Targeting cancer metabolism: a therapeutic window opens

Matthew G. Vander Heiden[*‡]

Abstract | Genetic events in cancer activate signalling pathways that alter cell metabolism. Clinical evidence has linked cell metabolism with cancer outcomes. Together, these observations have raised interest in targeting metabolic enzymes for cancer therapy, but they have also raised concerns that these therapies would have unacceptable effects on normal cells. However, some of the first cancer therapies that were developed target the specific metabolic needs of cancer cells and remain effective agents in the clinic today. Research into how changes in cell metabolism promote tumour growth has accelerated in recent years. This has refocused efforts to target metabolic dependencies of cancer cells as a selective anticancer strategy.

Therapeutic window
A term describing the ability of a drug to treat a disease effectively without causing unacceptable toxicity.

Aerobic glycolysis
The metabolism of glucose to lactate in the presence of oxygen. This is sometimes also referred to as the 'Warburg effect'.

^{18}F-fluorodeoxyglucose positron emission tomography
(FDG–PET). A medical imaging test that is used in the clinic to visualize tissues with increased glucose uptake, including tumours.

*Koch Institute for Integrative Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
‡Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.
Correspondence to M.G.V.H.
e-mail: mvh@mit.edu
doi:10.1038/nrd3504

Proliferating cancer cells exhibit considerably different metabolic requirements to most normal differentiated cells[1]. For example, in order to support their high rates of proliferation, cancer cells consume additional nutrients and divert those nutrients into macromolecular synthesis pathways (FIG. 1a). Metabolic pathways must therefore be rewired in such a way that balances biosynthetic processes with sufficient ATP production to support cell growth and survival. As all cancer cells are dependent on this change in metabolism, these altered pathways represent attractive therapeutic targets[2,3]. However, because normal proliferating cells have the same metabolic requirements as cancer cells, finding a therapeutic window between proliferating cancer cells and proliferating normal cells remains a major challenge in the development of successful cancer therapies targeting metabolic pathways.

Unlike their normal counterparts, many cancer cells metabolize glucose by aerobic glycolysis[1,4,5]. This phenomenon, known as the Warburg effect, is characterized by increased glycolysis and lactate production regardless of oxygen availability. Aerobic glycolysis is often accompanied by increased glucose uptake, and this phenomenon may be visualized in tumours of patients using ^{18}F-deoxyglucose positron emission tomography (FDG–PET) imaging. FDG–PET is used clinically as a staging tool for diverse types of cancers, and experimental PET tracers can distinguish cancer cells from normal cells based on other aspects of cancer metabolism[6].

Differential uptake of ^{11}C-choline, ^{11}C-acetate, ^{11}C-methionine and ^{18}F-labelled amino acid analogues has been demonstrated in some human cancers[6,7]. Variable uptake of these molecules — as well as FDG — and variable secretion of lactate are all observed in cancers, even among tumours arising from the same tissue[6–9]. Why some cancers exhibit increased labelling with these tracers is not understood; however, these findings suggest that tumours exhibit heterogeneous metabolic alterations that extend beyond the Warburg effect (FIG. 1b). Nevertheless, all cancer cells must ultimately direct available nutrients into the synthesis of new biomass while maintaining adequate levels of ATP for cell survival. Therefore, it is likely that these phenotypic differences are manifestations of various metabolic solutions that enable the proliferation of cancer cells in individual tumours.

At least some of the metabolic heterogeneity that is observed in tumours is influenced by the tumour microenvironment[5]. Abnormal tumour vasculature can result in gradients of nutrients, oxygen and pH. Glucose, amino acids and lipids provide the substrates to supply metabolic pathways, and therefore metabolism is altered depending on the cellular availability of these nutrients. In addition, the signalling mechanisms of cells are linked to growth control pathways that sense conditions such as amino acid availability and oxygen levels; these signalling mechanisms also influence metabolism[5,10–12]. Genetic alterations that are associated with cancer often occur in these same signalling pathways, which suggests that both environmental and genetic factors influence the metabolic heterogeneity that is present across tumours[5].

Despite having an in-depth understanding of metabolic regulation, which has been built on almost a century of biochemistry research, our knowledge of how pathways are regulated to facilitate cell proliferation is incomplete[13]. Success in targeting cancer metabolism will emerge from

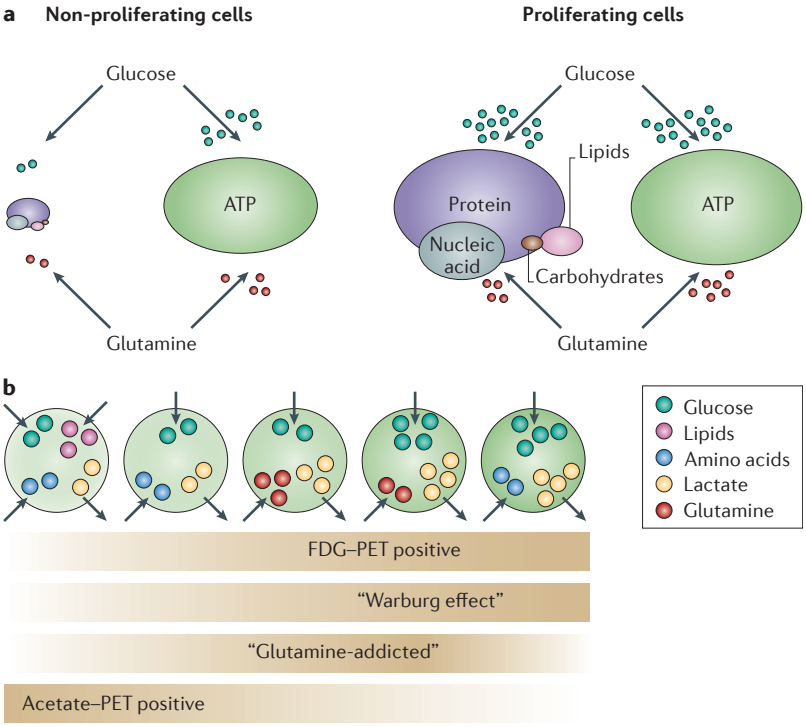

Figure 1 | **Cancer cell metabolism.** Proliferating cell metabolism involves a shift in nutrient metabolism towards biosynthesis. **a** | Mammalian cells are exposed to ~5 mM glucose and ~0.5 mM glutamine in serum, and these nutrients are the primary metabolic fuel for cancer cells and many normal cells. Additional nutrients, including lipids and other amino acids, can also be an important source of ATP and biosynthetic precursors for some cells. Most of the increased nutrient uptake in proliferating cells is used to support biosynthetic reactions. As a result, cancer cell metabolism involves many complex changes in metabolite flux beyond a switch in the amount of glucose metabolized by oxidative phosphorylation and aerobic glycolysis. Understanding how different cancer cells regulate metabolism to achieve a balance between ATP production and biosynthesis is vital for successfully targeting enzymes for cancer therapy. **b** | Not all tumours exhibit the same metabolic phenotype. Tracer uptake studies in patients and in model systems of cancer have demonstrated that cancer cells exhibit differential uptake of nutrients. This variety is seen even among different tumours that arise from the same normal tissue. This heterogeneity underlies observed differences in ^{18}F-deoxyglucose positron emission tomography (FDG–PET) and acetate–PET scans in patients, as well as why some tumour cells are 'glutamine-addicted' or exhibit the Warburg effect, and should be considered when stratifying patients for trials using novel therapies that target cancer metabolism.

a better understanding, in specific genetic contexts, of precisely how cells regulate the flux of nutrients into pathways that are required for biosynthesis. Understanding tumour cell metabolism requires the use of methods to assess metabolite flux and pathway regulation that are not often used in cancer drug discovery. However, akin to how antibiotics target the biosynthetic processes that are unique to microorganisms, the possibility of selectively targeting the biosynthetic processes of cancer cells holds promise as a strategy for improving cancer therapy.

