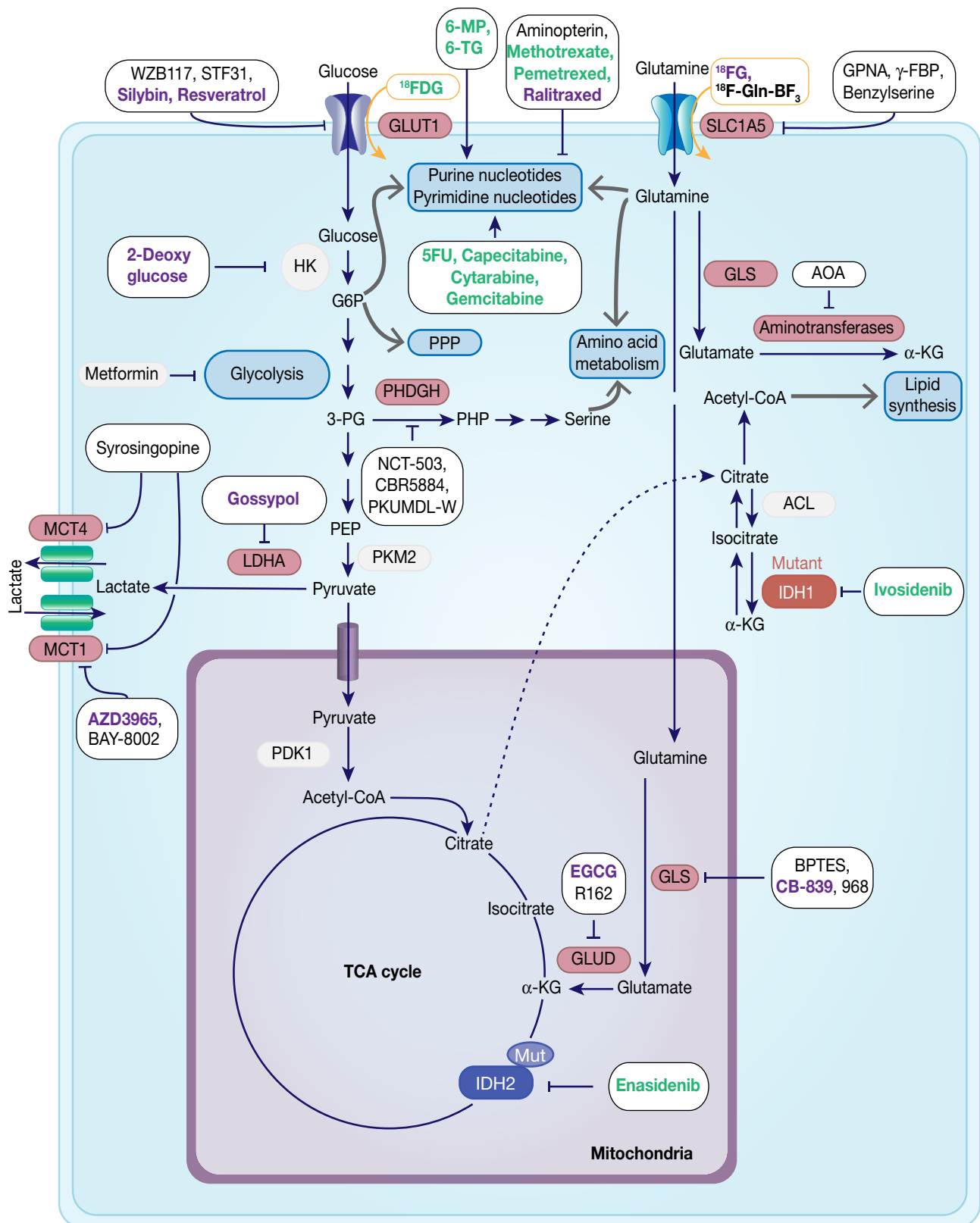
Fig. 7. Therapeutic targets in cancer metabolism. Metabolic enzymes which are under preclinical investigation are highlighted in red. Inhibitors are framed in black, whereas compounds used for imaging are framed in orange. Compounds which are under development are in black, compounds in clinical studies are in purple and FDA-approved drugs are in green.

been shown to lead to the accumulation of intracellular lactate resulting in a decrease in intracellular pH and disabling glycolysis and tumour cell growth (Ref. 72). In a few tumours, lactate import was also observed to be important for tumour growth under glucose-deprived conditions in which lactate fuels into the oxidative phosphorylation pathway (Ref. 149). The only promising small-molecule MCT inhibitors to date are BAY-8002 and AZD3965 (Refs 150, 151). Both are MCT1-specific inhibitors with AZD3965 showing potent antitumour activity in a Burkitt's lymphoma xenograft model, whereas treatment with

Table 2. List of approved drugs or preclinical tools among anti-folates and nucleotide analogues

Drug	Target(s)	Phase of development	NCT number ('https://www.clinicaltrials.gov')
Anti-folates			
Aminopterin	DHFR	No longer clinically used.	
Methotrexate	DHFR	Approved (ALL, different solid tumours)	
Pemetrexed	DHFR, TS, GARFT	Approved (malignant pleural mesothelioma, NSCLC)	
Raltitrexed	TS	Phase II (colorectal cancer)	NCT03053167
		Phase II (gastric cancer)	NCT03392103
Purine analogues			
6-Mercaptopurine	Phosphoribosyl pyrophosphate amidotransferase	Approved (ALL)	
6-Thioguanine		Approved (AML)	
Pyrimidine analogues			
5-Fluorouracil	TS	Approved (different solid cancers)	
Capecitabine	TS	Approved (breast and colorectal cancer)	
Cytarabine	DNA polymerase (cytarabine triphosphate gets incorporated into DNA causing chain termination)	Approved [ALL, AML, chronic myelogenous leukaemia (CML)]	
Gemcitabine	RNR (gemcitabine triphosphate gets incorporated into DNA causing chain termination)	Approved (breast, ovarian, pancreatic cancer, NSCLC)	

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; DHFR, dihydrofolate reductase; GARFT, glycinamide ribonucleotide formyltransferase; NSCLC, non-small cell lung cancer; RNR, ribonucleotide reductase, TS, thymidylate synthase.

