Targeting cancer metabolism: a therapeutic window opens

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Preface

Genetic driver events in cancer activate signaling pathways that alter cell metabolism. Clinical evidence has linked metabolism with cancer outcomes. Together, this has raised interest in targeting metabolic enzymes for cancer therapy, but also concerns that these therapies would have unacceptable effects on normal cells. However, some of the first cancer therapies target the specific metabolic needs of cancer cells and remain effective agents used in the clinic today. Research to understand how changes in cell metabolism promote tumor growth has accelerated in recent years. This has refocused efforts on targeting metabolic dependencies of cancer cells, an approach with the potential to have a major impact on patient care.

Proliferating cancer cells exhibit significantly different metabolic requirements than 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 (Figure 1a). Metabolic pathways must therefore be rewired in such a way that balances biosynthetic processes with sufficient ATP production to support growth and survival. Because 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, finding a therapeutic window between proliferating cancer cells and proliferating normal cells remains a major challenge to developing successful cancer therapies targeting metabolic pathways.

Many cancer cells, unlike their normal counterparts, metabolize glucose by aerobic glycolysis[1, 4, 5]. This phenomenon, known as the Warburg effect, is characterized by increased glycolysis and high lactate production regardless of oxygen availability. Aerobic glycolysis is often accompanied by increased glucose uptake, and this phenomenon may be visualized in patient tumors using ^{18}F-deoxyglucose positron emission tomography (FDG-PET) scanning. FDG-PET scanning is used clinically as a staging tool for diverse cancer types, and experimental PET tracers can distinguish cancer 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-labeled amino acid analogs has also 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 human cancers even among tumors arising from the same tissue[6-9]. Why some cancers exhibit increased labeling with these tracers is not understood, however these findings suggest that tumors exhibit heterogeneous metabolic alterations that extend beyond the Warburg effect (Figure 1b). Nevertheless, all cancer cells must ultimately direct available nutrients into the synthesis of new biomass while maintaining adequate ATP levels for cell survival. Therefore, it is likely these phenotypic differences are manifestations of various metabolic solutions individual cancers use to support proliferation.

At least some of the metabolic heterogeneity observed in tumors is influenced by the microenvironment[5]. Gradients of nutrients, oxygen, and pH can result from abnormal tumor vasculature. Glucose, amino acids and lipids provide the substrates available to supply metabolic pathways, and thus metabolism will change based on the concentrations of these metabolites available to cells. In addition, cells have signaling mechanisms linked to growth control pathways that sense conditions such as amino acid availability and oxygen levels and that influence metabolism[5, 10-12]. Genetic alterations associated with cancer often occur in these same signaling pathways, suggesting that both environmental and genetic factors influence the metabolic heterogeneity present across tumors[5].

Despite a deep understanding of metabolic regulation built upon 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 further understanding of precisely how cells regulate nutrient flux into pathways required for biosynthesis in specific genetic contexts. Understanding

tumor cell metabolism requires the use of methods to assess metabolite flux and pathway regulation that are not often employed in cancer drug discovery. However, akin to how antibiotics target the biosynthetic processes unique to microorganisms, selective targeting of the biosynthetic processes of cancer cells holds tremendous promise as a strategy to improve 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 to consider new targets in metabolism. We also discuss emerging evidence involving specific metabolic enzyme targets, and how they might be used to limit cell proliferation. To date only a handful of molecules targeting metabolic pathways have been tested as cancer therapy. However a growing body of evidence supports the notion that altered metabolism is a key consequence of important genetic drivers of cancer, 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, hyperglycemia, and insulin resistance are all associated with an increased risk of developing cancer and worse outcomes among 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 insulin and insulin-like growth factor levels have been linked with cancer progression, suggesting obesity and insulin resistance promote cancer at least in part by activating signaling pathways that drive cell growth[15]. These same signaling pathways also drive nutrient uptake into cells and regulate enzymes in glycolysis, implying that hormonal changes can have important indirect effects on cancer cell metabolism[16]. Furthermore, elevated glucose levels alone may promote increased glucose uptake in some cells, and lower circulating glucose levels are associated with better cancer treatment outcomes[19-22].