Here, we review existing evidence supporting the therapeutic potential of targeting the metabolic adaptations that are characteristic of cancer cells, discuss the associated challenges and limitations of this as an anticancer strategy, and outline a framework for considering new targets in cancer metabolism. We also discuss emerging evidence involving specific metabolic enzyme targets, and examine how they might be used to limit cell proliferation. To date, only a handful of molecules that target metabolic pathways have been tested as a form of cancer therapy. However a growing body of evidence supports the notion that altered metabolism is a key consequence of important genetic drivers of cancer, thus inciting renewed interest in exploring metabolic enzymes as therapeutic targets.

Why target cancer cell metabolism?

Metabolism may influence cancer initiation and progression. Clinical studies have linked altered whole-body metabolism to cancer development, progression and poor treatment outcomes. Indeed, obesity, hyperglycaemia and insulin resistance are all associated with an increased risk of developing cancer and are associated with worse clinical outcomes in patients with cancer[14–18]. However, how such changes in organismal metabolism influence metabolism at the cellular level to promote cancer is controversial.

Increased circulating levels of insulin and insulin-like growth factor (IGF) have been linked with cancer progression, which suggests that obesity and insulin resistance promote cancer at least in part by activating signalling pathways that drive cell growth[15]. These same signalling pathways also drive nutrient uptake into cells and regulate enzymes in glycolysis, which implies that hormonal changes can have important indirect effects on cancer cell metabolism[16]. Furthermore, elevated levels of glucose alone may promote increased glucose uptake in some cells, and lower circulating levels of glucose are associated with better cancer treatment outcomes[19–22].

As a result, antidiabetic drugs are being explored for antitumour activity, and retrospective clinical studies have shown a reduction in cancer-related mortality in patients with diabetes who are taking metformin[23,24]. However, this effect appears to be independent of blood glucose levels, as patients with diabetes whose blood glucose levels are controlled by other means do not derive the same anticancer effect as patients taking metformin[24]. Metformin is widely used for the treatment of type 2 diabetes and acts by inhibiting mitochondrial complex I in the liver to interfere with ATP production[25,26]. This causes energy stress, increased AMP-activated protein kinase (AMPK) activity and inhibition of gluconeogenesis, which results in lower blood glucose levels, improved insulin sensitivity and decreased insulin[27]. Thus, it is controversial whether metformin benefits patients with cancer by directly acting on the tumour or by indirectly decreasing levels of insulin and insulin-related growth factors.

Other antidiabetic therapies that act by raising insulin levels may therefore lead to worse clinical outcomes in patients with cancer. Dietary restriction, which has been known to prolong survival in cancer models, has no effect on tumours that proliferate in the absence of IGF signalling[28]. These findings are consistent with metformin providing an indirect benefit to patients with high circulating levels of IGF. However, high doses of metformin are toxic to cancer stem cells[29], and women taking metformin have an increased tumour response to neoadjuvant chemotherapy for breast cancer that may extend to patients with cancer who do not have diabetes[30].

Tumour microenvironment
The local conditions experienced by cells in a tumour, including the levels of nutrients, oxygen and signalling molecules such as growth factors and cytokines.

Metabolic enzymes
Proteins that catalyse the interconversion of two metabolites.

LKB1 (also known as STK11), a kinase that is important for AMPK activation in response to metformin[27], is frequently deficient in human cancers[5]. Thus, the administration of metformin to induce energy stress may be particularly beneficial for treating LKB1-deficient tumours because these cells are unable to activate AMPK and cope with this stress[31]. Planned adjuvant clinical trials of metformin in patients with breast cancer will provide additional insight into which patients benefit from metformin. Metformin could also be used as a form of chemoprevention in patients with a high risk of developing cancer[32,33], although the best strategy for identifying individuals to include in such trials has yet to be determined[34].

Regardless of whether the benefit that is observed with metformin involves a direct effect on cell metabolism, blocking the signals that link whole-body metabolism to cellular metabolism presents several therapeutic opportunities. Antibodies and small-molecule kinase inhibitors that target the IGF receptor (IGFR) have been well tolerated by patients[35]. Early studies with these agents have focused on sarcomas, based on preclinical evidence suggesting that these tumours are dependent on IGFR signalling. In fact, some patients with rare sarcomas develop tumour-associated hypoglycaemia that is related to increased production of an isoform of IGF, and dramatic anecdotal responses have been reported in these individuals[36]. Unfortunately, overall these agents have demonstrated limited efficacy in clinical trials, which suggests that their clinical utility has yet to be determined[35].

Further efforts to identify tumours with altered metabolism that is dependent on IGFR signalling may enable the selection of patients who could benefit from these therapies. IGFs are thought to increase tumour growth by activating the phosphoinositide 3-kinase (PI3K) signal transduction pathway, which influences metabolic pathways as one downstream consequence of increased signalling[4,37]. In addition, mammalian target of rapamycin (mTOR) — a major effector downstream of PI3K — is regulated by nutrient availability[12]. Activation of mTOR stimulates a metabolic programme to promote cell growth[38]; consequently, mTOR inhibitors are increasingly being used in the clinic to treat various cancers, and many compounds targeting the PI3K pathway are in clinical development[39,40]. A better understanding of how these drugs affect tumour metabolism may define mechanisms of resistance to these agents or identify synergistic targets in metabolism that could convert mTOR inhibitors from cytostatic to cytotoxic agents and thus increase their efficacy in patients.

Targeting metabolism could improve existing approaches. Many genetic alterations that are known to promote cancer lead to a single converging metabolic phenotype that is characterized by enhanced cell-autonomous nutrient uptake and reorganization of metabolic pathways to support biosynthesis[4,5,41]. Growth signalling pathways that are activated in cancer promote these metabolic changes, and compounds that target signal transduction pathways are available in the clinic. However, despite the considerable success of these agents in selective cancers[42], for many common malignancies it remains a challenge to identify which patients are likely to respond to these drugs.

Interestingly, a decrease in glucose uptake — as measured by FDG–PET — has been predictive of a response to compounds that target the PI3K pathway in animal models[43], and of a response to kinase inhibitors in patients[44]. These findings support the hypothesis that a major metabolic consequence of dysregulated PI3K or tyrosine kinase activation is an increase in nutrient uptake. There is also evidence that increased nutrient uptake is a crucial effect of oncogenic *RAS* mutations[45], and therefore decreased nutrient uptake can be predictive of a response to therapy in *KRAS*-driven lung cancer[43]. This underscores the potential value of FDG–PET as an early predictor of response to molecules that target signalling pathways in the treatment of cancer.

Despite the availability of creative approaches, effective agents targeting many of the common driver mutations in cancer are not available. For instance, mutations in *RAS* or dysregulated expression of *MYC* are frequent events in human cancer, yet no specific therapies exist to treat cancers based on either genetic event, and many *RAS*-driven cancers are refractory to existing therapies[46,47]. Enzymes that are involved in metabolism appear to be key effectors of RAS- and MYC-dependent pathways. *RAS*-mutant cells are dependent on sufficient glucose uptake[45], and *MYC*-dependent cells have a particular reliance on glutamine metabolism[48–50].

In preclinical models, targeting metabolic enzymes has been shown to be effective in the treatment of *KRAS*-mutant[45,51] and *MYC*-dependent tumours[52,53]. For instance, small-molecule inhibitors that disrupt glucose metabolism can decrease the growth of xenograft tumours that are derived from cells driven by these oncogenes[45,51,53]. This suggests that targeting metabolism as an effector of signal transduction pathways that are required for cell growth might be an effective way of targeting cancers that are driven by genetic alterations and cannot be targeted directly. Furthermore, because kinase inhibitor therapies can result in decreased glucose uptake[1,43], compounds that further impair glucose metabolism may be synergistic with these approaches. Cytotoxic therapies also compromise glucose metabolism[54], so targeting metabolism may sensitize cancers to these drugs as well.

Metabolism is a proven target of successful therapies. Given that all cancer cells rely on changes in metabolism to support their growth and survival, targeting metabolism has the potential to affect cancers arising from many different tissues[2]. In fact, the possibility that agents targeting cell metabolism could be effective across diverse cancer types has historical precedent. For example, antifolate drugs were developed before there was an understanding of how folic acid contributes to a metabolic cycle that allows single-carbon transfer reactions (BOX 1), which are critical for the generation of nucleic acids (FIG. 2). Consequently, the success of antifolate drugs led to the study of other metabolite analogues as potential anticancer agents that disrupt nucleotide synthesis[55,56].

