Targeting metabolic transformation for cancer therapy

Daniel A. Tennant, Raúl V. Durán‡ and Eyal Gottlieb**

Abstract | Cancer therapy has long relied on the rapid proliferation of tumour cells for effective treatment. However, the lack of specificity in this approach often leads to undesirable side effects. Many reports have described various 'metabolic transformation' events that enable cancer cells to survive, suggesting that metabolic pathways might be good targets. There are currently several drugs under development or in clinical trials that are based on specifically targeting the altered metabolic pathways of tumours. This Review highlights pathways against which there are already drugs in different stages of development and also discusses additional druggable targets.

Glycolysis
The pathway leading from intracellular glucose directly to pyruvate, resulting in the generation of two moles of pyruvate, ATP and NADH from one mole of glucose.

Glutaminolysis
The process by which glutamine is metabolised to α-ketoglutarate by glutamate.

**Cancer Research UK, The Beatson Institute for Cancer Research, Glasgow, UK.*
‡Growth & Development Biozentrum, University of Basel, Basel, Switzerland.
Correspondence to E.G.
e-mail:
e.gottlieb@beatson.gla.ac.uk
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Tumour cells have a remarkably different metabolism from that of the tissues from which they are derived. They exhibit an altered metabolism that allows them to sustain higher proliferative rates[1,2] and resist some cell death signals, particularly those mediated by increased oxidative damage[3]. However, this means that they are more nutrient hungry and excrete more waste products than normal tissues, resulting in a build-up of metabolites inside the cell and the formation of a more hostile environment outside the cell. In order to divide, a cell needs to both increase its size and replicate its DNA — processes that are hugely metabolically demanding and which require large quantities of proteins, lipids and nucleotides, as well as energy in the form of ATP. This anabolic drive requires cells to increase their uptake of the building blocks for this process, major factors being amino acids and glucose.

The metabolic alterations and adaptations of cancer cells have been extensively studied over the past century (TIMELINE). They create a phenotype that is essential for tumour cell growth and survival, altering the flux along key metabolic pathways, such as glycolysis and glutaminolysis[2]. Some of the mechanisms used by tumours to bring about these changes include the altered expression[4–6], mutation[7–10] and post-translational inactivation of an enzyme[11], or the substitution of a different enzyme isoform[12,13]. On the basis of these observations, the development of treatments that target tumour metabolism is receiving renewed attention, with several potential drugs targeting metabolic pathways currently in clinical trials, and many more at the bench (FIG. 1).

In this Review, we outline new chemotherapeutic approaches that are being used to target the aberrant metabolism that is observed in tumours. We split strategies that target metabolism in two: indirect and direct. Indirect targets include signalling pathways that are activated or repressed in tumours, resulting in aberrant metabolic control, and direct targets consist of the metabolic enzymes themselves. We also discuss the role of altering diet to subvert tumour metabolism.

Indirect inhibition of tumour metabolism

There are several therapeutic strategies being used to target upstream regulators of metabolic pathways. These regulators include hypoxia inducible factor (HIF), PI3K, Akt, mTOR and AMP-activated protein kinase (AMPK). Targeting HIF can prevent metabolic adaptation to hypoxia or the metabolic shift observed in pseudohypoxic tumours, which leads to tumour cell death[14] (BOX 1). The pathway linking insulin and insulin-like signalling to PI3K and Akt is often activated and is known to contribute to the metabolic transformation of cancer[15]. Akt increases glycolytic flux by raising plasma membrane occupancy of glucose transporters[16,17] and altering the expression or localization of glycolytic enzymes such as hexokinase and the family of 6-phosphofructo-2-kinase/fructose-2,6-biphosatases (PFKFBs)[18,19]. Akt also activates mTOR, another master regulator of metabolism, which itself contributes to the anabolic growth of cancer cells[20] (BOX 2). Another regulator of mTOR is AMPK. Under conditions with a low ATP/AMP ratio AMPK is activated, leading to metabolic adaptation (increasing catabolism and decreasing anabolism) partially through the inhibition of mTOR[21]. Interestingly, starvation (caloric restriction) can lead to cancer cell death *in vivo*, a process that is inhibited by the PI3K–Akt pathway[22]. In light of this evidence, it is

At a glance

- The metabolism observed in tumours is different from that of the normal tissues from which the tumours are derived. This altered metabolic phenotype allows cancer cells to accommodate increased metabolic demands and adapt to environmental changes.
- Specific alterations in metabolic pathways may generate opportunities to design new therapeutic approaches.
- Metabolic alterations in cancer can be driven by changes in signalling pathways involving kinases such as PI3K and mTOR, and transcription factors, including hypoxia inducible factor and MYC. These are important targets for cancer therapy in general and cancer metabolism in particular.
- Cancer cells increase their rate of glucose and glutamine metabolism for bioenergetic and anabolic purposes. These important external carbon sources are diverted to generate DNA, proteins and lipids that are required for cancer cell growth.
- Cancer-specific isoforms of enzymes involved in energy metabolism, anabolism and adaptation to low oxygen may be new druggable targets for cancer therapy with potentially improved therapeutic indices compared with current therapy.

clear that targeting these pathways could have important clinical benefits for tumour metabolic transformation and growth.

Insulin-like growth factor receptors (IGFRs), in particular IGF1R, are important signalling molecules that influence, among other things, glucose uptake and cell growth. They function upstream of several signalling pathways, including PI3K and Akt, and are thought to be potential new targets for cancer therapy (TABLE 1; reviewed in REF. 23). PI3K inhibitors were also shown to reverse some of the metabolic phenotypes of cancer, leading to cancer regression[24]. Blocking either IGF1R or PI3K is also likely to have an inhibitory effect on mTOR, although this is yet to be formally shown in cancer cells[15] (BOX 1). However, it is important to mention that the inhibition of IGF1R or the PI3K–Akt signalling cascade can also contribute to tumour regression in a metabolism-independent manner as these pathways are tumorigenic in several different ways, contributing to cell proliferation and survival[25].