As a result, anti-diabetic drugs are being explored for anti-tumor activity, and retrospective clinical studies have shown a reduction in cancer-related mortality for diabetic patients taking metformin[23, 24]. This effect appears to be independent of blood glucose, as diabetic patients who control glucose levels by other means do not derive the same benefit as individuals taking metformin[24]. Metformin is widely used for the treatment of type II 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 resulting in lower blood glucose levels and improved insulin sensitivity[27]. Because metformin lowers insulin levels, it is controversial whether metformin benefits cancer patients by directly acting on the tumor, or by indirectly decreasing levels of insulin-related growth factors. Other anti-diabetic therapies that act by raising insulin levels may therefore in fact lead to worse outcomes. Dietary restriction, which has been known to prolong survival in cancer

models, has no effect on tumors that proliferate in the absence of insulin-like growth factor signaling[28]. These findings are consistent with metformin providing an indirect benefit to patients with high circulating insulin-related growth factors. However, high doses of metformin are toxic to cancer stem cells[29], and women taking metformin have an increased tumor response to neoadjuvant chemotherapy for breast cancer that may extend to non-diabetics[30]. LKB1, a kinase important for AMPK activation in response to metformin[27], is frequently lost in human cancers[5]. Thus, metformin use to induce energy stress may be particularly beneficial for treating LKB1-deficient tumors because these cells are unable to activate AMPK and cope with this stress[31]. Planned adjuvant trials of metformin in breast cancer patients will provide additional insight. It is also possible that metformin could be used as chemoprevention in patients with a high risk of developing cancer[32, 33], although the best strategy to identify individuals to include in such trials has yet to be determined[34].

Regardless of whether the benefit observed with metformin involves a direct effect on cell metabolism, blocking the signals that link whole body metabolism to cellular metabolism presents therapeutic opportunities. Antibodies and small molecule kinase inhibitors directed against the insulin-like growth factor receptor (IGFR) have been well tolerated by patients[35]. Early studies with these agents have focused on sarcomas based on preclinical evidence suggesting these tumors are dependent on IGFR signaling. In fact, some rare sarcoma patients develop tumor-associated hypoglycemia related to increased production of an IGF isoform, and dramatic anecdotal responses have been reported in these individuals[36].

Unfortunately, overall these agents have demonstrated limited efficacy in trials suggesting that their clinical utility has yet to be determined[35]. Further efforts to identify those tumors with altered metabolism dependent on IGFR-signaling may allow selection of patients to benefit from these therapies. Insulin-like growth factors are thought to increase tumor growth by activating the phosphoinositide-3-kinase (PI3K) signal transduction pathway, which influences metabolic pathways as one of many downstream effectors of this signaling pathway[4, 37]. In addition, mTOR (mammalian target of rapamycin), a major effector downstream of PI3K is regulated by nutrient availability[12]. mTOR activation stimulates a metabolic program to promote cell growth[38], mTOR inhibitors are increasingly 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 tumor metabolism may define mechanisms of resistance to these agents or identify synergistic targets in metabolism that might convert mTOR inhibitors from cytostatic to cytotoxic agents and increase their efficacy in patients.

Targeting metabolism could improve existing approaches

Many genetic alterations known to promote cancer lead to a single converging metabolic phenotype characterized by enhanced cell autonomous nutrient uptake and metabolic pathway reorganization to support biosynthesis[4, 5, 41]. Growth signaling pathways activated in cancer promote these metabolic changes, and compounds that target signal transduction pathways are available in the clinic. Despite significant success with these agents in select cancers[42], for many common

malignancies challenges remain to identify which patients are likely to respond to these drugs. Interestingly, a decrease in glucose uptake as measured by FDG-PET scan has been predictive of response to compounds targeting the PI3K pathway in animal models[43] and kinase inhibitors in patients[44]. These findings support the hypothesis that a major metabolic consequence of inappropriate PI3K or tyrosine kinase activation is promoting nutrient uptake. There is also evidence that increased nutrient uptake is a critical effect of oncogenic *RAS* mutations[45], and decreased nutrient uptake can predict therapy response in *KRAS*-driven lung cancer[43]. This underscores the potential value of FDG-PET scanning as an early predictor of response to molecules targeting signaling pathways.

Despite creative approaches, effective agents targeting many of the common driver mutations in cancer are not available. For instance, mutations in *RAS* or inappropriate 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 in metabolism appear to be key effectors of both 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 effective for treating *KRAS*-mutant[45, 51] and *MYC*-dependent tumors [52, 53]. For instance, small molecule inhibitors that disrupt glucose metabolism can decrease growth of xenograft tumors derived from cells driven by these oncogenes[45, 51, 53]. This suggests that targeting metabolism as an effector of signal transduction pathways required for growth might be a way to attack cancers driven by genetic

alterations that 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], and 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 impact 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, the development of anti-folates took place prior to an understanding of how folic acid contributes to a metabolic cycle that allows single carbon transfer reactions (Box 1). These reactions are critical for the generation of nucleic acids (Figure 2), and the success of anti-folates led to the study of other metabolite analogues as potential anti-cancer agents that disrupt nucleotide synthesis[55, 56]. Today, the anti-metabolite class of nucleoside analogs including 5-flurouracil, gemcitibine, and fludarabine, along with hydroxyurea and a newer generation of antifolates (e.g. pemetrexed) are widely used in the treatment of diverse human tumors. While these drugs are not considered by most to be "targeted therapies," they have clear targets in metabolism such as dihydrofolate reductase and thymidylate synthase (Figure 2, Table 1), and remain effective therapies for many human cancers.