Today, the antimetabolite class of nucleoside analogues — which includes 5-flurouracil, gemcitabine (Gemzar; Lilly) and fludarabine (Fludara; Bayer/Genzyme), along with hydroxyurea and a newer generation of antifolate

Cancer cell metabolism
The enzymes and pathways used by cancer cells to transform nutrients into the chemical precursors that make up a cell, and to generate ATP and reducing equivalents that support cellular processes.

Box 1 | **The discovery of antifolate drugs as effective anticancer agents**

Targeting metabolism has been a prominent feature of some of the first efforts to treat cancer with drugs. Shortly after the discovery of folic acid as a nutrient that is needed to prevent anaemia in pregnancy, Sidney Farber[152] noted that the administration of folic acid conjugates appeared to stimulate leukaemic cell proliferation in patients[56]. This led to one of the first examples of rational drug design as Farber, working with Yellapragada Subbarao and chemists at the Lederle Laboratories, developed the folate analogue aminopterin for use in humans. Aminopterin could antagonize the ability of folic acid to stimulate the growth of bacteria, and this compound was the first drug to induce remission in children with acute lymphoblastic leukaemia[153]. Another folate analogue, methotrexate (amethopterin), replaced aminopterin as the agent used for cancer chemotherapy and resulted in one of the first cures of a solid tumour (choriocarcinoma) by chemotherapy in the late 1950s[154]. Methotrexate was also the first successful adjuvant therapy for osteosarcoma[155], and is still used for the management of several cancers in the clinic today.

drugs (for example, pemetrexed (Alimta; Lilly)) — is widely used in the treatment of diverse human tumours. Although these drugs are not considered by most to be 'targeted therapies', they have clear targets in metabolism, such as dihydrofolate reductase and thymidylate synthase (FIG. 2; TABLE 1), and remain effective therapies for many human cancers.

The use of the enzyme L-asparaginase to treat acute lymphoblastic leukaemia (ALL) and related lymphomas is another example of how the unique metabolism of tumour cells has been successfully exploited for cancer therapy. Like antifolate drugs, the potential utility of L-asparaginase in treating cancer was discovered by accident and represents another example of rational drug design that was later revealed to exploit a metabolic difference between cancer cells and normal cells (BOX 2). It was found that ALL cells are functional asparagine (and glutamine) auxotrophs[57]. L-asparaginase deaminates asparagine to aspartic acid, thereby limiting the availability of asparagine for cancer cells (FIG. 3). The bacterial L-asparaginase that is used in the clinic has preferential selectivity for asparagine over the structurally related amino acid glutamine[58]; however, the enzyme retains some ability to degrade glutamine, and this activity may have a role in the dose-limiting coagulopathy caused by the imbalanced synthesis of pro- and anticoagulant proteins[58,59]. However, glutamine is a crucial nutrient for many cancer cells and glutamine depletion may contribute to the effectiveness of the drug in ALL[3,60]. L-asparaginase has little utility in the clinic outside of ALL treatment, but other therapeutic uses of this enzyme have not been explored since the early days of chemotherapy.

Glutamine is the most abundant amino acid in serum and a key component of mammalian tissue culture media[60], and several studies have identified a dependence of some cancer cells on the nutrient[48–50]. Thus, L-asparaginase — or analogous agents that are designed to specifically reduce levels of glutamine — may be effective for treating cancers other than ALL. A rational approach to identify other auxotrophies of cancer cells could lead to the development of similar treatment strategies. Indeed, several types of cancer cells have low levels of arginosuccinate synthetase, which is required for endogenous arginine synthesis[3], and early experiments have suggested that tumours may be sensitive to arginase[61]. Arginine deiminase conjugated to polyethylene glycol (PEG) is an agent that lowers extracellular levels of arginine and is currently in clinical trials for various solid tumours[62]. Early phase (Phase I/II) trials have shown that this drug can be administered safely, and some positive responses have been observed in both hepatocellular carcinoma and melanoma[62,63].

Auxotroph
A term describing the inability of a cell (or organism) to synthesize a chemical compound that is required for growth or survival.

Key issues in targeting cancer cell metabolism

Challenges of directly targeting metabolic pathways. Because all cells rely on the same metabolic pathways to generate ATP, it is often assumed that drugs that target metabolic pathways would have detrimental effects on normal tissues. Although this is the case for some metabolic targets, the success of cytotoxic agents that target folate metabolism and DNA synthesis illustrates that a therapeutic window can exist for anticancer drugs that target metabolic pathways.

These chemotherapies have side effects that are related to on-target inhibition of the same enzymes in rapidly proliferating normal tissues such as the gut epithelium and bone marrow[64]. The common assumption that the therapeutic window obtained by these agents is due to the more rapid proliferation of cancer cells is not necessarily true. Proliferating cells in the gut have a cell-cycle time that is estimated to be 30–40 hours and these cells may proliferate as frequently as every 10 hours[65,66]. Haematopoiesis is also very fast as humans generate 2 million red blood cell precursors per second[67]. Cancer cells can proliferate at similar rates under optimal tissue culture conditions, but most cancer cells proliferate more slowly *in vivo*[66,68]. Despite this difference in the rate of proliferation, sensitive cancers can be targeted using these therapies.

Tumour sensitivity to these agents can be accounted for in part by the loss of cell cycle checkpoints that accompany the transformation of normal cells into cancer cells (chemotherapy-based killing mechanisms reviewed in REF. 69). However, the fact that folinic acid can selectively rescue dihydrofolate reductase inhibition in normal proliferating tissues (FIG. 2) and enhance the efficacy of 5-fluorouracil in colon cancer therapy[70] suggests that additional metabolic differences exist in cancer cells that also contribute to the therapeutic window. A better understanding of the molecular mechanisms underlying why some cancer cells are more dependent on specific metabolic pathways could result in more effective ways of targeting metabolic pathways, with fewer side effects on normal proliferating cells.

Unwanted toxicity caused by the effects of agents targeting metabolic pathways in normal proliferating cells is likely to be a major challenge in the development of drugs that target proliferative cell metabolism. Several pathways often exist to generate the same metabolic end product, and redundant pathways that are present in normal cells but absent in cancer cells may provide a therapeutic window. However, this same redundancy may also impair the efficacy of drugs in tumours that can use more than one pathway. For instance, the success of targeting ATP citrate lyase as a means of blocking cytoplasmic levels of acetyl-CoA is limited in part by the generation of acetyl-CoA via

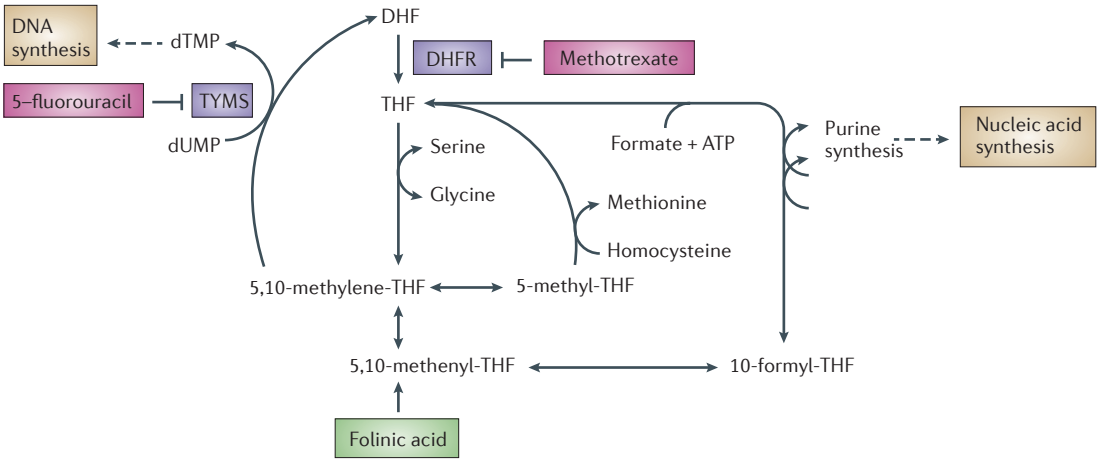

Figure 2 | **Existing chemotherapies targeting specific metabolic enzymes.** 5-fluorouracil inhibits thymidylate synthase (TYMS), an enzyme that is required to generate thymidine for DNA synthesis. Methotrexate inhibits dihydrofolate reductase (DHFR), which catalyses the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF) — a key step in folate metabolism. Interrupting folate metabolism compromises thymidine synthesis, but also interferes with purine synthesis and other reactions involving single-carbon transfers. Folinic acid can enter the folate pool downstream of DHFR and rescue the effects of DHFR inhibition in some cells. Glycine can also be used to convert THF to 5,10-methylene-THF. dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate.

another route[71]. Nevertheless, there is mounting evidence that genetic changes that are associated with cancer create addictions to specific metabolic pathways[4,5], and cancer cells often have chromosomal deletions that could eliminate enzymes that are necessary for the use of redundant pathways. Combining agents to target complementary metabolic pathways might therefore be another strategy for reducing the dose of individual drugs and limiting unwanted effects on normal cells.