The AMPK activator metformin has also been proposed as a potential anticancer drug. This drug is first and foremost used to treat patients with type 2 diabetes, but a study of patients with diabetes found that those treated with metformin were more likely to be cancer free over 8 years than those on other treatment regimes[26]. A further study on diabetic patients with breast cancer as well as studies on breast cancer cells *in vitro* and a mouse model of breast cancer also found an anti-tumour effect of metformin[27–29]. Metformin is currently being tested in Phase I and II clinical trials (TABLE 1).

Targeting nucleotide biosynthesis

Some of the first anticancer drugs targeting tumour metabolism were aimed at inhibiting DNA synthesis (TIMELINE). Known as antimetabolites, these drugs include 5-fluorouracil (5-FU), cytarabine (Ara-C) and methotrexate (FIG. 1; TABLE 1) and have been used as chemotherapeutic agents for several years. Most of these drugs target the final stages in the nucleotide synthetic pathway, leading to nucleotide shortage, incomplete DNA synthesis and cell death[30]. However, owing to their lack of tumour specificity there are few new agents in the clinic that directly target this pathway. Interest is instead being directed towards the pathways that supply intermediates to be used in nucleotide biosynthesis, namely the pentose phosphate pathway (PPP), glutaminolysis and the tricarboxylic acid cycle (TCA cycle) (discussed below; FIG. 1). It is possible that the nucleotide building blocks necessary for normal proliferating cells can be supplied by the exogenous pool of nutrients, such as nucleosides. But owing to poor vascularization and the high proliferative burden of cancer cells, tumours might rely more on endogenous synthesis from glucose and glutamine[31]. Therefore, blocking early stages of nucleotide biosynthesis such as ribose-5-phosphate (R5P) production could provide a better therapeutic window than that shown by previous antimetabolic therapies.

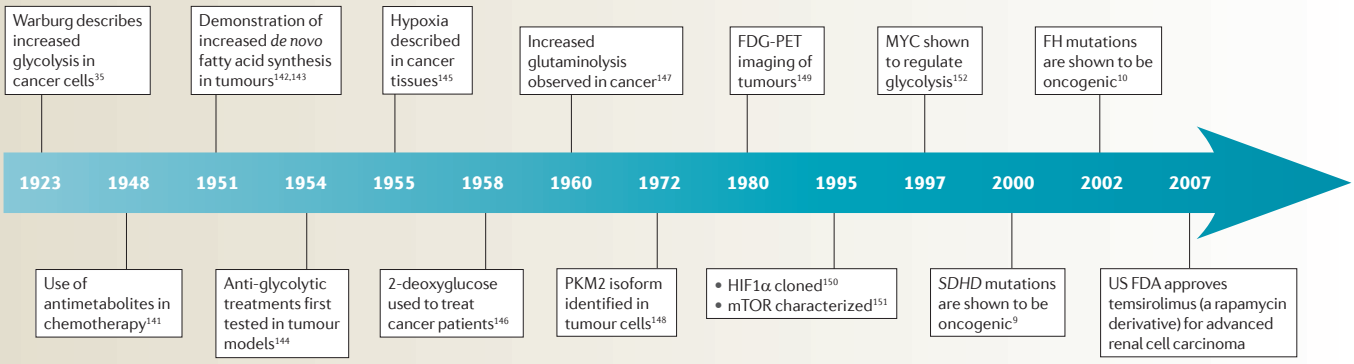
Glycolysis and the PPP

Most of the metabolic pathways used by tumours are ubiquitous in the body and so may not initially seem to be valid drug targets. However, it is the enzyme isoform

Pentose phosphate pathway
A bypass of glycolysis with both biosynthetic and antioxidant outcomes. It can generate NADPH and/or ribose-5-phosphate, which can be used for glutathione reduction and anabolic processes.

Tricarboxylic acid cycle
A set of interconnected pathways in the mitochondrial matrix. It produces reducing equivalents (NADH and $FADH_2$) for the electron transport chain and precursors for amino acid and fatty acid synthesis.

Timeline | **Some important advances in understanding and targeting tumour metabolism**



FDA, Food and Drug Administration; FDG-PET, [18F]-fluorodeoxyglucose positron emission tomography; FH, fumarate hydratase; HIF1α, hypoxia inducible factor 1α; PKM2, pyruvate kinase isozyme M2; SDHD, succinate dehydrogenase complex, subunit D, integral membrane protein.