Table 3. List of preclinical tools and compounds in clinical trials for targets within glucose metabolism

Drug	Target(s)	Phase of development	NCT number ('https://www.clinicaltrials.gov')
Inhibitors of glucose transport or metabolism			
WZB117	GLUT 1	Preclinical tool (lung cancer) (Ref. 156)	
STF31	GLUT 1	Preclinical tool (VHL-deficient kidney cancer) (Ref. 157)	
Silybin	GLUT 1/4	Phase I (hepatocellular carcinoma)	NCT01129570
		Phase II (prostate cancer)	NCT00487721
Resveratrol	GLUT 1	Phase I (colon cancer)	NCT00256334
		Phase II (multiple myeloma)	NCT00920556
2-Deoxyglucose	Phosphoglucoseisomerase (PGI), hexokinase (HK)	Phase I/II (prostate cancer)	NCT00633087
		Phase I (advanced solid tumours)	NCT00096707
Inhibitors of lactate production or excretion			
Gossypol	LDHA, Bcl-2 family proteins	Phase II (small cell lung cancer)	NCT00773955
		Phase II (prostate cancer)	NCT00666666
AZD3965	MCT-1	Phase I (adult solid tumour, diffuse large B cell and Burkitt's lymphoma)	NCT01791595
BAY-8002		Preclinical (haematopoietic tumour cells) (Ref. 150)	
Syrosingopine	MCT-1 and MCT-4	Preclinical (in combination with metformin) (Ref. 153)	

GLUT, glucose transporter 1; LDHA, lactate dehydrogenase; MCT, monocarboxylate transporter.

BAY-8002 only resulted in tumour stasis (Refs 150, 151). AZD3965 is currently undergoing phase I clinical trials in patients with advanced solid tumours, B cell lymphoma or Burkitt's lymphoma (Table 3). A challenge in the development of MCT inhibitors for the treatment of cancer is the potential of drug resistance because of overlapping functions within the MCT family, such as functional redundancy of MCT1 and 4. In this context, it has been shown that MCT4 overexpression leads to AZD3965 resistance, whereas MCT4 knockdown resulted in AZD3965 sensitisation (Ref. 152). Recently, syrosingopine has been reported to be a dual inhibitor of MCT1 and MCT4 and to be synthetically lethal in combination with metformin in cancer cells (Refs 153, 154). Initially developed and widely used for the treatment of Type II diabetes, metformin has been shown

Table 4. List of preclinical tools and compounds in clinical trials for IDH and PHGDH

Drug	Target(s)	Phase of development	NCT number ('https://www.clinicaltrials.gov')
Inhibitors of mutant isocitrate dehydrogenase (IDH)			
Ivosidenib /Tibsovo®	Mutant IDH-1	Approved (relapsed/refractory AML)	
		Phase I (IDH mutation myeloid neoplasms)	NCT03564821
Enasidenib/Idhifa®	Mutant IDH-2	Approved (relapsed/refractory AML)	
		Phase I (AML, CML – maintenance therapy)	NCT03515512
Inhibitors of phosphoglycerate dehydrogenase (PHGDH)			
NCT-503	PHGDH	Preclinical tool (breast cancer and melanoma) (Ref. 164)	
CBR-5884	PHGDH	Preclinical tool (in vitro) (Ref. 165)	
PKUMDL-WQ-2101/2201	PHGDH	Preclinical tool (breast cancer xenografts) (Ref. 166)	

AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; IDH, isocitrate dehydrogenase; PHGDH, 3-phosphoglycerate dehydrogenase.

Table 5. List of preclinical tools and compounds in clinical trials for targets within glutaminolysis

Drug	Target(s)	Phase of development	NCT number ('https://www.clinicaltrials.gov')
GPNA	Glutamine transporters: SLC1A5, SNAT1/2, LAT1	Preclinical tool	
γ-FBP	SLC1A5	Preclinical tool	
Benzylserine, benzylcysteine	SLC1A5	Preclinical tool	
BPTES	GLS	Preclinical tool	
CB-839	GLS	Phase I (haematological tumours)	NCT02071888
		Phase I (solid tumours)	NCT02071862 NCT02771626
		Phase I (in combination with carfilzomib and dexamethasone) (multiple myeloma)	NCT03798678
		Phase II (in combination with cabozantinib/everolimus) (Renal cell carcinoma)	NCT03428217 NCT03163667
		Phase II (in combination with paclitaxel) (TNBC)	NCT03057600
968	GLS	Preclinical tool	
EGCG	GLUD	Early phase I (colorectal cancer)	NCT02891538
R162		Preclinical tool	
AOA	Aminotransferase	Palliative in tinnitus Preclinical tool (breast cancer xenografts)	

AOA, aminooxyacetate; BPTES, bi-2-(5-phenylacetamido-12,4-thiadiazol-2-yl)ethyl sulphide; EGCG, pigallocatechin gallate; γ-FBP, γ-folate-binding protein; GPNA, L-γ-glutamyl-p-nitroanilide.

to have multiple targets in glycolysis and is now repurposed and being investigated for the treatment of different cancers, such as in a currently recruiting phase II clinical trial for prostate cancer (NCT03137186) (Ref. 155).

Oncoenzymes: IDH and PHGDH

The 'oncoenzymes' presented here: IDH and PHGDH also present interesting targets for cancer therapy that are currently been exploited. Ivosidenib (Tibsovo®) and enasidenib (Idhifa®) are orally available, selective, small-molecule inhibitors of mutated cytosolic IDH1 or IDH2, respectively, which, in line with the presented biology on IDH, prevent the formation of the oncometabolite 2-HG (Refs 158, 159). Evosidenib and ivosidenib have both recently received US Food and Drug Administration (FDA)-approval for treatment of patients with relapsed or refractory AML and are still under further clinical investigation for solid tumours, cholangiocarcinoma and haematological

malignancies (Refs 160, 161). To date several other targeted inhibitors of mutant IDH1 and/or IDH2 are under development and some are currently investigated in clinical phases I–III (Table 4) (Refs 162, 163).