A therapeutic window does not exist for some targets in cancer metabolism, but drugging alternative targets in the same metabolic pathway may be feasible. Although it has never been used to treat cancer, the mitochondrial uncoupling agent 2,4-dinitrophenol (DNP) was used as a weight-loss agent in the 1930s[72]. By uncoupling mitochondrial electron transport from ATP synthesis (FIG. 3), agents like DNP cause energy that is released from nutrient oxidation to be lost as heat and also induce energy stress in cells. Unfortunately, only slight overdoses of DNP result in lethal hyperthermia. However, metformin also targets oxidative phosphorylation but in a different way (FIG. 3), is well tolerated and one of the most commonly prescribed drugs in the world. By slowing mitochondrial ATP generation, metformin causes mild cellular energy stress[27]. Metformin has an on-target, dose-dependent side effect of inducing lactic acidosis. Complex I inhibition by metformin decreases mitochondrial oxidation of NADH to NAD^+. Regenerating NAD^+ is necessary to allow continued glycolytic flux, and lactate synthesis allows the regeneration of NAD^+ from NADH in the absence of mitochondrial electron transport. Thus, increased lactate production is an inevitable consequence of increased complex I inhibition, and this defines the therapeutic window for this class of drugs. However, whether this window is large enough to achieve doses that have direct growth-inhibitory effects on tumours *in vivo* remains to be determined.

Lactic acidosis
A condition of low blood pH (metabolic acidosis) that is caused by the accumulation of lactate.

Metabolism is often viewed as a housekeeping function for cells, whereas signalling pathways are viewed as unique pathways that act only in specific cell types and physiological situations. However, with the exception of gain-of-function mutations, no target is unique to cancer cells. Successful targeted therapies take advantage of the dependence of cancer cells on specific pathways. Similarly, cancer cells depend on specific metabolic pathways, and identifying these dependencies is crucial for generating drugs that can successfully target metabolic enzymes and have minimal effects on normal tissues.

Metabolic flux in cancer cells is not well understood. Resistance to therapy is an issue with all cancer treatments, and metabolism is a complex network with built-in plasticity that may allow the cell to overcome inhibition at a single enzymatic step. This further highlights the importance of understanding precisely how metabolic pathways are regulated in cancer cells *in vivo*. Flux through metabolic pathways, rather than levels of individual pathway metabolites, provides the cell with the ability to continually generate ATP (to support cell survival) or crucial biosynthetic precursors (for cell growth). Thus, rather than focusing on levels of individual metabolites, determining flux through the cancer cell metabolic network is likely to provide a better insight into successful enzyme targets.

Recent advances in metabolite profiling methodologies are providing new tools for understanding flux through pathways, and will enhance our understanding of cancer metabolism. Furthermore, increased application of techniques such as magnetic resonance spectroscopy can allow direct visualization of how metabolism is altered in patients as a result of new therapies[73,74]. These techniques include the use of dynamic nuclear polarization to generate hyperpolarized ^{13}C-labelled metabolites to track metabolism in tumours. These approaches for

Table 1 | **Strategies to target metabolic enzymes for cancer therapy**

Target (or targets)	Agent (or agents)	Stage of development	Indications/key preclinical findings	Refs
Nucleic acid synthesis				
Folate metabolism (DHFR)	Methotrexate, pemetrexed	Approved agents	Effective therapies for various cancers	-
Thymidine synthesis (TYMS)	5-Fluorouracil	Approved agent	An effective therapy for various cancers	-
Deoxynucleotide synthesis (RNR)	Hydroxyurea	Approved agent	An effective therapy for leukaemia	-
Nucleotide incorporation (DNA polymerase/ RNR)	Gemcitabine, Fludarabine	Approved agents	Effective therapies for various cancers	-
Ribose synthesis (TKTL1, G6PD)	Preclinical data only	Preclinical data only	TKTL1 allows non-oxidative ribose production and its expression correlates with poor prognosis; TKTL1 depletion via RNAi inhibits cell proliferation; G6PD is necessary for oxidative ribose production; high levels of G6PD seen in some cancers and its expression can transform fibroblasts	148, 161
Amino acid metabolism/protein synthesis				
Asparagine availability	L-asparaginase	Approved agent	An effective therapy for leukaemia	-
Arginine availability	Arginine deiminase conjugated to PEG	Phase II clinical trials	Arginine auxotrophy is thought to be related to low levels of arginosuccinate synthase expression in some tumours; the clinical efficacy of this agent is being explored in hepatocellular carcinoma, melanoma, small-cell lung cancer and mesothelioma	62
Glutamine availability (GLS1)	Preclinical data only	Preclinical data only	GLS1 converts glutamine to glutamate, and is likely to be important as a means of generating anapleurotic carbon for the TCA cycle	162
PHGDH	Preclinical data only	Preclinical data only	PHGDH is in a region of copy-number gain that is most commonly observed in melanoma and breast cancer, and cell lines with copy-number gain are dependent on PHGDH expression to proliferate	163, 164
Lipid synthesis				
FASN	Preclinical data only	Preclinical data only	FASN is a key enzyme in *de novo* lipogenesis; the growth of human xenograft tumours in mice is inhibited by tool compounds	165
ACLY	Preclinical data only	Preclinical data only	ACLY is necessary for exporting citrate from the mitochondria to the cytosol for *de novo* lipogenesis, and is important for cell proliferation and growth of human xenograft tumours	71,166
ACC	Preclinical data only	Preclinical data only	ACC is necessary for *de novo* lipogenesis and is required for the growth of cancer cells in culture in the absence of exogenous lipids	161, 167
Glycolysis				
Glucose transport	Preclinical data only	Preclinical data only	Efforts to inhibit glucose transport are ongoing	-
Hexokinase	2-deoxyglucose	Clinical data and preclinical data	Unacceptable toxicity observed at high doses; clinical trials at lower doses are currently on hold. Inhibition of hexokinase blocks proliferation, and is a rationale for the development of selective HK2 inhibitors	88,89, 91,92
PFK2	Preclinical data only	Preclinical data only	PFK2 controls a key regulatory step in glycolysis; tool compounds targeting the FB3 isoform (PFKFB3) inhibit the growth of xenograft tumours	51
PGAM	Preclinical data only	Preclinical data only	PGAM1 was identified in a screen as the target of a molecule that kills cancer cells	168
PKM2	Preclinical data only	Preclinical data only	Ongoing studies on both enzyme activation and inhibition; cancer cells expressing the PKM1 isoform do not grow as xenografts	99
LDHA	Preclinical data only	Preclinical data only	LDHA is responsible for lactate production; tool compounds inhibit the growth of xenograft tumours	53
Lactate excretion (MCT4)	Preclinical data only	Preclinical data only	Lactate is excreted from cells via MCTs; MCT4 is used by some cancer cells, and small-molecule MCT inhibitors can block cell proliferation	111, 114
TCA cycle/mitochondrial metabolism				
PDK	DCA	Phase II clinical trials	DCA is available in the clinic for treating lactic acidosis resulting from inborn errors of metabolism; can modulate mitochondrial metabolism in human gliomas, and its clinical efficacy is being studied	81
IDH1, IDH2	Preclinical data only	Preclinical data only	2-hydroxyglutarate production by mutated enzymes is linked to cancer pathogenesis Decreased expression of wild-type enzyme using RNAi can impair proliferation of wild-type IDH-expressing cancer cells	128–130