The use of the enzyme L-asparaginase to treat acute lymphoblastic leukemia (ALL) and related lymphomas is another example of how the unique metabolism of tumor cells has been successfully exploited for therapy. Like anti-folates, the potential utility of L-asparaginase to treat cancer was discovered by accident and represents another example of rational drug design that was later revealed to exploit a metabolic difference between cancer 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 asparagine availability for cancer cells (Figure 3). The bacterial L-asparaginase 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 play a role in the dose limiting coagulopathy caused by imbalanced synthesis of pro- and anti-coagulant 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-aspariginase has little utility in the clinic outside of ALL, but other uses 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, use of L-asparaginase, or analogous agents designed specifically to lower glutamine levels, may be effective to treat cancers other than ALL. A rational approach to identify other auxotrophies of cancer cells could lead to similar treatment strategies. Indeed, several types of cancer have low levels of arginosuccinate synthetase required for endogenous

arginine synthesis[3], and early experiments have suggested that tumors may be sensitive to arginase[61]. PEGylated arginine deiminase is an agent that lowers extracellular arginine levels and is currently in clinical trials for various solid tumors [62]. Early phase trials have shown this drug can be given safely, and some responses have been observed in both hepatocellular carcinoma and melanoma[62, 63].

Key issues in targeting cancer cell metabolism

Challenges in targeting metabolic pathways directly

Because all cells rely on the same metabolic pathways to generate ATP, it is often assumed that drugs targeting metabolism would have detrimental effects on normal tissues. While this will be the case for some metabolic targets, the success of cytotoxic agents targeting folate metabolism and DNA synthesis illustrate that a therapeutic window can exist for drugs targeting metabolic pathways. These chemotherapies have side effects 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 estimated at 30-40 hours and may proliferate as frequently as every 10 hours[65, 66]. Hematopoiesis is also very fast as humans generate 2 million red 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, sensitive cancers can be cured using these therapies. Tumor sensitivity to these agents can be

accounted for in part by the loss of cell cycle checkpoints that accompany transformation (see [69] for review of chemotherapy killing mechanisms). However, the fact that folinic acid can rescue dihydrofolate reductase inhibition selectively in normal proliferating tissues (Figure 2), and enhance the efficacy of 5-fluorouracil in colon cancer therapy[70], argues 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 metabolic targeting with fewer effects on normal proliferating cells.

Unwanted toxicity caused by effects on proliferating normal cells is likely to be a major challenge in developing drugs that target proliferative cell metabolism. Often more than one pathway exists to generate the same metabolic end product, and redundant pathways present in normal cells that are lost in cancer cells may allow for a therapeutic window. However, this same redundancy may also impair efficacy of drugs in tumors that can use more than one pathway. For instance, the success of targeting ATP citrate lyase as a means to block cytoplasmic acetyl-coA levels is limited in part by the generation of acetyl-coA via another route[71]. Nevertheless, there is mounting evidence that genetic changes associated with cancer create addictions to specific metabolic pathways[4, 5], and cancer cells often have chromosomal deletions that could eliminate enzymes necessary for the use of redundant pathways. Combining agents to target complementary metabolic pathways might be another strategy to reduce the dose of individual drugs and limit unwanted effects on normal cells.

A therapeutic window does not exist for some targets in metabolism, but drugging alternative targets in the same pathway may be feasible. While 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 (Figure 3), agents like DNP cause energy released from nutrient oxidation to be lost as heat and induce energy stress in cells. Unfortunately, only slight overdoses of DNP result in lethal hyperthermia. However, metformin also targets oxidative phosphorylation in a different way (Figure 3), is well tolerated, and is 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 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 defines the therapeutic window for this class of drugs. Whether this window is large enough to achieve doses *in vivo* that have direct growth inhibitory effects on tumors remains to be determined.