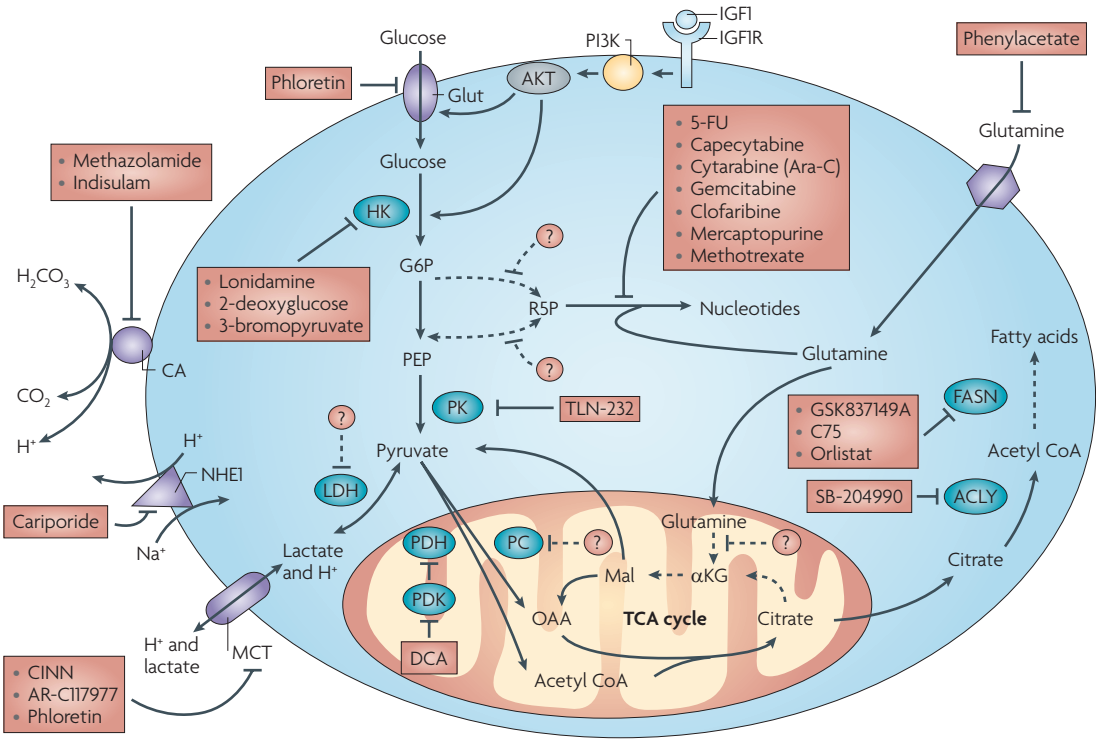


Figure 1 | **Targeting tumour metabolism.** Metabolic pathways and enzymes against which compounds are already in the clinic. Dashed lines indicate possible areas of inhibition for which suitable compounds have not yet been tested. 5-FU, 5-fluorouracil; αKG, α-ketoglutarate; ACLY, ATP citrate lyase; CA, carbonic anhydrase; CINN, α-cyano-4-hydroxycinnamate; DCA, dichloroacetate; FASN, fatty acid synthase; G6P, glucose-6-phosphate; Glut, glucose transporter; HK, hexokinase; IGF1, insulin-like growth factor 1; IGF1R, IGF1 receptor; LDH, lactate dehydrogenase; Mal, malate; MCT, monocarboxylate transporter; NHE1, Na^+/H^+ exchanger 1; OAA, oxaloacetate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PEP, phosphoenol pyruvate; PK, pyruvate kinase; R5P, ribose 5-phosphate; TCA, tricarboxylic acid cycle.

selection or altered activity of a pathway that could allow them to be targeted. One example of this is the metabolism of glucose by tumours.

Glycolysis is the metabolic process that converts glucose to pyruvate, generating two moles of ATP, two moles of NADH and two moles of pyruvate per mole of glucose. The fate of pyruvate depends on many factors among which oxygen availability is one of the most important. In anaerobic conditions pyruvate is reduced to lactate by lactate dehydrogenase. In the presence of oxygen, the mitochondrion can completely oxidize the pyruvate and NADH from glycolysis, resulting in the production of up to a further 36 moles of ATP per mole of glucose using oxidative phosphorylation[32]. Although the glycolytic pathway is ubiquitous in the human body, its physiological roles can change between different cell types and in response to altering environmental cues. For instance, the heart requires large amounts of ATP for contraction, and uses both glycolysis and oxidative phosphorylation to maximize ATP production. Conversely, glycolysis is not used in the liver as a major source of energy but is mostly a source of substrates for biosynthetic processes. In general, proliferating normal cells are thought to have high rates of glycolysis owing to their need for the biosynthetic intermediates that are derived from glucose[33]. Anabolic pathways that branch from glycolysis are responsible for producing some amino acids, as well as both lipid and nucleotide precursors. When flux through these anabolic pathways is increased, glycolytic flux and therefore glucose uptake must increase to maintain normal ATP levels (from both glycolysis and oxidative phosphorylation).

Most tumours seem to use glucose at similar or even higher rates than normal organs. This is particularly evident in images recorded using [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET), in which tumours often appear as PET-positive as some of the most metabolically active organs (such as the brain and heart)[34]. Although they retain their ability to increase glucose uptake under anaerobic conditions (the Pasteur effect), cancer cells show an increased glucose uptake in the presence of oxygen (aerobic glycolysis). However, in contrast to normal tissues, a substantial amount of pyruvate is reduced to lactate instead of being directed into the mitochondrion, a phenotype first described by Warburg[35].

Despite early indications[36], many glycolysis inhibitors (such as 2-deoxyglucose) do not seem to have as significant an effect on tumour growth as monotherapy, but can resensitize tumours to other chemotherapeutic agents (such as paclitaxel[37]), presumably through reducing tumour ATP levels. Agents used in combination with glycolysis inhibitors include both chemotherapy and radiotherapy[37–39], with some glycolytic inhibitors being

Pasteur effect
First used to describe the inhibitory effect of oxygen on yeast fermentation. It is now described as the inhibition of glycolysis by mitochondria-generated ATP that is observed in eukaryotic cells.

used in Phase II and III clinical trials in combination with other agents against breast, ovarian and lung cancers, as well as glioblastoma multiforme[40–43].

The facultative glucose transporter GLUT1 is upregulated in many cancer types by the transcription factor HIF (BOX 1) and by AKT, among others[2]. There are also agents that target glucose transport across the plasma membrane, such as phloretin[44]. This compound has been shown to inhibit leukaemia cell growth[45] and trigger apoptosis in melanoma cells *in vitro*[46], and *in vivo* phloretin was been shown to inhibit xenograft tumour growth[47]. However, to our knowledge there are no GLUT inhibitors in the clinic. Direct inhibitors of the glycolytic pathway are numerous, with compounds targeting almost all of the enzymes in the pathway (FIG. 1; TABLE 1). The first step in glycolysis, the phosphorylation of glucose to form glucose-6-phosphate (G6P), is controlled by hexokinase. This is one of many enzymes in glycolysis that is upregulated by both HIF and MYC, both of which are highly expressed in most tumour types. Two inhibitors of hexokinase, lonidamine and a glucose mimetic, 2-deoxyglucose, are currently in clinical trials in combination with other agents in several different solid tumours[40,41] (TABLE 1). A further inhibitor, 3-bromopyruvate, is not currently in clinical trials but has shown promise in *in vivo* studies[48,49].