With PHGDH's proposed role in tumourigenesis and tumour progression in several solid cancers, the focus has been on the development of small-molecule inhibitors targeting PHGDH. Fragment-based approaches have been reported to target the substrate and cofactor-binding pockets of PHGDH, yielding novel starting points for the development of PHGDH inhibitors (Ref. 167) as well as a more elaborate inhibitor from AstraZeneca with a reported K_d (SPR) of 0.18 μM (Ref. 168). In addition, two non-competitive inhibitors, CBR-5884 (Ref. 165) and NCT-503 (Ref. 164) have been proposed with IC_{50} values of 33 and 2.5 μM, respectively. Of these inhibitors, only NCT-503 was investigated in vivo because of poor plasma stability of CBR-5884. In the in vivo study with NCT-503 in mice bearing MDA-MB-468 (PHGDH-high expressing breast cancer cells)

orthotopic xenografts, the tumour burden was reduced by 50% following daily treatment (40 mg/kg, IP) (Refs 164, 165). In addition, a series of pyrazole-5 carboxamide with $IC_{50} < 50 \mu M$ derivatives have been disclosed in a patent (WO2016/040449A1) as well as PHGDH inhibitors, PKUMDL-WQ-2101/2201, that bind to allosteric sites of PHGDH and could significantly inhibit tumour growth in breast cancer xenografts (Ref. 166).

Glutamine metabolism

With glutamine being an important nutrient for cancer cells, but at the same time a non-essential amino acid under normal growth conditions, preventing glutamine import into cells provides a valid cancer targeting strategy (Table 5, Fig. 7). Among the transporters for glutamine, SLC1A5 has been predominantly studied in cancer and shown to be frequently upregulated. The glutamine analogue L-γ-glutamyl-p-nitroanilide (GPNA) was among the first SLC1A5 inhibitors to be proposed (Ref. 169) and has been shown to reduce glutamine uptake in several cancer types, such as NSCLC (Ref. 170), myeloma cells (Ref. 171) or prostate cancer (Ref. 172). However, recent studies suggest that GPNA is a non-selective SLC1A5 inhibitor that also affects other sodium-dependent carriers of the SNAT family (Ref. 173) as well as the sodium-independent neutral amino acid transporter LAT1 (Ref. 174).

Other reported SLC1A5 inhibitors are benzylserine and benzylcysteine, which have been shown to are weak (mM-affinity for SLC1A5), competitive inhibitors of SLC1A5 (Ref. 175), but are also non-selective and in the case of benzylserine also affect other amino acid transporters, such as LAT1 (Ref. 104). Despite weak inhibitory affinity, benzylserine has been shown to decrease breast cancer cell viability and cell progression (Ref. 176).

The proline derivative γ-folate-binding protein is a newly described SLC1A5 inhibitor, obtained from a virtual screening campaign and shown to decrease melanoma cancer cell viability using compound doses in the low-mM range (Ref. 177).

Glutaminase 1 (GLS1) isoforms, especially GAC, have been found to be upregulated in different cancers and suppression by genetic knockdown showed antitumour activity, thus validating glutaminases as promising targets for cancer therapy. In this context, bi-2-(5-phenylacetamido-12,4-thiadiazol-2-yl)ethyl sulphide (BPTES) has been reported and identified as an allosteric KGA and GAC inhibitor that blocks the respective tetramer in an inactive conformation, but does not affect the GLS2 isoforms (Refs 178, 179, 180). Although several studies report antitumour activity of BPTES in different tumour types, its usage in clinical studies is limited by moderate potency, metabolic instability and low solubility (Ref. 181). In order to improve these properties, BPTES derivatives were investigated and led to CB-839, a potent, selective and orally bioavailable inhibitor of the two main splice variants of GLS1: KGA and GAC, that is currently investigated in phase I and II clinical trials (Table 5) (Refs 181, 182). In search of glutaminase inhibitors with a different modus operandi, compound 968 was reported to bind to the inactive, monomeric GAC and preventing it from undergoing conformational changes to an activated state (Refs 183, 184).

The metabolism of glutamate to α-KG is performed by GLUD or aminotransferases and as α-KG is not only a precursor for the oncometabolite 2-HG, but also fuels into the TCA cycle, its production is another attractive target for cancer therapy.

The green tea polyphenol, epigallocatechin gallate (EGCG) was reported to inhibit GLUD in the nM-range and to inhibit the growth of N-Myc amplified neuroblastoma cells and c-Myc overexpressing glioma cells (Refs 185, 186, 187). However, as EGCG inhibits a variety of NADPH-requiring enzymes, a screening for more selective GLUD inhibitors revealed the purpurin analogue R162 (Ref. 188). R162 was subsequently shown to significantly reduce cell viability in human lung, breast and leukaemia cell lines (Ref. 188). Aminooxyacetate (AOA) is a non-specific transaminase inhibitor that initially has been investigated as palliative treatment in tinnitus (Refs 189, 190) and had failed in a small trial with seven patients in Huntington's disease (Ref. 191). In preclinical studies for cancer therapy, AOA has been used to inhibit the anaplerotic use of glutamine in the TCA cycle and has shown to decrease tumour growth in breast cancer xenograft studies (Refs 192, 193).

Concluding remarks and future directions

The discovery of aerobic glycolysis by Warburg was certainly a milestone discovery in the area of cancer metabolism, but also just one aspect of a complex metabolic network. There is an increasing body of literature and evidence showing that cancer is a metabolic disease and that metabolic dependencies in cancer offer an exciting new area of research with great potential for the development of novel therapeutics, such as the renewed focus on glutamine metabolism shows. As a tumour represents a heterogeneous mixture of cells and accumulates further mutations during division and expansion, it will be difficult to erase a tumour completely by targeting individual mutated gene products. In this regard, targeting metabolic dependencies of cancers, such as the Warburg effect or glutamine dependence, could provide promising new avenues for developing new cancer treatments.

However, the metabolic network is also very intricate and complex so that a clear understanding of the metabolic alterations in tumours within their context is necessary. As metabolic pathways are evolutionarily conserved, it is important to consider potential toxicities when developing metabolic inhibitors. Nevertheless, for other diseases, metabolic inhibitors, such as metformin for Type II diabetes, have successfully been developed with sufficient safety profiles, indicating that targeting cancer metabolism with limited toxicities should be achievable.

Next to the development of novel inhibitors, it is necessary to establish non-invasive methods for patient stratification as not all tumours rely on the same metabolic pathways. Progression biomarkers and early detection of resistance mechanism will be a key factor as cancer metabolism has been shown to be an adaptive and dynamic system influenced by the tumour micro-environment.