Table 1 cont. | **Strategies to target metabolic enzymes for cancer therapy**

Target (or targets)	Agent (or agents)	Stage of development	Indications/key preclinical findings	Refs
Malic enzyme	Preclinical data only	Preclinical data only	Key enzyme involved in NADPH production	1,139
Mitochondrial complex I	Metformin	Approved agent (not for cancer)	Improved survival in patients with diabetes who have cancer; increased response rate observed in patients with breast cancer taking metformin; prospective trials planned to explore efficacy in several cancers	24,30
Glutamine availability (GLS1, GDH)	Preclinical data only	Preclinical data only	GLS1 converts glutamine to glutamate, and GDH converts glutamate to αKG as a source of anapleurotic carbon for the TCA cycle; GDH is required for proliferation of some cells; inhibition of GLS1 impairs proliferation of some cells	142, 162, 169
PC	Preclinical data only	Preclinical data only	PC provides an alternative route to replenish the TCA cycle when GLS is inhibited, which suggests that PC inhibition could synergize with GLS inhibition in glutamine-addicted cells	170
Fatty acid metabolism				
MGLL	Preclinical data only	Preclinical data only	Inhibition of MGLL impairs the growth of xenograft tumours	147
CPT1C	Preclinical data only	Preclinical data only	Tool compounds inhibit the growth of xenograft tumours	171
NAD metabolism				
NAMPT	Various	Phase II clinical trials	FK866 had a dose-limiting toxicity of thrombocytopaenia in Phase I trials, and NAMPT inhibitors are being considered for further development as a cancer therapy	119, 123, 124

αKG, α-ketoglutarate; ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; CPT1C, carnitine palmitoyltransferase 1C; DCA, dichloroacetate; DHFR, dihydrofolate reductase; FASN, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; GLS, glutaminase 1; HK2, hexokinase 2; IDH, isocitrate dehydrogenase; LDHA, lactate dehydrogenase A; MCT4, monocarboxylate transporter 4; MGLL, monoacylglycerol lipase; NAMPT, nicotinamide phosphoribosyltransferase; PC, pyruvate carboxylase; PDK, pyruvate dehydrogenase kinase; PEG, polyethylene glycol; PFK2, phosphofructokinase 2; PGAM, phosphoglycerate mutase; PHGDH, phosphoglycerate dehydrogenase; PKM2, pyruvate kinase M2 isoform; RNAi, RNA interference; RNR, ribonucleotide reductase; TCA, tricarboxylic acid; TKTL1, transketolase-like protein 1; TYMS, thymidylate synthase.

tracking metabolism *in vivo* will be especially vital for understanding how cell metabolism is influenced by the tumour microenvironment[5], and will help with the selection of the right patients for specific drugs that target cancer cell metabolism.

Potential of metabolic enzymes as drug targets. Mutations in oncogenes or tumour suppressor genes result in the addiction of cancer cells to downstream signalling events[2,75]. These genetic events define an ideal set of possible targets for cancer therapy, but unfortunately many of the gene products are transcription factors or signalling molecules that rely on protein–protein interactions and present challenges to drug development. As a result, efforts have focused on targeting other tractable signalling molecules in a key pathway that is associated with the genetic event. These strategies have had limited success in the clinic, which suggests that blocking single downstream signalling targets is insufficient for inhibiting the transforming effects of some driver mutations.

Altered expression of metabolic enzymes or changes in the regulation of metabolic pathways also occurs downstream of many oncogenes and tumour suppressor genes, and cancers with specific genetic lesions are addicted to at least some of these metabolic changes[1,4,76]. In addition, ATP is necessary for the survival of all cells, and the ability to convert nutrients into biomass is crucial for all cancer cells. Thus, attacking metabolism as a downstream consequence of driver mutations is an attractive strategy because it is central to the growth and survival of cancer cells. Furthermore, many metabolic enzymes are amenable to targeting with small molecules.

Metabolite profiling
The measurement of multiple metabolite levels in cells or in body fluid. This is sometimes also referred to as metabolomics. Metabolites are usually detected using nuclear magnetic resonance spectroscopy or mass spectrometry.

Tumour metabolism can be safely targeted

It is possible to safely target central metabolic pathways in patients. The small molecule dichloroacetate (DCA) is used to treat patients with lactic acidosis resulting from rare inborn errors of mitochondrial metabolism. At least one target of DCA is pyruvate dehydrogenase kinase (PDK) (FIG. 3). The expression of PDK is increased in many cancers as a result of increased activation of the transcription factor hypoxia-inducible factor (HIF)[77,78]. PDK is a negative regulator of the pyruvate dehydrogenase complex (PDH)[79]. PDH catalyses oxidative decarboxylation of pyruvate to acetyl-CoA, which allows the entry of pyruvate into the tricarboxylic acid (TCA) cycle and away from lactate production. Thus, DCA-mediated inhibition of PDK leads to the activation of PDH, increased metabolism of pyruvate to acetyl-CoA and decreased lactate production. DCA can alter the mitochondrial membrane potential and inhibit lactate production in cancer model systems[80], and has been shown to alter mitochondria in patients with glioblastoma[81]. Importantly, even at doses that influence the mitochondrial membrane potential, DCA is well tolerated by patients[81]. Although there are insufficient data to determine whether DCA will provide clinical benefit, these studies demonstrate that a sufficient therapeutic window can exist to target cancer cell metabolism in patients.

Approaches for targeting cancer cell metabolism

Despite a renewed interest in exploring metabolic enzymes as targets for cancer therapy, very few molecules that target central carbon metabolism are currently in clinical trials (TABLE 1). However, mounting evidence

supports several metabolic enzymes as candidate targets, and studies using tool compounds have yielded encouraging results in preclinical models of cancer. New molecules directed against metabolic enzymes are likely to enter clinical studies in the next few years. Such compounds have the potential to limit macromolecular synthesis needed for cell growth, a strategy that is employed by existing drugs that target nucleic acid synthesis. Alternatively, targeting metabolism can limit pathways that are important for supplying nutrients to the cell and impair bioenergetics, thus preventing an adaptive response to cell stress. This latter approach is more likely to be synergistic with non-metabolic therapies that also impair nutrient uptake[54]. Enzyme targets that fall into both classes are summarized in TABLE 1.

These approaches could have seemingly opposite effects on some metabolic phenotypes. For instance, both DCA and metformin target mitochondrial physiology, yet DCA decreases lactate production and is used to treat lactic acidosis, whereas metformin increases lactate production and lactic acidosis is an important side effect of metformin treatment. Although paradoxical, there is evidence to suggest that both drugs are potentially beneficial in cancer treatment. By increasing glucose entry into the TCA cycle, DCA directs carbon away from lactate production[80] (FIG. 3) and, as a consequence, it may direct metabolism away from efficient biosynthetic reactions[1]. Conversely, metformin inhibits the transfer of electrons from NADH in the mitochondria to the electron transport chain (FIG. 3). This increases reliance on lactate production as a means to regenerate NAD^+ from NADH, impairs mitochondrial production of ATP and causes cellular energy stress[26,31]. Both approaches to impair metabolism could have therapeutic benefit in the right context. The former strategy (DCA) is likely to be more effective in tumours with increased reliance on high glucose uptake and lactate production, whereas the latter strategy (metformin) might synergize with other therapies that induce energy stress.

Directly targeting glucose metabolism. Various agents have been shown to block glucose uptake by cancer cells, but so far no specific glucose transport inhibitors have been reported. Glucose transporter 1 (GLUT1; also known as SLC2A1) is the glucose transporter with the largest tissue distribution and is thought to be responsible for basal glucose uptake in most cancer cells and normal cells[82,83] (FIG. 3). Although GLUT1 is expressed at much higher levels in cancer cells than in normal cells, it may be difficult to directly inhibit glucose uptake in tumours without having an effect on normal tissues. Nevertheless, partial inhibition of glucose uptake may still sensitize cancer cells to other drugs (reviewed in REF. 84).