G6P is an important intermediate, as it can be diverted from glycolysis into the PPP. This allows for the production of two important molecules: NADPH, necessary for lipid and nucleotide biosynthesis as well as protection against oxidative stress; and R5P, a nucleotide precursor. When R5P is not required by the cell, these carbons can be recycled back into glycolysis through the non-oxidative arm of the PPP. There is, however, an energetic cost to using this pathway. For every three G6P diverted into the PPP, only five each of NADH, ATP and pyruvate are produced, instead of the six of each that are produced if only glycolysis is used. This obvious deficit can be considered a reasonable price to pay for the production of six NADPH. There are currently no inhibitors of the PPP in clinical trials, even though it may be an attractive target. However, transketolase-like protein 1 (TKTL1), an enzyme in the non-oxidative arm of the PPP, is of particular interest. It has been found to be upregulated in several tumour types[50,51], and furthermore, knocking it down was shown to reduce the proliferation of tumour cells[52], as well as decrease lactate production and resensitize cells to reactive oxygen species (ROS)-generating compounds[53]. Inhibition of the PPP is not only likely to mimic the effect of antimetabolites, but it could also alter the cellular redox balance and inhibit lipid synthesis (owing to the decrease in NADPH levels).

A second phosphate group is added to fructose-6-phosphate by 6-phosphofructo-1-kinase (PFK1; also known as PFKM). This enzyme, which is under considerable allosteric regulation, is a rate-limiting step in glycolysis and highly activated in tumour cells. One potent allosteric activator of PFK1 is fructose-2,6-bisphosphate (F2,6BP), the product of the PFKFBs. A recent study using a small molecule inhibitor (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one) against a member of the PFKFB family that is upregulated in tumours, PFKFB3, demonstrated the importance of PFK1 regulation in the proliferation of cancer cells. Inhibition of PFKBP3 decreased F2,6BP levels and therefore in turn decreased PFK1 activity and glycolytic flux, with cytostatic effects[54]. This suggests that PFKFB3 is a valid target for chemotherapy, although as with many other glycolytic inhibitors it may not be suitably efficacious as monotherapy.

The final enzyme in glycolysis, converting phosphoenolpyruvate (PEP) into pyruvate, is pyruvate kinase (PK). This is an important energy-producing step in glycolysis and is highly regulated — both by isoform selection (the alternatively spliced form PKM2 replaces PKM1 in all highly proliferative cells, including tumour cells[55]) and by allosteric regulation. The upstream glycolytic intermediate, fructose-1,6-bisphosphate (F1,6BP), which is the product of PFK1, binds to and allosterically activates PKM2 but not PKM1 (REF. 56). This is an important feedforward mechanism, linking these two rate-limiting steps in glycolysis and thereby enables coordinated glycolytic flux in PKM2-expressing cells. An inhibitor to PK (TLN-232) is currently in Phase II clinical trials. However, data from Christofk *et al.*[12] highlight an interesting, and potentially important difference between PK that is expressed by tumour cells and that of normal tissue. The PKM1 isoform, which seems to be expressed only in normal tissues, was shown to be incompatible with tumour growth. If an inhibitor could be designed that is specific for the tumour-expressed PKM2 isoform this might specifically inhibit glycolysis in tumour cells, killing them by energy deficit.

Box 1 | **Hypoxia, HIF and cancer therapy**

Hypoxia-inducible factors (HIFs) are transcription factor complexes comprised of a HIFα and HIFβ subunit and function as an integral part of the hypoxia response, allowing both the organism and its constituent cells to survive periods of low oxygen supply. Although an important positive factor during development and physiological stress, HIFs have been shown to accelerate tumorigenesis and promote the development of a more malignant phenotype. HIF activity is high in most, if not all, tumours either owing to hypoxia or conditions leading to HIF stabilization under normoxia (pseudohypoxia)[116].

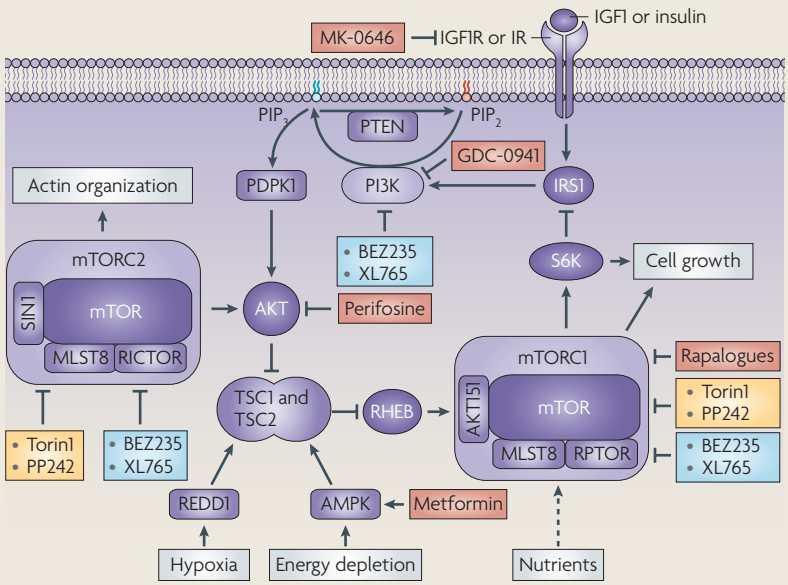
The HIF transcription factors are therefore seen as important targets against which agents are being generated. Currently, there are several agents targeting HIF or its downstream effectors at various stages of validation, and some are showing very good anti-tumour effects (TABLE 1). In particular, PX-478 reduces HIF1α levels both *in vitro* and *in vivo*[117–119], and exhibits potent anti-tumour effects[118,120,121]. More recently, the drug acriflavine has been shown to block the dimerization of HIF1α and HIF1β subunits, reducing xenograft tumour growth and vascularization[122]. Agents that decrease the levels of reactive oxygen species (ROS), such as superoxide dismutase mimetics, have also been shown to reduce HIF[123]. There have also been agents designed to target some of the downstream effectors of HIF: bevacizumab (targeting vascular endothelial growth factor) and carbonic anhydrase IX (see the text).