One has to remember that several anticancer chemotherapeutic agents used to date are drugs targeting tumour metabolism, such as the antifolates or nucleotide analogues. Together with a strong research effort that has led to several clinical trials for inhibitors targeting metabolic pathways to date, there is substantial potential in pursuing these new avenues for effective new cancer treatments.

References

1. **Altenberg B and Greulich KO** (2004) Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. *Genomics* **84**, 1014–1020.
2. **Muir A and Vander Heiden MG** (2018) The nutrient environment affects therapy. *Science* **360**, 962–963.
3. **Warburg O** (1925) Über den Stoffwechsel der Carcinomzelle. *Klinische Wochenschrift* **4**, 534–536.
4. **Warburg OH** (1915) Notizen zur Entwicklungsphysiologie des Seeigeleies. *Pflügers Archiv – European Journal of Physiology* **160**, 324–332.
5. **Warburg O and Minami S** (1923) Versuche am überlebenden Carcinomgewebe. *Klinische Wochenschrift* **2**, 776–777.
6. **Warburg O, Posener K and Negelein E** (1924) Über den Stoffwechsel der Carcinomzelle. *Biochemische Zeitschrift* **152**, 309–344.
7. **Warburg O** (1924) Verbesserte Methode zur Messung der Atmung und Glykole. *Biochemische Zeitschrift* **152**, 51–63.

8. **Cori CF and Cori GT** (1925) The carbohydrate metabolism of tumors: i. the free sugar, lactic acid, and glycogen content of malignant tumors. *Journal of Biological Chemistry* **64**, 11–22.

9. **Cori CF and Cori GT** (1925) The carbohydrate metabolism of tumors: ii. changes in the sugar, lactic acid, and co2-combining power of blood passing through a tumor. *Journal of Biological Chemistry* **65**, 397–405.

10. **Warburg OH and Ernst H** (1952) Versuche mit Ascites-Tumorzellen. *Zeitschrift für Naturforschung* **7b**, 193–194.

11. **Warburg O** (1956) On respiratory impairment in cancer cells. *Science* **124**, 269–270.

12. **Warburg O** (1956) On the origin of cancer cells. *Science* **123**, 309–314.

13. **Weinhouse S** (1956) On respiratory impairment in cancer cells. *Science* **124**, 267–269.

14. **Racker E and Spector M** (1981) Warburg effect revisited: merger of biochemistry and molecular biology. *Science* **213**, 303–307.

15. **Cooper GM** (2000) The mechanism of oxidative phosphorylation. In *The Cell: A Molecular Approach*, 2nd edition. Sunderland (MA): Sinauer Associates. Available from https://www.ncbi.nlm.nih.gov/books/NBK9885/

16. **Hanahan D and Weinberg R** (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.

17. **Stehelin D** *et al.* (1976) DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* **260**, 170–173.

18. **Tabin CJ** *et al.* (1982) Mechanism of activation of a human oncogene. *Nature* **300**, 143.

19. **Nowell PC** (1976) The clonal evolution of tumor cell populations. *Science* **194**, 23–28.

20. **Greaves M** (2015) Evolutionary determinants of cancer. *Cancer Discovery* **5**, 806–820.

21. **Sever R and Brugge JS** (2015) Signal transduction in cancer. *Cold Spring Harbor Perspectives in Medicine* **5**(4), a006098.

22. **Jackson RC** *et al.* (1980) Purine and pyrimidine nucleotide patterns of normal, differentiating, and regenerating liver and of hepatomas in rats. *Cancer Research* **40**, 1286.

23. **Natsumeda Y** *et al.* (1984) Enzymic capacities of purine de novo and salvage pathways for nucleotide synthesis in normal and neoplastic tissues. *Cancer Research* **44**, 2475.

24. **Tomlinson IP** *et al.* (2002) Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nature Genetics* **30**, 406–410.

25. **Baysal BE** *et al.* (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* **287**, 848–851.

26. **Parsons DW** *et al.* (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**, 1807–1812.

27. **Yan H** *et al.* (2009) IDH1 and IDH2 mutations in gliomas. *New England Journal of Medicine* **360**, 765–773.

28. **Mardis ER** *et al.* (2009) Recurring mutations found by sequencing an acute myeloid leukemia genome. *New England Journal of Medicine* **361**, 1058–1066.

29. **Dang L, Yen K and Attar EC** (2016) IDH mutations in cancer and progress toward development of targeted therapeutics. *Annals of Oncology* **27**, 599–608.

30. **Dang L** *et al.* (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **462**, 739.

31. **Ward PS** *et al.* (2010) The common feature of leukemia-associated IDH1 and IDH2 mutations Is a neomorphic enzyme activity converting α-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* **17**, 225–234.

32. **Noushmehr H** *et al.* (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* **17**, 510–522.

33. **Turcan S** *et al.* (2012) IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* **483**, 479.

34. **Figueroa ME** *et al.* (2010) Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* **18**, 553–567.

35. **Xu W** *et al.* (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* **19**, 17–30.

36. **Watanabe T** *et al.* (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *The American Journal of Pathology* **174**, 1149–1153.

37. **Lai A** *et al.* (2011) Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. *Journal of Clinical Oncology* **29**, 4482–4490.

38. **Johnson BE** *et al.* (2014) Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science* **343**, 189–193.

39. **Lass U** *et al.* (2012) Clonal analysis in recurrent astrocytic, oligoastrocytic and oligodendroglial tumors implicates IDH1- mutation as common tumor initiating event. *PLoS ONE* **7**, e41298.

40. **Kosmider O** *et al.* (2010) Mutations of IDH1 and IDH2 genes in early and accelerated phases of myelodysplastic syndromes and MDS/myeloproliferative neoplasms. *Leukemia* **24**, 1094.

41. **Pardanani A** *et al.* (2010) IDH1 and IDH2 mutation analysis in chronic- and blast-phase myeloproliferative neoplasms. *Leukemia* **24**, 1146.

42. **Kats L** *et al.* (2014) Proto-oncogenic role of mutant IDH2 in leukemia initiation and maintenance. *Cell Stem Cell* **14**, 329–341.

43. **Chen C** *et al.* (2013) Cancer-associated IDH2 mutants drive an acute myelocytic leukemia that is susceptible to Brd4 inhibition. *Genes & Development* **27**, 1974–1985.