Many of the studies exploring glucose dependence rely on the withdrawal of glucose from cells in culture, which illustrates the need for pharmacological agents that inhibit glucose uptake. There are at least thirteen passive glucose transporters, most of which have poorly understood functions. Interestingly, some of these, such as GLUT3 (also known as SLC2A3), are not expressed in most normal cells but they can be expressed at high levels in cancer cells, which suggests that these transporters could be possible therapeutic targets[82]. Antibodies that selectively target GLUT3 or other nutrient transporters with restricted expression may represent another way of blocking nutrient uptake and starving cancer cells.

2-deoxy-D-glucose (2DG) is an inhibitor of glucose metabolism as it is phosphorylated in cells by hexokinase to produce 2-deoxyglucose-6-phosphate, which is a competitive inhibitor of enzymes that metabolize glucose-6-phosphate. Cells that are exposed to sufficient amounts of 2DG undergo growth arrest and/or apoptosis[85], and 2DG may potentiate the effects of standard cytotoxic chemotherapy[84,86]. 2DG has been tested as an anticancer agent in patients[87], but when it was administered to patients with glioblastoma at doses that were sufficient to limit glucose metabolism in cancer cells, unacceptable toxicity was observed[88,89]. Lower doses of 2DG are better tolerated by patients, but limited efficacy has been observed at these doses[90]. However, because 2DG is a competitive inhibitor of glucose, and glucose is present at millimolar concentrations in the blood, it remains to be determined whether a sufficient therapeutic window exists to competitively inhibit glucose uptake or the downstream enzymes in glycolysis.

It appears that cancer cells preferentially rely on specific isoforms of glycolytic enzymes. Therefore, isoform-selective targeting may provide an alternative approach for modulating glucose metabolism in cancer cells. Hexokinase is responsible for trapping glucose in cells (FIG. 3) and at least some cancers are specifically dependent on the hexokinase 2 (HK2) isoform of this enzyme[91,92]. HK2 is normally expressed in skeletal muscle and adipose tissue, which provides a therapeutic window to target HK2 without risking on-target side effects in other normal tissues that express another isoform. The properties of HK2 that select for its expression over other hexokinase isoforms in cancers are not clear. Nevertheless, the fact that HK2 is specifically required by some cancers suggests that re-expression of another hexokinase isoform is unlikely to provide an escape mechanism for tumours that are treated with an HK2-selective inhibitor.

An association between hexokinase and mitochondria influences the regulation of apoptosis[93], and compounds isolated from plants that disrupt this association are toxic to cancer cells in culture[94]. Hexokinase is also a target of 3-bromopyruvate, a compound that has been shown to

Mitochondrial membrane potential
The electrochemical proton gradient across the inner mitochondrial membrane that is generated by the mitochondrial electron transport chain. This gradient is used to synthesize ATP and transport molecules across the inner mitochondrial membrane.

Central carbon metabolism
The core metabolic pathways used by cells to generate ATP, reducing equivalents and the main precursors for amino acid, nucleic acid and lipid biosynthesis.

Bioenergetics
A term referring to how energy flows through living systems.

Box 2 | Development of L-asparaginase to treat ALL

The potential utility of L-asparaginase in the treatment of cancer was first discovered when it was noted that guinea pig serum, but not the serum of other animals, had an inhibitory effect on the proliferation of lymphoma cells in mice[156]. Guinea pigs are unique among mammals as their serum has L-asparaginase activity[157], and this L-asparaginase activity was found to be responsible for the antilymphoma effect that was observed in mice[55,158]. L-asparaginase was found to be a particularly effective agent in the treatment of acute lymphoblastic leukaemia (ALL) and associated high-grade lymphomas, and it induced remission as a single agent in more than 50% of children with the disease[159]. Although these remissions were not durable, when L-asparaginase has been used as part of a combination chemotherapy regimen, it has contributed to a >80% cure rate for children with ALL, and its inclusion in adult chemotherapy regimens has contributed to improved clinical outcomes[160].

be toxic to cancer cells[91,95]. However, 3-bromopyruvate is also toxic to some cancer cells at concentrations that are too low to inhibit hexokinase; it has therefore been argued that the combined inhibition of several metabolic enzymes accounts for the toxic effects of this compound on cancer cells[96].

Pyruvate kinase is another glycolytic enzyme for which isoform-selective targeting may be therapeutically beneficial (FIG. 3). There are two pyruvate kinase genes in mammals, and both produce two distinct gene products by alternative splicing[97,98]. Most tissues express a product of the pyruvate kinase M (*PKM*) gene that is alternatively spliced to produce either the PKM1 or PKM2 isoform. All cancer cells express PKM2, whereas many differentiated tissues express PKM1 (REF. 97). The expression of PKM2 promotes aerobic glycolysis, and PKM2 expression is selected for during growth of xenograft tumours in mice[99]. PKM1 is a constitutively active enzyme, whereas