Hypoxia itself can also be used to specifically target chemotherapies to hypoxic regions of tumours. The hypoxic environment is strongly reducing, allowing the use of bioreductive drugs, which are showing encouraging signs of reversing hypoxia-mediated resistance to both radiotherapy and chemotherapy[124]. Other ways of targeting hypoxia include the use of cytotoxic expression vectors downstream of hypoxia responsive elements, and modified anaerobic bacterial spores expressing death receptor proteins, or enzymes such as cytosine deaminase (which will convert exogenous 5-fluorocytosine to the genotoxic 5-fluorouracil[125] and is reviewed in REFS 126,127).

Box 2 | **mTOR and cancer therapy**

mTOR is regulated by the PI3K–PTEN–AKT pathway (see the Figure), which is overactivated in many types of both sporadic and hereditary cancer[128]. Therefore, the involvement of mTOR in tumorigenesis has been extensively investigated during the past decade[129]. Several mTOR inhibitors (rapamycin and derivatives; TABLE 1) have been studied in preclinical and clinical trials, including renal cell carcinoma[130], mantle cell lymphoma[131], hepatocellular cancer[132], glioblastoma multiforme[133] and breast cancer[134]. Despite the fact that mTOR is believed to have a crucial role in tumour development, the clinical outcome in trials using mTOR inhibitors as monotherapy has shown only modest results.

So far, renal cell carcinoma has been the cancer with the most promising results when using an mTOR-targeted therapy[130]. As a consequence, the rapamycin derivatives temsirolimus (Wyeth Pharmaceuticals) and everolimus (Novartis) have been approved by the US Food and Drug Administration for the treatment of patients with renal cell carcinoma. However, even in this tumour type mTOR inhibition gives only moderate effects on patient survival[130,135]. This limited success can be explained by the negative feedback loop downstream of mTOR and by the specificity of rapamycin for mTOR complex 1 (mTORC1)[136]. As a consequence, rapamycin treatment leads to the overactivation of the PI3K–PTEN–AKT pathway[137]. New approaches should focus on inhibiting both mTORC1 and mTORC2 complexes[129], and on treatments that combine mTOR inhibitors with other signalling pathway inhibitors (particularly the PI3K–PTEN–AKT pathway)[138]. However, it is still unclear whether mTOR inhibition could have any additive effect to an AKT-targeted therapy. A new generation of drugs has recently been developed that can target both mTOR complexes and the PI3K pathway, including BEZ235 (Novartis)[139] and XL765 (Exelixis)[140]. These are already in Phase I clinical trials (FIG. 1; TABLE 1). Further investigation is still required to establish the role and the limits of mTOR in cancer therapies.



AKT1S1, AKT1 substrate 1; AMPK, AMP-activated protein kinase; IGF, insulin-like growth factor; IGF1R, IGF1 receptor; IR, insulin receptor; IRS1, IR substrate 1; MLST8, target of rapamycin complex subunit LST8; PDPK1, 3-phosphoinositide-dependent protein kinase 1; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; RPTOR, regulatory-associated protein of mTOR; RHEB, Ras homologue enriched in brain; RICTOR, RPTOR-independent companion of mTOR, complex 2; SIN1, stress-activated map kinase-interacting protein 1 (also known as MAPKAP1); TSC, tuberous sclerosis protein. Inhibitors and activators are in red. Inhibitors for both mTORC1 and mTORC2 are highlighted in yellow. Dual inhibitors for PI3K and mTORC1 and mTORC2 are highlighted in blue.

A further complexity in the regulation of PKM2 highlights another possible approach for targeting this isoform. Christofk *et al.*[57] demonstrated that phosphotyrosine residues can inhibit the feedforward effect of F1,6BP by binding to the same allosteric regulatory site. This allosteric effect was found to be more prevalent in tumour cells than normal cells, resulting in a perhaps counterintuitive decrease in flux through this enzyme. Assuming that the interaction between phosphotyrosine residues and PKM2 contributes to the metabolic phenotype of tumour cells, interfering with it may well inhibit tumour cell growth, even though it should increase PKM2 activity and the production of pyruvate. This strategy is also likely to be specific for tumour cells as the phosphotyrosine-containing proteins do not interact with the PKM1 isoform.

The inhibition of glycolysis seems to be tolerated remarkably well in normal tissues, but could have a disappointingly limited effect on tumorigenesis as monotherapy[37]. One possible explanation for this might be the strong increase in glutamine use (through glutaminolysis) in tumours, and therefore the ability of tumours with functional mitochondrial respiration to produce ATP by oxidative phosphorylation (FIG. 1). This would, in turn, predict that anti-glycolysis treatment could lead to increased glutaminolysis and perhaps muscle wastage (cachexia) owing to increased tumour demand for muscle-derived glutamine in patients under chronic anti-glycolysis dosing regimens[58].

The fate of pyruvate

Most pyruvate is produced from the oxidation of glucose using glycolysis. If glucose is fully directed into glycolysis, two moles of pyruvate are produced for every mole of glucose consumed. In normal tissues, most of this pyruvate is directed into the mitochondrion to be converted into acetyl-CoA by the action of pyruvate dehydrogenase (PDH; FIG. 1) or transaminated to form alanine. In an anaerobic environment, pyruvate is redirected into lactate production owing to the increase in the cytosolic NADH/NAD^+ ratio as a consequence of the decreased NADH oxidation by the mitochondria. The reduction of pyruvate to lactate is also facilitated by the increased activity of two key enzymes: pyruvate dehydrogenase kinase 1 (PDK1), which blocks PDH activity, and lactate dehydrogenase A (LDHA), which converts cytosolic pyruvate to lactate[4,11,59]. In tumour cells, this effect is observed even under aerobic conditions (the Warburg effect). The decrease in the rate of pyruvate entering the TCA cycle and the concurrent increase in lactate production is vital for the growth and survival of tumours (FIG. 1), as knocking down LDHA or inhibiting PDK1 (using RNAi or dichloroacetate (DCA)) leads to a reduction in tumour growth in xenograft models[60–63]. DCA showed remarkable anticancer effects in preclinical studies and is already in clinical trials (TABLE 1). The outcomes of these trials will allow for a much anticipated thorough evaluation of this compound[64]. At present, there are no therapies that specifically target LDHA, but this could be a highly attractive target if predicted side effects on muscle metabolism, for example, can be avoided.