44. **Sasaki M** *et al.* (2012) D-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. *Genes & Development* **26**, 2038–2049.

45. **Rohle D** *et al.* (2013) An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* **340**, 626–630.

46. **Davis JL, Fallon HJ and Morris HP** (1970) Two enzymes of serine metabolism in rat liver and hepatomas. *Cancer Research* **30**, 2917–2920.

47. **Snell K and Weber G** (1986) Enzymic imbalance in serine metabolism in rat hepatomas. *The Biochemical Journal* **233**, 617–620.

48. **Snell K** *et al.* (1988) Enzymic imbalance in serine metabolism in human colon carcinoma and rat sarcoma. *British Journal of Cancer* **57**, 87–90.

49. **Locasale JW** *et al.* (2011) Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. *Nature Genetics* **43**, 869.

50. **Possemato R** *et al.* (2011) Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* **476**, 346.

51. **Pollari S** *et al.* (2011) Enhanced serine production by bone metastatic breast cancer cells stimulates osteoclastogenesis. *Breast Cancer Research and Treatment* **125**, 421–430.

52. **Adams CM** (2007) Role of the transcription factor ATF4 in the anabolic actions of insulin and the anti-anabolic actions of glucocorticoids. *Journal of Biological Chemistry* **282**, 16744–16753.

53. **Nilsson LM** *et al.* (2012) Mouse genetics suggests cell-context dependency for Myc-regulated metabolic enzymes during tumorigenesis. *PLoS Genetics* **8**, e1002573.

54. **Ding J** *et al.* (2013) The histone H3 methyltransferase G9A epigenetically activates the serine-glycine synthesis pathway to sustain cancer cell survival and proliferation. *Cell Metabolism* **18**, 896–907.

55. **Beroukhim R** *et al.* (2010) The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899.

56. **DeNicola GM** *et al.* (2015) NRF2 regulates serine biosynthesis in nonsmall cell lung cancer. *Nature Genetics* **47**, 1475.

57. **Chen J** *et al.* (2013) Phosphoglycerate dehydrogenase is dispensable for breast tumor maintenance and growth. *Oncotarget* **4**, 2502–2511.

58. **Grant GA** (2018) D-3-Phosphoglycerate dehydrogenase. *Frontiers in Molecular Biosciences* **5**, 110–110.

59. **Mattaini KR** *et al.* (2015) An epitope tag alters phosphoglycerate dehydrogenase structure and impairs ability to support cell proliferation. *Cancer & Metabolism* **3**, 5.

60. **Fan J** *et al.* (2015) Human phosphoglycerate dehydrogenase produces the oncometabolite d-2-hydroxyglutarate. *ACS Chemical Biology* **10**, 510–516.

61. **Samanta D** *et al.* (2016) PHGDH expression is required for mitochondrial redox homeostasis, breast cancer stem cell maintenance, and lung metastasis. *Cancer Research* **76**, 4430–4442.

62. **Liu J** *et al.* (2013) Phosphoglycerate dehydrogenase induces glioma cells proliferation and invasion by stabilizing forkhead box M1. *Journal of Neuro-Oncology* **111**, 245–255.

63. **Murphy JP** *et al.* (2018) The NAD + salvage pathway supports PHGDH-driven serine biosynthesis. *Cell Reports* **24**, 2381–2391, e5.

64. **Meyer N and Penn LZ** (2008) Reflecting on 25 years with MYC. *Nature Reviews Cancer* **8**, 976–990.

65. **Kim JW** *et al.* (2004) Evaluation of myc E-box phylogenetic footprints in glycolytic genes by chromatin immunoprecipitation assays. *Molecular and Cellular Biology* **24**, 5923–5936.

66. **Le A** *et al.* (2012) Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. *Cell Metabolism* **15**, 110–121.

67. **Osthus RC** *et al.* (2000) Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. *Journal of Biological Chemistry* **275**, 21797–21800.

68. **Shen L** *et al.* (2015) Metabolic reprogramming in triple-negative breast cancer through Myc suppression of TXNIP. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 5425–5430.

69. **David CJ** *et al.* (2010) HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature* **463**, 364–368.

70. **Sun L** *et al.* (2015) cMyc-mediated activation of serine biosynthesis pathway is critical for cancer progression under nutrient deprivation conditions. *Cell Research* **25**, 429.

71. **Shim H** *et al.* (1997) c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. *Proceedings of the National Academy of Sciences* **94**, 6658–6663.

72. **Doherty JR** *et al.* (2014) Blocking lactate export by inhibiting the Myc target MCT1 disables glycolysis and glutathione synthesis. *Cancer Research* **74**, 908–920.

73. **Sonveaux P** *et al.* (2012) Targeting the lactate transporter MCT1 in endothelial cells inhibits lactate-induced HIF-1 activation and tumor angiogenesis. *PLoS ONE* **7**, e33418.

74. **Lu H, Forbes RA and Verma A** (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *Journal of Biological Chemistry* **277**, 23111–5.

75. **Maxwell PH, Pugh CW and Ratcliffe PJ** (2001) Activation of the HIF pathway in cancer. *Current Opinion in Genetics & Development* **11**, 293–299.

76. **Sun Q** *et al.* (2011) Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 4129–4134.

77. **Maxwell PH** *et al.* (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**, 271–275.

78. **Gimenez-Roqueplo A-P** *et al.* (2001) The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. *The American Journal of Human Genetics* **69**, 1186–1197.

79. **Pollard PJ** *et al.* (2005) Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. *Human Molecular Genetics* **14**, 2231–2239.

80. **Maxwell PH** *et al.* (1997) Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 8104–8109.

81. **Semenza GL** *et al.* (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *Journal of Biological Chemistry* **269**, 23757–23763.

82. **Kim J-W** *et al.* (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metabolism* **3**, 177–185.

83. **Iurlaro R, Leon-Annicchiarico CL and Munoz-Pinedo C** (2014) Regulation of cancer metabolism by oncogenes and tumor suppressors. *Methods in Enzymology* **542**, 59–80.

84. **Cole AJ** *et al.* (2017) Comprehensive analyses of somatic TP53 mutation in tumors with variable mutant allele frequency. *Scientific Data* **4**, 170120–20.