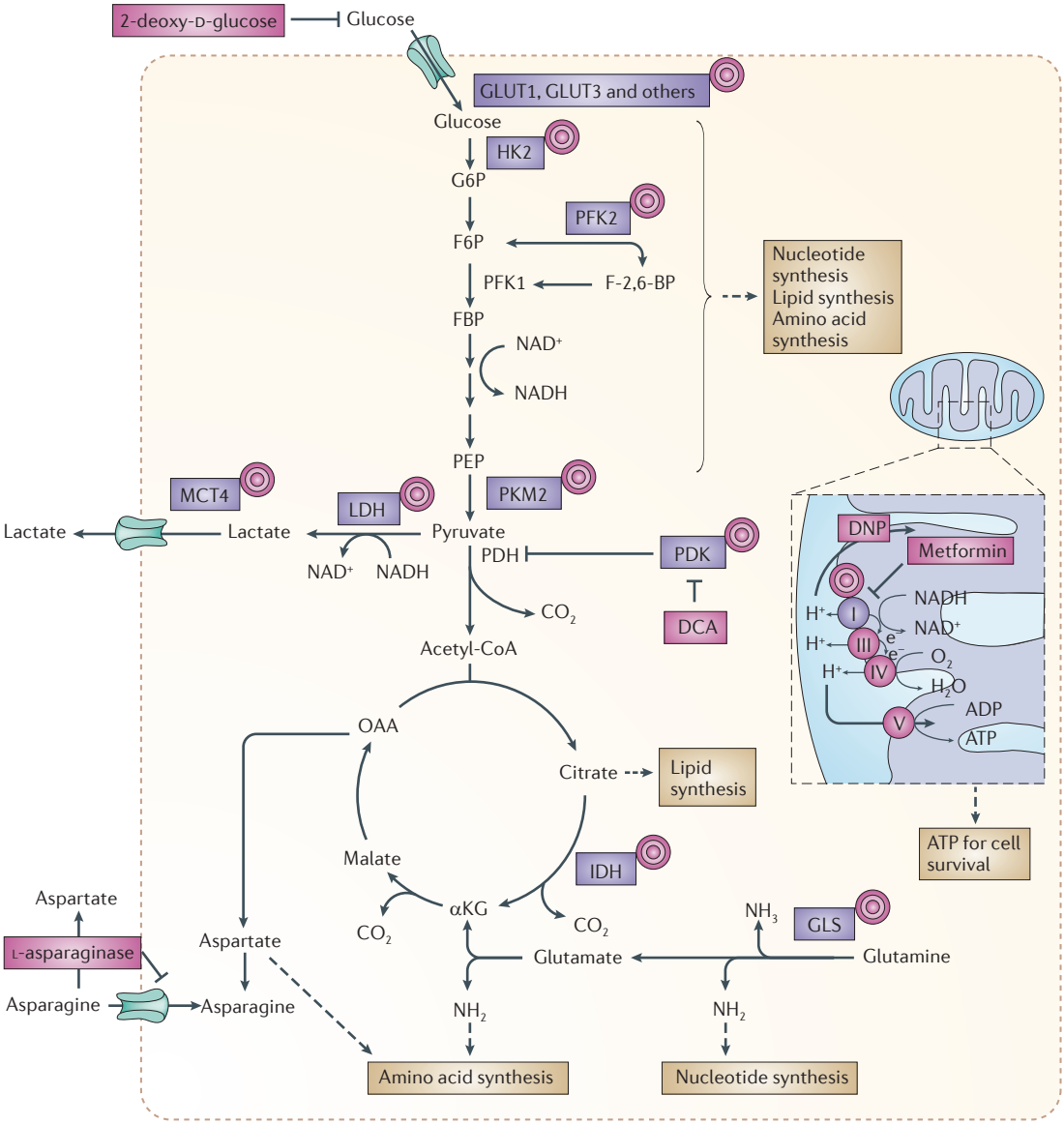

Figure 3 | **Targeting metabolic enzymes as a strategy to block biosynthesis or induce energy stress.** The pathways of central carbon metabolism are presented. Some of the metabolic enzymes that are currently being considered as therapeutic targets for cancer are marked with a target (shown as a pink circle in the figure). Five drugs that influence metabolism and have been tested in humans are shown in pink boxes. This figure illustrates how these enzyme targets are involved in the synthesis of important macromolecules (shown in brown boxes) that are needed for cell growth. αKG, α-ketoglutarate; DCA, dichloroacetate; DNP, 2,4-dinitrophenol; F-2,6-BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; G6P, glucose-6-phosphate; GLS, glutaminase; GLUT1, glucose transporter type 1; HK2, hexokinase 2; I, complex I; IDH, isocitrate dehydrogenase; III, complex III; IV, complex IV; LDH, lactate dehydrogenase; MCT4, monocarboxylate transporter 4; OAA, oxaloacetate; PDH, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PEP, phosphoenolpyruvate; PFK1, phosphofructokinase 1; PFK2, phosphofructokinase 2; PKM2, pyruvate kinase M2 isoform; V, complex V.

PKM2 is unique among pyruvate kinase isoforms in that its enzyme activity is inhibited following its binding to tyrosine-phosphorylated proteins downstream of cellular growth signals[100]. Surprisingly, it is this ability to inhibit the PKM2 enzyme that appears to be important for the promotion of aerobic glycolysis and cell proliferation in tumours.

Selection for a less active form of pyruvate kinase may help to divert glucose metabolites upstream of pyruvate kinase into biosynthetic pathways[5,97,100]. Efforts have been made to selectively inhibit PKM2 (REFS 101,102). Peptide aptamers that promote the less active form of pyruvate kinase have been shown to cause energy stress and cell death in cultured cancer cells[101], and more modest effects were observed using small-molecule inhibitors of PKM2 (REF. 102). Targeting PKM2 with short hairpin RNA can slow cell proliferation in cell culture[99]; however, these cells retain the ability to proliferate even in the near-complete absence of pyruvate kinase activity. These findings suggest that the activation of PKM2 to restore the high activity state of pyruvate kinase found in normal tissues may be an alternative strategy for targeting pyruvate kinase in cancer.

Isoform-specific small-molecule activators of PKM2 have been reported[103,104]. However, whether these compounds can induce the same growth disadvantage *in vivo* that is observed in PKM1-expressing cells remains to be determined. PKM2 is unique among pyruvate kinase isoforms as it has the ability to switch between a low and high activity state; therefore, it is possible that disrupting this dynamic capability with either enzyme inhibitors or activators could be therapeutically beneficial in cancer. However, PKM2 is also expressed in many normal tissues[97], and it remains to be determined whether the activation or inhibition of PKM2 in these tissues will result in unacceptable toxicity.

Another example of a regulatory enzyme in glycolysis with isoform selectivity in some cancers is phosphofructokinase 2 (PFK2) (FIG. 3). By generating fructose-2,6-bisphosphate (F-2,6-BP), PFK2 activates phosphofructokinase 1 (PFK1) to increase flux through this step of glycolysis. Most isoforms of PFK2 are bifunctional enzymes with both kinase and phosphatase activity, and can therefore also catalyse the destruction of F-2,6-BP and decrease PFK1 activity[105]. The FB3 isoform of PFK2 (PFKFB3) is expressed in many cancers and is required for anchorage-independent growth of *RAS*-driven tumours[106,107]. PFKFB3 has almost no phosphatase activity, and its kinase activity is influenced by several factors that are implicated in controlling cancer metabolism, including metabolite levels as well as RAS, MYC and AMPK signalling[105,108,109]. Small-molecule inhibitors of PFKFB3 have been reported to have a cytostatic effect on RAS-transformed cancer cells[51]. The compound targeting PFKFB3 decreases levels of F-2,6-BP and impairs the growth of xenograft tumours[51], thus raising interest in this enzyme as a target for cancer therapy.

Inhibiting lactate production or transport. Because lactate is excreted from the cell, inhibiting lactate production or lactate transport out of the cell are two strategies that directly target the Warburg effect in cancer. The family of monocarboxylate transporters (MCTs) comprises the major proteins that are responsible for lactate export in glycolytic cells, including cancer cells[110–112] (FIG. 3). There is evidence that a symbiotic relationship exists among different cells within a tumour whereby some cells rely on the lactate produced by other cells as a fuel source, and so disrupting lactate transport can starve cells that are dependent on lactate for survival[113]. However, targeting MCTs using small molecules also inhibits the proliferation of lymphocytes that rely on aerobic glycolysis[114,115]. This suggests that impaired immune function could be a side effect of targeting lactate export in cancer, and that drugs targeting cancer metabolism may have applications as immunosuppressive therapies.

Additional potential side effects of inhibiting lactate transport include negative effects on other normal tissues — such as the liver, muscles and brain — that rely on lactate as a fuel in certain physiological situations[116]. Lactate dehydrogenase (LDH) is the enzyme that interconverts pyruvate and NADH with lactate and NAD^+, respectively (FIG. 3). When LDHA is knocked down using RNA interference, cancer cell proliferation is severely impaired both *in vitro* and *in vivo*[52,117]. LDHA is the form of LDH that is expressed in many cancer cells, and inhibitors of this enzyme are being developed. Most non-cancerous tissues are not dependent on LDHA, and LDHA can be selectively inhibited over other forms of LDH[118]. Furthermore, LDHA inhibitors slow the growth of xenograft tumours in mice and can induce tumour regression when they are combined with nicotinamide phosphoribosyltransferase (NAMPT) inhibitors[53], which indicates that LDHA could be a promising therapeutic target for cancer therapy.

Targeting NAD^+ metabolism. Cells possess a limited pool of NAD^+ and NADH, yet these molecules exist as important cofactors in metabolic oxidation–reduction reactions. They are also substrates for enzymes such as NAD-dependent deacetylase sirtuins and poly(ADP-ribose) polymerases that are involved in the regulation of numerous processes related to cancer, including DNA repair, inflammation and protein acetylation[119]. Unlike oxidation–reduction reactions, these latter reactions consume NAD^+ and deplete the cellular pool of this important cofactor.

Interestingly, NAMPT, the enzyme that is involved in regenerating NAD^+ from nicotinamide and phosphoribosyl pyrophosphate via a salvage pathway, was identified as the target of a molecule that was discovered in a screen to find novel cytotoxic compounds[120]. Cells that are treated with NAMPT inhibitors die as a result of NAD^+ depletion, and NAMPT inhibition has shown activity as an anticancer agent in preclinical models of cancer[119].

Because NAD^+ is a required cofactor for the step of glycolysis that is catalysed by glyceraldehyde-3-phosphate dehydrogenase, cells must regenerate NAD^+ from NADH to enable the continued flow of glucose carbon via glycolysis (FIG. 3). Consistent with NAMPT inhibitors limiting glucose metabolism in cells with a high activity of NAD^+-consuming enzymes, NAMPT inhibition in cells primarily has an effect on the cytosolic rather than the mitochondrial NAD^+ pool[121]. NAMPT inhibition can

also be toxic to lymphocytes[122], which suggests that the use of NAMPT inhibitors in patients might be limited by immunosuppression. Mild lymphopaenia was observed in early clinical trials of NAMPT inhibitors, but thrombocytopaenia was the dose-limiting toxicity[123]. Limited clinical efficacy has been observed so far with NAMPT inhibitors, although there is ongoing research to develop more potent compounds and define those patients who are most likely to benefit from NAMPT inhibition[124].