The cell exports lactate with H^+ using monocarboxylate transporter 4 (MCT4), allowing the cell to preserve normal cellular pH (for efficient cellular

Table 1 | **Compounds targeting tumour metabolism currently in clinical studies**

Compound	Target	Effect	Stage as anti-tumour therapy	Tumour types targeted	Study number(s)
2-deoxyglucose	Hexokinase	Inhibits glycolysis	Phase I/II	Advanced solid tumours (e.g. lung, breast, prostate and gastric)	• NCT00633087 • NCT00096707 • NCT00247403
Lonidamine	Hexokinase	Inhibits glycolysis	Phase III	Benign prostatic hyperplasia	• NCT00435448 • NCT00237536
3-bromopyruvate	Hexokinase	Inhibits glycolysis	Preclinical	N/A	N/A
TLN-232	Pyruvate kinase	Inhibits glycolysis	Phase II	Metastatic melanoma and renal cell carcinoma	NCT00735332
Dichloroacetate	PDK1	Reactivates PDH	Phase I/II	Metastatic solid tumours, glioma and GBM	• NCT00540176 • NCT00566410 • NCT00703859
Phenylacetate	Glutamine	Reduces plasma glutamine levels	Phase II	Brain tumours (e.g. glioma, astrocytoma and medulloblastoma)	• NCT00003241 • NCT00006450 • NCT00001565
Asparaginase and Pegasparaginase	Asparagine	Reduces plasma asparagine levels	Phase II/III	ALL, TCL and BCL	• NCT00400946 • NCT00004034 • NCT00165178 • Others
Arginine deiminase	Arginine	Reduces plasma arginine levels	Phase I/II	Metastatic melanoma and hepatocellular carcinoma	• NCT00450372 • NCT00029900 • NCT00056992
Acetazolamide, Indisulam and other sulfonamides	Carbonic anhydrases	pH regulation	Phase II	Solid tumours (e.g. pancreatic, lung, melanoma and metastatic breast)	• NCT00060567 • NCT00165594 • NCT00165880 • Others
Cariporide	NHE1	pH regulation	Preclinical	N/A	N/A
SB-204990	ATP-citrate lyase	Inhibits fatty acid synthesis	Preclinical	N/A	N/A
Orlistat, GSK837149A and C75	FASN	Inhibits fatty acid synthesis	Preclinical	N/A	N/A
Temsirolimus and Everolimus	mTORC1	Inhibits mTORC1	US FDA approved	Solid tumours (both metastatic and non-metastatic)	>100
Ridaforolimus and other rapalogues	mTORC1	Inhibits mTORC1	Phase I/II	Solid tumours (e.g. pancreatic, endometrial and glioblastoma) and lymphoma	• NCT00110188 • NCT00086125 • NCT00122343 • Others

metabolic processes), but with the creation of an acidic tumour environment. Protons can also be exported using the Na^+/H^+ exchangers (NHEs). There are small molecule inhibitors targeting NHEs, such as cariporide, which are in clinical trials as cardioprotective agents. However, despite some *in vitro* studies showing decreased tumour cell invasion and proliferation[65,66] these are not currently being tested as anti-tumour agents in the clinic (TABLE 1). Interestingly, a recent report suggested that there is a swapping of lactate between oxygenated and hypoxic cells in tumours, much like that found in muscle[67]. Hypoxic cells produce large amounts of lactate using LDHA and export it using MCT4. The oxygenated cells can then remove lactate from the extracellular fluid using MCT1, and convert it back to pyruvate for further oxidation, perhaps using the LDHB isoform, conserving glucose for use by the hypoxic cells. It was shown that inhibition of MCT1, either pharmacologically or using RNAi, results in the inhibition of xenograft tumour growth and resensitization of tumour cells to radiation[67]. However, strong reservations to this possible therapeutic route have already been voiced, as the use of lactate as an energy source is important in some metabolically active tissues such as muscle and brain[68]. To design therapies and treatment regimens directed towards such ubiquitous transporters, such as the MCTs or NHEs, it is likely that the effect must be either extremely rapid so that normal tissues are relatively unaffected, or they must be used in combination with other treatments so that the doses required are below those that will adversely affect other tissues.

Targeting tumour acidification may have more than one benefit: in the short term it will inhibit glycolytic energy production, but in the longer term it may well inhibit tumour cell invasion[69,70]. Extracellular acidification has been shown to increase the motility of cells both *in vitro* and *in vivo*, and lactate itself has been shown to directly increase cell motility[71–73]. Indeed, neutralizing extra-tumoural pH using bicarbonate was

Warburg effect
Originally described as the large increase in aerobic production of lactate by cancer cells and suggested to be a consequence of defects in oxidative phosphorylation. Today, it is defined as an increase in 'aerobic glycolysis' that is not necessarily correlated with permanent mitochondrial dysfunction.