85. **Schwartzenberg-Bar-Yoseph F, Armoni M and Karnieli E** (2004) The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Cancer Research* **64**, 2627–2633.

86. **Kawauchi K** *et al.* (2008) P53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. *Nature Cell Biology* **10**, 611–618.

87. **Bensaad K** *et al.* (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* **126**, 107–120.

88. **Boidot R** *et al.* (2012) Regulation of monocarboxylate transporter MCT1 expression by p53 mediates inward and outward lactate fluxes in tumors. *Cancer Research* **72**, 939–948.

89. **Contractor T and Harris CR** (2012) P53 negatively regulates transcription of the pyruvate dehydrogenase kinase Pdk2. *Cancer Research* **72**, 560–567.

90. **Liu K** *et al.* (2016) Parkin regulates the activity of pyruvate kinase M2. *Journal of Biological Chemistry* **291**, 10307–10317.

91. **Matoba S** *et al.* (2006) P53 regulates mitochondrial respiration. *Science* **312**, 1650–1653.

92. **Stambolsky P** *et al.* (2006) Regulation of AIF expression by p53. *Cell Death & Differentiation* **13**, 2140–2149.

93. **Bergstrom J** *et al.* (1974) Intracellular free amino acid concentration in human muscle tissue. *Journal of Applied Physiology* **36**, 693–697.

94. **Krebs HA** (1935) Metabolism of amino-acids: the synthesis of glutamine from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissues. *Biochemical Journal* **29**, 1951–1969.

95. **Lacey JM and Wilmore DW** (1990) Is glutamine a conditionally essential amino acid? *Nutrition Reviews* **48**, 297–309.

96. **Krebs H** (1980) 18 – special lecture: glutamine metabolism in the animal body. In Mora J and Palacios R (eds), *Glutamine: Metabolism, Enzymology, and Regulation*. New York: Academic Press, pp. 319–329.

97. **DeBerardinis RJ and Cheng T** (2010) Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. *Oncogene* **29**, 313–324.

98. **Eagle H** (1955) Nutrition needs of mammalian cells in tissue culture. *Science* **122**, 501–514.

99. **Abu Aboud O** *et al.* (2017) Glutamine addiction in kidney cancer suppresses oxidative stress and can be exploited for real-time imaging. *Cancer Research* **77**, 6746–6758.

100. **Son J** *et al.* (2013) Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* **496**, 101–105.

101. **Lampa M** *et al.* (2017) Glutaminase is essential for the growth of triple-negative breast cancer cells with a deregulated glutamine metabolism pathway and its suppression synergizes with mTOR inhibition. *PLoS ONE* **12**, e0185092.

102. **Bhutia YD and Ganapathy V** (2016) Glutamine transporters in mammalian cells and their functions in physiology and cancer. *Biochimica et Biophysica Acta* **1863**, 2531–2539.

103. **van Geldermalsen M** *et al.* (2016) ASCT2/SLC1A5 controls glutamine uptake and tumour growth in triple-negative basal-like breast cancer. *Oncogene* **35**, 3201–3208.

104. **Wang Q** *et al.* (2014) Targeting glutamine transport to suppress melanoma cell growth. *International Journal of Cancer* **135**, 1060–1071.

105. **Ren P** *et al.* (2015) ATF4 and N-Myc coordinate glutamine metabolism in MYCN-amplified neuroblastoma cells through ASCT2 activation. *The Journal of Pathology* **235**, 90–100.

106. **Huang F** *et al.* (2014) Upregulated SLC1A5 promotes cell growth and survival in colorectal cancer. *International Journal of Clinical and Experimental Pathology* **7**, 6006–6014.

107. **Commisso C** *et al.* (2013) Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. *Nature* **497**, 633–637.

108. **Mates JM, Campos-Sandoval JA and Marquez J** (2018) Glutaminase isoenzymes in the metabolic therapy of cancer. *Biochimica et Biophysica Acta, Reviews on Cancer* **1870**, 158–164.

109. **Martin-Rufian M** *et al.* (2012) Mammalian glutaminase Gls2 gene encodes two functional alternative transcripts by a surrogate promoter usage mechanism. *PLoS ONE* **7**, e38380.

110. **Cassago A** *et al.* (2012) Mitochondrial localization and structure-based phosphate activation mechanism of glutaminase C with implications for cancer metabolism. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 1092–1097.

111. **van den Heuvel AP** *et al.* (2012) Analysis of glutamine dependency in non-small cell lung cancer: GLS1 splice variant GAC is essential for cancer cell growth. *Cancer Biology & Therapy* **13**, 1185–1194.

112. **Huang F** *et al.* (2014) Expression of glutaminase is upregulated in colorectal cancer and of clinical significance. *International Journal of Clinical and Experimental Pathology* **7**, 1093–1100.

113. **Pan T** *et al.* (2015) Elevated expression of glutaminase confers glucose utilization via glutaminolysis in prostate cancer. *Biochemical and Biophysical Research Communications* **456**, 452–458.

114. **Moreadith RW and Lehninger AL** (1984) The pathways of glutamate and glutamine oxidation by tumor cell mitochondria. Role of mitochondrial NAD(P) + -dependent malic enzyme. *Journal of Biological Chemistry* **259**, 6215–6221.

115. **Coloff JL** *et al.* (2016) Differential glutamate metabolism in proliferating and quiescent mammary epithelial cells. *Cell Metabolism* **23**, 867–880.

116. **Gaglio D** *et al.* (2011) Oncogenic K-Ras decouples glucose and glutamine metabolism to support cancer cell growth. *Molecular Systems Biology* **7**, 523.

117. **Wise DR *et al.*** (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 18782–7.

118. **Gao P *et al.*** (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* **458**, 762–765.

119. **Bott AJ *et al.*** (2015) Oncogenic Myc induces expression of glutamine synthetase through promoter demethylation. *Cell Metabolism* **22**, 1068–1077.

120. **Hu W *et al.*** (2010) Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 7455–7460.

121. **DeBerardinis RJ *et al.*** (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 19345–19350.

122. **Yang C *et al.*** (2014) Glutamine oxidation maintains the TCA cycle and cell survival during impaired mitochondrial pyruvate transport. *Molecular Cell* **56**, 414–424.

123. **Metallo CM *et al.*** (2011) Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* **481**, 380–384.