Metabolism is often viewed as a housekeeping function for cells, while signaling pathways are viewed as unique pathways acting only in specific cell types and physiological situations. However, with the exception of gain-of-function gene mutations, there is no target unique to cancer cells. Successful targeted therapies

take advantage of a relative dependence of cancer cells on specific pathways. Similarly, cancer cells depend on specific metabolic pathways, and identifying these dependencies is the key to generating drugs against metabolic enzymes that 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 pathways, rather than levels of individual metabolites, provide the cell with the ability to continually generate ATP to support cell survival, or to provide critical biosynthetic precursors for cell growth. Thus, understanding flux through the cancer cell metabolic network is likely to provide more insight into successful enzyme targets than ascertainment of individual metabolite levels alone. Recent advances in metabolite profiling methodologies provide new tools to understand flux through pathways, and will enhance our understanding of cancer metabolism. Furthermore, increased application of techniques such as MR spectroscopy, including dynamic nuclear polarization to generate hyperpolarized ^{13}C-labeled metabolites whose metabolism can be tracked in tumors, can allow direct visualization of how metabolism is altered as a result of new therapies in patients[73, 74]. These approaches to track metabolism *in vivo* will be especially critical to understand how cell metabolism is influenced by the tumor microenvironment[5],

and will help select the right patients for specific drugs targeting metabolism.

Potential of Metabolic Enzymes as Drug Targets

Mutations in oncogenes or tumor suppressor genes result in addiction of cancer cells to downstream signaling 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 signaling molecules that rely on protein-protein interactions and present challenges to drug development. As a result, efforts have focused on targeting other tractable signaling molecules in a key pathway associated with the genetic event. These strategies have had limited success in the clinic, arguing that blocking single downstream signaling targets is insufficient to block the transforming effects of some driver mutations. Altered expression of metabolic enzymes or changes in metabolic pathway regulation are also downstream of many oncogenes and tumor 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 survival of all cells, and the ability to convert nutrients into biomass is critical 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.

Tumor 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) (Figure 3). PDK expression is increased in many cancers as a result of increased activation of the HIF transcription factor[77, 78]. PDK is a negative regulator of the pyruvate dehydrogenase complex (PDH)[79]. PDH catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, allowing pyruvate entry into the tricarboxylic acid cycle and away from lactate production. Thus, DCA-mediated inhibition of PDK leads to activation of PDH, increased metabolism of pyruvate to acetyl-CoA, and decreased lactate production. DCA can alter mitochondrial membrane potential and inhibit lactate production in cancer model systems[80], and has been shown to alter mitochondria in human glioblastoma[81]. Importantly, even at doses that influence mitochondrial membrane potential, DCA is well tolerated by patients[81]. While there is insufficient data to know whether DCA will provide clinical benefit, these studies demonstrate that a sufficient therapeutic window can exist to target cell metabolism in patients.

Approaches to target cancer 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 cancer models. New molecules directed against metabolic enzymes will likely enter clinical studies in the next several years.

Such compounds have the potential to limit macromolecular synthesis needed for cell growth, a strategy employed by existing drugs targeting nucleic acid synthesis. Alternatively, targeting metabolism can limit pathways important to supply nutrients to the cell and impair bioenergetics to prevent 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 while metformin increases lactate production and has lactic acidosis as an important side effect. While paradoxical, there is evidence to support both as potentially beneficial in cancer treatment. By increasing glucose entry into the TCA cycle, DCA directs carbon away from lactate production[80] (Figure 3), and as a consequence may direct metabolism away from efficient biosynthetic reactions[1]. Metformin on the other hand, inhibits the transfer of electrons from NADH in the mitochondria to the electron transport chain (Figure 3). This increases reliance on lactate production as a means to cycle NADH back to NAD^+, 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 is likely to be more effective in tumors with increased reliance on high glucose uptake with lactate production, while the latter might synergize with other therapies that induce energy stress.

Directly targeting glucose metabolism

Various agents have been shown to block glucose use by cancer cells, but to date no specific glucose transport inhibitors have been reported. GLUT1 is the glucose transporter with the largest tissue distribution and is thought to be the transporter responsible for basal glucose uptake in most cancer and normal cells[82, 83] (Figure 3). Although GLUT1 is expressed at much higher levels in cancer cells than in normal cells it may be difficult to inhibit glucose uptake directly in tumors without an effect on normal tissues. Nevertheless, partial inhibition of glucose uptake may still sensitize cancer cells to other drugs (see [84] for review of various studies). Many of these studies rely on the withdrawal of glucose from cells in culture, illustrating 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, are not expressed in most normal tissues but can be expressed at high levels in cancer suggesting these transporters as possible targets[82]. Antibodies that selectively target GLUT3 or other nutrient transporters with restricted expression may represent another way to block nutrient uptake and starve cancer cells.