Table 1 (cont.) | **Compounds targeting tumour metabolism currently in clinical studies**

Compound	Target	Effect	Stage as anti-tumour therapy	Tumour types targeted	Study number(s)
Torin1 and PP242	mTORC1 and mTORC2	Inhibits mTORC1 and mTORC2	Preclinical	N/A	N/A
PX-478	HIF1α	Inhibits HIF signalling	Phase I	Advanced solid tumours and lymphoma	NCT00522652
Acriflavine	HIF1α	Inhibits HIF signalling	Preclinical	N/A	N/A
Tirapazamine and other bioreductive compounds	Hypoxia	Resensitizes cells to other treatments	Phase III	Solid tumours (e.g. cervical, SCLC and NSCLC)	• NCT00033410 • NCT00098995 • NCT00017459
Bevacizumab and related compounds	Hypoxia, VEGF and VEGFR	Blocks angiogenesis	US FDA approved	Solid tumours (e.g. malignant glioma, NSCLC, ovarian and colorectal)	>100
MK-0646, BIIB022, AVE1642 and others	IGF1R	Blocks IGF signalling	Phase I/II	Solid tumours (e.g. NSCLC, pancreatic, hepatocellular carcinoma and metastatic breast)	• NCT00799240 • NCT00555724 • NCT00791544 • Others
BEZ235, XL765, SF1126 and BGT226	PI3K and mTOR	Inhibits signalling from PI3K and mTORC1 and mTORC2	Phase I/II	Advanced solid tumours (e.g. malignant glioma and NSCLC)	• NCT00485719 • NCT00777699 • NCT00704080 • NCT00907205 • NCT00600275 • Others
GDC-0941 and PX866	PI3K	Inhibits PI3K signalling	Phase I	Advanced solid tumours (metastatic breast and non-Hodgkin's lymphoma)	• NCT00876109 • NCT00726583
Perifosine and GSK690693	AKT	Inhibits AKT	Phase I/II	Solid tumours (e.g. renal cancer and NSCLC) and lymphoma	• NCT00399789 • NCT00399152 • NCT00493818
Metformin	AMPK and Complex I (mitochondrial)	Activates AMPK	Phase I/II	Solid tumours and lymphoma	• NCT00659568 • NCT00881725 • NCT00984490 • NCT00909506 • Others
Antimetabolites (e.g. 5-FU, cytarabine and methotrexate)	Nucleotide biosynthetic pathway	Inhibits cell proliferation	US FDA approved	Many tumour types	>100

5-FU, 5-fluorouracil; ALL, acute lymphoblastic leukaemia; AMPK, AMP-activated protein kinase; BCL, B cell lymphoma; FASN, fatty acid synthase; FDA, Food and Drug Administration; GBM, glioblastoma multiforme; HIF, hypoxia inducible factor; IGF, insulin-like growth factor; IGF1R, IGF1 receptor; mTORC, mTOR complex; N/A, not applicable; NHE1, Na^+/H^+ exchanger 1; NSCLC, non-small-cell lung cancer ; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; SCLC, small-cell lung cancer; TCL, T cell lymphoma; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

recently shown to decrease the metastatic potential of tumour cells in both orthotopic (mammary fat pad) and a metastatic (intra-splenic injection) tumour models[74]. One strategy for directly targeting the acidification of the tumour is to inhibit members of the carbonic anhydrase (CA) family, and specifically the hypoxia-associated protein, CAIX[75]. There are already many compounds that have been shown to inhibit the CAs, with several having the greatest efficacy against CAIX (over other isoenzymes)[76–79]. One such inhibitor, Indisulam, is currently in Phase II clinical trials treating various tumour types, including stage IV melanoma, renal clear cell carcinoma and metastatic breast cancer (TABLE 1).

Targeting amino acid metabolism

It is rapidly becoming clear that amino acid metabolic pathways could also be chemotherapeutic targets. Tumours seem to require high levels of exogenous essential and non-essential amino acids. The amino acid found at highest concentrations in the plasma is glutamine[80]. It has several uses in the tumour cell: as well as being a substrate for protein synthesis, its amine groups can be used to generate most of the non-essential amino acids (through transamination), it can replenish TCA cycle metabolite levels (through anapleurosis) to enable their use in anabolic processes, and is an essential starting point for nucleotide biosynthesis (FIG. 1). Tumour cells use large amounts of glutamine, often leading to the depletion of glutamine in the plasma[81]. Therapies that further decrease glutamine concentrations may well rapidly induce tumour regression, owing to its importance as an energetic substrate. Phenylacetate is a drug that reduces the biological availability of glutamine in the blood. It achieves this by condensing with the γ-amino group of glutamine resulting in its excretion in urine, and seems not to be toxic in humans[82–84] (TABLE 1). Phenylacetate inhibits the proliferation of tumour cells and promotes their differentiation[82,85,86]: a phenotype that is usually associated with less aggressive tumours. However, the removal of glutamine directly from the plasma may also increase the rate at which the body cannibalises its own muscles (cachexia)[58].

Anapleurosis
From the Greek 'ana' meaning 'up' and 'plerotikos' meaning 'to fill', this term describes the replenishment of TCA cycle intermediates.

Glutaminolysis is the stepwise conversion of glutamine into glutamate then α-ketoglutarate (FIG. 1). The first enzyme in this pathway, glutaminase, converts glutamine to glutamate, and has been reported to have increased activity in several tumour types, and is often upregulated in MYC-transformed cells[87], highlighting it as a potential chemotherapeutic target. However, although various anti-glutaminolysis compounds have been developed, they were found to be toxic or raised an immune reaction[82,88,89]. The recent renaissance of interest in the glutaminolytic pathway has led to the re-evaluation of this as a potential clinical target. If more specific inhibitors of this enzyme were to be designed, they may well avoid the side effects observed in the past, and are likely to be important in treating glutamine-dependent tumours.