Targeting metabolic enzymes that are mutated in cancer. The idea that metabolic alterations are not the same across all cancers is supported by the discovery of a novel metabolic flux that is dictated by mutations in isocitrate dehydrogenase (IDH). Point mutations in isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* that are found in cancer always involve a residue in the active site of only one allele[125–127], and lead to the production of D-2-hydroxyglutarate (2HG) — a metabolite that is only found at very low levels in normal cells[128–130]. Mutations in IDH define a clinically distinct subset of both glioma and leukaemia, which suggests that these mutations contribute to a unique biology within each tumour type[125,127,131,132].

It is not clear how mutations in IDH and the production of 2HG promote cancer, nor is it clear whether existing cancers remain dependent on the abnormal enzyme activity; however, 2HG is an inhibitor of α-ketoglutarate (αKG)-dependent dioxygenases[133–135]. αKG-dependent dioxygenases are involved in an oxygen-sensing pathway that leads to the stabilization of the HIF transcription factor, which controls the expression of many genes that have an important role in cancer progression and metabolic regulation[10,11]. αKG-dependent dioxygenases are also involved in demethylation reactions that affect chromatin structure, and they have pleiotropic effects on global transcription and cellular differentiation[136,137]; this methylation pattern is altered in cells with *IDH* mutations[133,134]. Thus, the development of small molecules that inhibit the production of 2HG by mutated IDH may restore normal αKG-dependent dioxygenase function and normalize both HIF levels and chromatin structure. In addition, because αKG-dependent dioxygenases are influenced by the αKG/succinate ratio, the delivery of αKG analogues may be another way to restore normal dioxygenase activity. These cell-permeable esters of αKG can increase both αKG levels and dioxygenase activity[138]. This latter strategy to increase αKG has shown some success in models of human cancer with abnormal αKG/succinate ratios that are caused by loss-of-function mutations in succinate dehydrogenase or fumarate hydratase[138].

Additional strategies for targeting glutamine metabolism. As discussed above, glutamine is an important nutrient for some cancer cells. Glutamine is the major source of nitrogen for nucleotide and amino acid synthesis, but many cells metabolize glutamine in excess of their nitrogen requirement. Glutamine also has an important role in replenishing intermediates of the TCA cycle that are depleted by biosynthetic reactions[139] (anapleurosis) (FIG. 3). The enzyme glutaminase catalyses the conversion of glutamine to glutamate in a pathway involved in producing αKG. Glutaminase has two major isoforms in mammals, glutaminase 1 (GLS1) and GLS2, and the expression of these enzymes can have opposite effects on cell proliferation[140]. GLS1 is an important downstream effector of MYC and promotes the entry of glutamine into the TCA cycle[49,50], whereas GLS2 is regulated by the tumour suppressor p53 and influences the cellular redox state[141].

These different functions of GLS1 and GLS2 are likely to have key roles in cancer metabolism, and the growth of transformed cells can be selectively inhibited by targeting glutaminase activity[142,143]. Blocking GLS1 activity can prevent the entry of glutamine into cells as a source of 2HG production by mutated IDH1, and can therefore slow the growth of these cells[143]. GLS1 has also been identified as the target of a molecule that blocks cell transformation by RHO GTPases; this molecule can slow the growth of RHO GTPase-transformed fibroblasts and breast cancer cells[142]. However, lymphocytes are also dependent on glutamine metabolism[144], which suggests that immunosuppression may be a side effect of drugs that target glutamine metabolism for cancer therapy.

Targeting other metabolic dependencies in cancer cells. Therapies that target cancer metabolism should attack those metabolic pathways that meet the specific needs of cancer cells. This approach is analogous to targeting nucleic acid metabolism with antimetabolites, but need not be limited to approaches that interfere with DNA replication. Many cancer cells rely on *de novo* fatty acid synthesis to generate new membranes for cell growth, and the enzymes that are directly involved in fatty acid synthesis have been suggested as cancer targets[145,146]. Lipids also have important signalling functions in cells, and chemical genetic screens have identified lipases that release fatty acyl chains from glycerol as therapeutic targets in some cancers[147]. However, it remains to be determined whether targeting lipid synthesis to alter signal transduction or to structurally interfere with cell growth will have a better therapeutic index.

NADPH is the major cofactor carrying electrons for reductive biosynthesis and must constantly be regenerated from $NADP^+$ to maintain reducing conditions in the cell and feed biosynthetic reactions. Targeting the major sites of NADPH production in cancer cells could limit biosynthesis and lead to cellular damage by promoting a more oxidizing intracellular environment[1]. The pentose phosphate pathway is a source of NADPH production and may represent a target for cancer therapy[148]. However, decreased NADPH production via the pentose phosphate pathway is a characteristic of patients with glucose-6-phosphate dehydrogenase deficiency, and this deficiency has not been found to be protective against cancer[149]. Furthermore, some cancers do not have a large pentose phosphate pathway flux[150]. Cells can generate NADPH via other pathways, and malic enzyme — which can be involved in the conversion of glutamine to lactate — has been suggested both as a therapeutic target and a major source of NADPH in glioblastoma cells[139]. Whether there are other targets that are important for NADPH generation remains to be determined.

Lymphopaenia
A clinical term referring to an abnormally low number of lymphocytes in the blood.

Metabolic flux
The rate by which molecules flow through a metabolic pathway. Flux through metabolic pathways is regulated by cells to support cellular processes, and is the composite outcome of: enzyme levels; genetic, allosteric and post-translational regulation of enzymes; and concentrations of metabolites.

Anapleurosis
A term describing the requirement of metabolites to replenish a metabolic cycle when the metabolic intermediates that are involved in the cycle are depleted for use in reactions outside the cycle. The classic example of this process is replenishing those intermediates that are depleted from the tricarboxylic acid cycle for biosynthesis, in order to allow the cycle to continue functioning.

Redox state
A term capturing the reduction–oxidation state of a system. For cells this refers to the propensity of redox couples — such as reduced and oxidized glutathione or NADH and NAD^+ — to be in one state or the other.

Conclusions and future directions

It is clear that instead of a single tumour-specific metabolism, several metabolic programmes exist to support the proliferation of cancer cells. This may explain why current antimetabolite chemotherapies that target DNA synthesis are efficacious in some cancers but not in others, despite the need for all tumour cells to synthesize nucleotides. It may also highlight why a therapeutic window exists for these agents despite the fact that the same pathways are required in normal proliferating cells. A better understanding of how metabolism is altered in specific genetic contexts that lead to cancer will provide an insight into which enzymes — or combination of enzymes — represent promising targets in certain cancers, and this understanding will arise from an analysis of cancer metabolism that extends beyond the levels of expression of various enzymes in a metabolic pathway.

Despite the success observed by targeting enzymes that are involved in nucleotide synthesis, efforts to target other enzymes and pathways involved in cellular metabolism are in their infancy. As targets become better defined, targeting these enzymes could result in the delivery of effective therapies that spare normal tissues but effectively target cancer tissues. Structural information, together with a basic understanding of enzyme properties, already exists for many potential targets in metabolism. Building a conceptual framework to understand metabolic regulation in cancer, however, remains a challenge for the development of successful therapies. Efforts to model human metabolism and select rational target combinations are ongoing[151]. Complementing these models with a more complete understanding of pathway biochemistry in cancer cells will help to determine the best targets for possible intervention. The development of new methods to study tumour metabolism *in vivo* will be crucial. Ultimately, these efforts will determine whether a sufficient therapeutic window exists to spare normal tissues from unwanted toxicity and whether further investigation of the anticancer potential of agents that target these enzymes is warranted.

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Acknowledgements
Thanks to D. Schenkein, L. Whitesell, B. Wolpin, K. Courtney, P. Ward and members of the Vander Heiden Laboratory for helpful discussions and comments on the manuscript. Special thanks to B. Bevis and S. Y. Lunt for advice and help with the generation of the figures. The author acknowledges support from the Burrough's Wellcome Fund, the Smith Family Foundation, the Starr Cancer Consortium, the Damon Runyon Cancer Research Foundation and the US National Institutes of Health.

Competing interests statement
The author declares competing financial interests: see Web version for details.

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