124. **Hatzivassiliou G *et al.*** (2005) ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell* **8**, 311–321.

125. **Yuneva M *et al.*** (2007) Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells. *Journal of Cell Biology* **178**, 93–105.

126. **Jiang L *et al.*** (2016) Reductive carboxylation supports redox homeostasis during anchorage-independent growth. *Nature* **532**, 255–258.

127. **Conti PS *et al.*** (1996) PET and [18F]-FDG in oncology: a clinical update. *Nuclear Medicine and Biology* **23**, 717–735.

128. **Zhu L *et al.*** (2017) Metabolic imaging of glutamine in cancer. *Journal of Nuclear Medicine* **58**, 533–537.

129. **Li C *et al.*** (2018) Preclinical study of an (18)F-labeled glutamine derivative for cancer imaging. *Nuclear Medicine and Biology* **64–65**, 34–40.

130. **Farber S *et al.*** (1948) Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (aminopterin). *New England Journal of Medicine* **238**, 787–793.

131. **Purcell WT and Ettinger DS** (2003) Novel antifolate drugs. *Current Oncology Reports* **5**, 114–125.

132. **Gonen N and Assaraf YG** (2012) Antifolates in cancer therapy: structure, activity and mechanisms of drug resistance. *Drug Resistance Updates* **15**, 183–210.

133. **Karran P and Attard N** (2008) Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. *Nature Reviews Cancer* **8**, 24.

134. **Longley DB, Harkin DP and Johnston PG** (2003) 5-fluorouracil: mechanisms of action and clinical strategies. *Nature Reviews Cancer* **3**, 330–338.

135. **Parker WB** (2009) Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. *Chemical Reviews* **109**, 2880–2893.

136. **Labak CM *et al.*** (2016) Glucose transport: meeting the metabolic demands of cancer, and applications in glioblastoma treatment. *American Journal of Cancer Research* **6**, 1599–1608.

137. **Flaig TW *et al.*** (2007) A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. *Investigational New Drugs* **25**, 139–146.

138. **Woodward GE and Hudson MT** (1954) The effect of 2-desoxy-d-glucose on glycolysis and respiration of tumor and normal tissues. *Cancer Research* **14**, 599–605.

139. **Wick AN *et al.*** (1957) Localization of the primary metabolic block produced by 2-deoxyglucose. *Journal of Biological Chemistry* **224**, 963–969.

140. **Crane RK and Sols A** (1954) The non-competitive inhibition of brain hexokinase by glucose-6-phosphate and related compounds. *Journal of Biological Chemistry* **210**, 597–606.

141. **Voss M *et al.*** (2018) Rescue of 2-deoxyglucose side effects by ketogenic diet. *International Journal of Molecular Sciences* **19**, 2462.

142. **Zhang D *et al.*** (2014) 2-Deoxy-D-glucose targeting of glucose metabolism in cancer cells as a potential therapy. *Cancer Letters* **355**, 176–183.

143. **Goldman RD, Kaplan NO and Hall TC** (1964) Lactic dehydrogenase in human neoplastic tissues. *Cancer Research* **24**(3 Part 1), 389–399.

144. **Xie H *et al.*** (2014) Targeting lactate dehydrogenase-a inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor-initiating cells. *Cell Metabolism* **19**(5), 795–809.

145. **Fantin VR, St-Pierre J and Leder P** (2006) Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* **9**(6), 425–434.

146. **Rani R and Kumar V** (2016) Recent Update on Human Lactate Dehydrogenase Enzyme 5 (hLDH5) Inhibitors: A Promising Approach for Cancer Chemotherapy. *Journal of Medicinal Chemistry* **59**(2), 487–496.

147. **Wang J *et al.*** (2015) AT-101 inhibits hedgehog pathway activity and cancer growth. *Cancer Chemotherapy and Pharmacology* **76**(3), 461–469.

148. **Izumi H *et al.*** (2011) Monocarboxylate transporters 1 and 4 are involved in the invasion activity of human lung cancer cells. *Cancer Science* **102**(5), 1007–1013.

149. **Sonveaux P *et al.*** (2008) Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *Journal of Clinical Investigation* **118**, 3930–3942.

150. **Quanz M *et al.*** (2018) Preclinical efficacy of the novel monocarboxylate transporter 1 inhibitor BAY-8002 and associated markers of resistance. *Molecular Cancer Therapeutics* **17**, 2285–2296.

151. **Curtis NJ *et al.*** (2017) Pre-clinical pharmacology of AZD3965, a selective inhibitor of MCT1: DLBCL, NHL and Burkitt's lymphoma antitumor activity. *Oncotarget* **8**, 69219–69236.

152. **Polanski R *et al.*** (2014) Activity of the monocarboxylate transporter 1 inhibitor AZD3965 in small cell lung cancer. *Clinical Cancer Research* **20**, 926–937.

153. **Benjamin D *et al.*** (2016) Syrosingopine sensitizes cancer cells to killing by metformin. *Science Advances* **2**, e1601756.

154. **Benjamin D *et al.*** (2018) Dual inhibition of the lactate transporters MCT1 and MCT4 is synthetic lethal with metformin due to NAD + depletion in cancer cells. *Cell Reports* **25**, 3047–3058, e4.

155. **Kourelis TV and Siegel RD** (2012) Metformin and cancer: new applications for an old drug. *Medical Oncology (Northwood, London, England)* **29**, 1314–1327.

156. **Liu Y *et al.*** (2012) A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth in vitro and in vivo. *Molecular Cancer Therapeutics* **11**, 1672–1682.

157. **Chan DA *et al.*** (2011) Targeting GLUT1 and the Warburg effect in renal cell carcinoma by chemical synthetic lethality. *Science Translational Medicine* **3**, 94ra70.

158. **Popovici-Muller J *et al.*** (2018) Discovery of AG-120 (Ivosidenib): a first-in-class mutant IDH1 inhibitor for the treatment of IDH1 mutant cancers. *ACS Medicinal Chemistry Letters* **9**, 300–305.

159. **Shih AH *et al.*** (2014) AG-221, a small molecule mutant IDH2 inhibitor, remodels the epigenetic state of IDH2-mutant cells and induces alterations in self-renewal/differentiation in IDH2-mutant AML model in vivo. *Blood* **124**, 437–437.