Interestingly, glutamine may not be the only amino acid the plasma levels of which are important for tumour growth. Asparaginase, the enzyme that converts asparagine to aspartate and ammonia, has been used to reduce plasma levels of asparagine in patients as a treatment for childhood acute lymphoblastic leukaemia (ALL) for several years[90]. Asparagine is not usually an essential amino acid in humans, owing to the presence of the asparagine-synthesizing enzyme, asparagine synthetase (ASNS). However, certain tumour types, including some types of leukaemia, have little ASNS activity and so require asparagine uptake from the plasma to survive[91,92]. There are currently various studies being carried out using asparaginase (TABLE 1), and there are new data indicating that this enzyme may also decrease plasma glutamine levels[93]. One side effect of asparaginase treatment is the potential for the development of acute hypersensitivity, including anaphalaxis[94]. It is therefore likely that although this approach shows good proof of principle, other modulators of this pathway might have to be developed. A further enzyme, arginine deiminase, has more recently been shown to have anti-tumour effects[95–97]. When administered to patients, this enzyme converts arginine to citrulline and ammonia, resulting in the depletion of arginine in the plasma. Although in normal tissues arginine is not an essential amino acid, at least two tumour types are auxotrophic for (require exogenous supply of) arginine: hepatocellular carcinoma (HCC) and melanoma[96]. Both of these tumour types do not express argininosuccinate synthetase 1 (ASS1), which is vital for endogenous arginine synthesis, and therefore depletion of plasma levels in patients adversely affects the growth of HCC or melanoma tumours[97,98]. Arginine deiminase is in Phase I and II clinical trials (TABLE 1).

It seems that we are only just scratching the surface in our understanding of the potential importance of limiting amino acid availability for tumours. It is highly likely that other tumour types are also auxotrophic for different amino acids, and it is only by thorough metabolomic screening that we will be able to identify many of these pathways.

Lipid metabolism

Fatty acid synthesis was first shown to be increased in tumours as early as 1951 (TIMELINE). Endogenous fatty acids are synthesized from citrate, which is derived from the TCA cycle from the condensation of oxaloacetate and acetyl-CoA, and large amounts of NADPH, which can be produced by both glutaminolysis and the PPP. Once exported from the mitochondrion, citrate is converted to cytosolic acetyl-CoA by the action of ATP citrate lyase (ACLY). SB-204990 has been shown to inhibit ACLY (TABLE 1), and was also reported to limit both tumour cell proliferation and survival *in vitro*[99]. Acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA, the substrate for fatty acid synthase (FASN). FASN is a multifunctional protein which, over multiple steps, converts malonyl-CoA to palmitate. Many tumours express high levels of FASN, including breast, colorectal and endometrial cancers[5,6,100]. Several FASN inhibitors are being used in *in vitro* and xenograft studies, including C75, orlistat and GSK837149A[101–104]. In these systems, FASN seems to be an excellent target, killing tumour cells directly, as well as sensitizing them to other therapies such as 5-FU and trastuzumab[105–108]. It has been suggested that the increase in malonyl-CoA, the substrate for FASN, is important for FASN inhibition[106]. It is therefore possible that it is the action of malonyl-CoA on another pathway that exerts its anti-tumour effect. One possible negative aspect of anti-FASN therapy might be its effect on food intake and body weight. When C75 was infused into rodent brains, they exhibited decreased eating (hypophagia) and consequent weight loss[109], thought to be through the inhibition of carnitine palmotoyltransferase 1A (CPT1A) in the hypothalamus. Therefore, although current data suggest that FASN may well be a good clinical target for anti-tumour therapies, the effect of its inhibition on the central nervous system should be monitored.

Altering diet to reduce cancer growth

A further strategy targeting energy generation in tumours is the use of the ketogenic diet: a low-carbohydrate and high-fat (specifically medium-chain triglyceride) diet. In the past this has been used to treat children with seizures, but has more recently been suggested as a potential anti-tumour therapy. The diet relies on the consumption of food that does not increase plasma glucose levels, but produces ketone bodies that can be used as a carbon source for energy production. These bypass glycolysis and are metabolised by mitochondria in the presence of oxygen. Although normal cells can adapt to using ketone bodies to produce ATP, tumour cells that rely on high glycolytic flux would not be expected to survive on this alternative fuel source. Findings have been mixed, with several studies reporting decreased tumour growth, both in animal models and patients, and even positive results in cachexic patients[110–114]. However, another study reported no inhibition of tumour development[115]. Although this change in diet is not likely to be a monotherapy, it may well prove to be a useful addition to other antimetabolic therapies.

Conclusions

The extent to which metabolism plays a part in tumorigenesis should not be underestimated and drugs that can selectively target the phenotype of the tumour and

tumour microenvironment are likely to at least delay if not halt tumour progression. The basis of several mechanisms of tumour resistance to both radiotherapy and chemotherapy is in the aberrant metabolism of a tumour, and so the reactivation of a more 'normal' metabolism could revert tumours to being sensitive to these agents. Cell metabolism is inextricably linked to its differentiated state, so it follows that if we can reverse the metabolism of a de-differentiated, aggressive tumour it may well be forced into a more quiescent state and therefore become more amenable to other interventions. As with most other chemotherapies, it is the ability of a drug to distinguish between tumour cells and the proliferating normal cells in the body that often determines its therapeutic index. This is still likely to be an issue with therapies targeting tumour metabolism, but by targeting tumour-specific enzyme isoforms or by supressing the activity of an enzyme or the concentration of a substrate instead of completely abolishing it, it may be possible to more selectively attack tumour cells. However, one must exercise caution when basing anti-tumour therapy design on established textbook data. Such studies are mostly based on one of several different metabolically active organs, most of which are not tumours. We require more analytical work to understand which pathways are activated in different tumour types, so that we can more efficiently identify targets that are efficacious and specific for tumours with minimal toxicity for normal organs.

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This is the first paper to show that MYC in tumours can also impinge on glycolytic control.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
National Cancer Institute Drug Dictionary:
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5-fluorouracil | BEZ235 | cytarabine | DCA | everolimus | metformin | methotrexate | paclitaxel | PX-478 | rapamycin | temsirolimus | trastuzumab | TLN-232 | XL765
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ACLY | asparaginase | ASNS | ASS1 | CAIX | CPT1A | FASN | GLUT1 | HIF1α | HIF1β | IGF1R | LDHA | mTOR | MCT1 | MCT4 | MYC | PDK1 | PFK1 | PFKFB3 | PKM2 | TKTL1

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