160. **Dhillon S** (2018) Ivosidenib: first global approval. *Drugs* **78**, 1509–1516.

161. **Dugan J and Pollyea D** (2018) Enasidenib for the treatment of acute myeloid leukemia. *Expert Review of Clinical Pharmacology* **11**, 755–760.

162. **Raineri S and Mellor J** (2018) IDH1: linking metabolism and epigenetics. *Frontiers in Genetics* **9**(493), 1–8.

163. **Buege MJ, DiPippo AJ and DiNardo CD** (2018) Evolving treatment strategies for elderly leukemia patients with IDH mutations. *Cancers (Basel)* **10**(6), 1–20.

164. **Pacold ME *et al.*** (2016) A PHGDH inhibitor reveals coordination of serine synthesis and one-carbon unit fate. *Nature Chemical Biology* **12**, 452.

165. **Mullarky E *et al.*** (2016) Identification of a small molecule inhibitor of 3-phosphoglycerate dehydrogenase to target serine biosynthesis in cancers. *Proceedings of the National Academy of Sciences* **113**, 1778–1783.

166. **Wang Q *et al.*** (2017) Rational design of selective allosteric inhibitors of PHGDH and serine synthesis with anti-tumor activity. *Cell Chemical Biology* **24**, 55–65.

167. **Unterlass JE *et al.*** (2016) Validating and enabling phosphoglycerate dehydrogenase (PHGDH) as a target for fragment-based drug discovery in PHGDH-amplified breast cancer. *Oncotarget* **9**, 13139–13153.

168. **Fuller N *et al.*** (2016) An improved model for fragment-based lead generation at AstraZeneca. *Drug Discovery Today* **21**, 1272–1283.

169. **Esslinger CS, Cybulski KA and Rhoderick JF** (2005) Ngamma-aryl glutamine analogues as probes of the ASCT2 neutral amino acid transporter binding site. *Bioorganic & Medicinal Chemistry* **13**, 1111–1118.

170. **Hassanein M** *et al.* (2015) Targeting SLC1a5-mediated glutamine dependence in non-small cell lung cancer. *International Journal of Cancer* **137**, 1587–1597.

171. **Bolzoni M** *et al.* (2016) Dependence on glutamine uptake and glutamine addiction characterize myeloma cells: a new attractive target. *Blood* **128**, 667–679.

172. **Wang Q** *et al.* (2015) Targeting ASCT2-mediated glutamine uptake blocks prostate cancer growth and tumour development. *The Journal of Pathology* **236**, 278–289.

173. **Broer A, Rahimi F and Broer S** (2016) Deletion of amino acid transporter ASCT2 (SLC1A5) reveals an essential role for transporters SNAT1 (SLC38A1) and SNAT2 (SLC38A2) to sustain glutaminolysis in cancer cells. *Journal of Biological Chemistry* **291**, 13194–13205.

174. **Chiu M** *et al.* (2017) GPNA inhibits the sodium-independent transport system L for neutral amino acids. *Amino Acids* **49**, 1365–1372.

175. **Grewer C and Grabsch E** (2004) New inhibitors for the neutral amino acid transporter ASCT2 reveal its Na + -dependent anion leak. *Journal of Physiology* **557**(Pt 3), 747–759.

176. **van Geldermalsen M** *et al.* (2018) Benzylserine inhibits breast cancer cell growth by disrupting intracellular amino acid homeostasis and triggering amino acid response pathways. *BMC Cancer* **18**, 689.

177. **Colas C** *et al.* (2015) Ligand discovery for the alanine-serine-cysteine transporter (ASCT2, SLC1A5) from homology modeling and virtual screening. *PLoS Computational Biology* **11**, e1004477.

178. **Newcomb RW** (2002) *Patent US6451828B1*, Selective inhibition of glutaminase by bis-thiadiazoles.

179. **DeLaBarre B** *et al.* (2011) Full-length human glutaminase in complex with an allosteric inhibitor. *Biochemistry* **50**, 10764–10770.

180. **Robinson MM** *et al.* (2007) Novel mechanism of inhibition of rat kidney-type glutaminase by bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES). *Biochemical Journal* **406**, 407–414.

181. **Gross MI** *et al.* (2014) Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Molecular Cancer Therapeutics* **13**, 890–901.

182. **Liang Y, Bromley SD and Orford K** (2018) *Patent WO2018039442A1*, Treatment of cancer with inhibitors of glutaminase.

183. **Wang JB** *et al.* (2010) Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. *Cancer Cell* **18**, 207–219.

184. **Stalnecker CA** *et al.* (2015) Mechanism by which a recently discovered allosteric inhibitor blocks glutamine metabolism in transformed cells. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 394–399.

185. **Li C** *et al.* (2006) Green tea polyphenols modulate insulin secretion by inhibiting glutamate dehydrogenase. *Journal of Biological Chemistry* **281**, 10214–10221.

186. **Qing G** *et al.* (2012) ATF4 regulates MYC-mediated neuroblastoma cell death upon glutamine deprivation. *Cancer Cell* **22**, 631–644.

187. **Yang C** *et al.* (2009) Glioblastoma cells require glutamate dehydrogenase to survive impairments of glucose metabolism or Akt signaling. *Cancer Research* **69**, 7986–7993.

188. **Jin L** *et al.* (2015) Glutamate dehydrogenase 1 signals through antioxidant glutathione peroxidase 1 to regulate redox homeostasis and tumor growth. *Cancer Cell* **27**, 257–270.

189. **Reed HT** *et al.* (1985) Amino-oxyacetic acid as a palliative in tinnitus. *Archives of Otolaryngology* **111**, 803–805.

190. **Guth PS** *et al.* (1990) Evaluation of amino-oxyacetic acid as a palliative in tinnitus. *Annals of Otology Rhinology & Laryngology* **99**, 74–79.

191. **Perry TL** *et al.* (1980) Failure of aminooxyacetic acid therapy in Huntington disease. *Neurology* **30**(7 Pt 1), 772–775.

192. **Thornburg JM** *et al.* (2008) Targeting aspartate aminotransferase in breast cancer. *Breast Cancer Research* **10**, R84.

193. **Korangath P** *et al.* (2015) Targeting glutamine metabolism in breast cancer with aminooxyacetate. *Clinical Cancer Research* **21**, 3263–3273.