2-deoxyglucose (2DG) is an inhibitor of glucose metabolism because it is phosphorylated in cells by hexokinase (HK) to make 2DG-6-phosphate, a competitive inhibitor of enzymes that metabolize glucose-6-phosphate. Cells exposed to sufficient amounts of 2DG undergo growth arrest and/or apoptosis[85], and 2DG may potentiate standard cytotoxic chemotherapy[84, 86]. 2DG has been tested

as an anti-cancer agent in patients[87], but when given to glioblastoma patients at doses sufficient to limit glucose metabolism in cancer cells, significant toxicity was observed[88, 89]. Lower dose 2DG is 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 proximal 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 to modulate glucose metabolism in cancer cells. HK is responsible for trapping glucose in cells (Figure 3) and at least some cancers are specifically dependent on the HK2 isoform of this enzyme [91, 92]. HK2 is normally expressed in skeletal muscle and adipose tissue, suggesting a 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 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 tumors treated with an HK2-selective inhibitor. An association between hexokinase and mitochondria influences apoptosis regulation[93], and compounds isolated from plants that disrupt this association are toxic to cancer cells in culture[94]. HK is also a target of 3-bromopyruvate, a compound shown to 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 HK and it has been argued that the

combined inhibition of multiple 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 (Figure 3). There are two pyruvate kinase genes in mammals, each producing two distinct gene products by alternative splicing[97, 98]. Most tissues express a product of the PK-M gene that is alternatively spliced to produce either the PKM1 or PKM2 isoform. All cancer cells express PKM2, while many differentiated tissues express PKM1[97]. PKM2 expression promotes aerobic glycolysis and is selected for during growth of xenograft tumors in mice[99]. PKM1 is a constitutively active enzyme, while PKM2 is unique among pyruvate kinase isoforms in that its enzyme activity is inhibited by binding to tyrosine-phosphorylated proteins downstream of cell growth signals[100]. Surprisingly, it is this ability to inhibit the enzyme that appears important for promotion of aerobic glycolysis and cell proliferation. Selection for a less active form of pyruvate kinase may help divert glucose metabolites upstream of pyruvate kinase into biosynthetic pathways[5, 97, 100]. Efforts have been made to selectively inhibit PKM2[101, 102]. Peptide aptamers that promote the less active form of pyruvate kinase have been shown cause energy stress and cell death in cultured cancer cells[101], and more modest effects were observed using small molecule inhibitors of PKM2[102]. Targeting PKM2 with shRNA can slow cell proliferation in cell culture[99], however these cells retain the ability to proliferate even with the near complete absence of pyruvate kinase activity. These findings suggest that activation of PKM2 to restore the high pyruvate kinase activity state found in normal tissues may be an alternative strategy to target

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

Another example of a regulatory enzyme in glycolysis with isoform selectivity in some cancers is fructose-6-phosphate-2-kinase (PFK2) (Figure 3). By generating fructose-2,6-bisphosphate (F-2,6-BP), PFK2 activates phosphofructokinase (PFK1) to increase flux through this step of glycolysis. Most PFK2 isoforms are bifunctional enzymes with both kinase and phosphatase activity, and thus can also catalyze the destruction of F-2,6-BP and decrease PFK1 activity[105]. PFK2FB3 is the isoform expressed in many cancers and is required for anchorage independent growth of RAS-driven tumors[106, 107]. PFK2FB3 has almost no phosphatase activity, and kinase activity is influenced by several factors implicated in controlling cancer metabolism including metabolite levels as well as RAS, MYC, and AMPK signaling[105, 108, 109]. Small molecule inhibitors of PFK2FB3 have been reported to have a cytostatic effect on Ras-transformed cancer cells[51]. The compound decreases F-2,6-BP levels and impairs growth of xenograft tumors[51], 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 to directly target the Warburg effect in cancer. The family of monocarboxylate transporters (MCT) are the major proteins responsible for lactate export in glycolytic cells including cancer cells (Figure 3)[110-112]. There is evidence that a symbiotic relationship exists among different cells within a tumor whereby some cells rely on lactate produced by other cells as a fuel source, and disrupting lactate transport can starve cells dependent on lactate for survival[113]. Targeting MCTs using small molecules also inhibits proliferation of lymphocytes that rely on aerobic glycolysis[114, 115]. This suggests impaired immune function could be a side effect of targeting lactate export in cancer. It also suggests drugs targeting cancer metabolism may have a role as immunosuppressive therapies. Additional potential side effects from inhibiting lactate transport include negatively impacting 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^+ (Figure 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 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 tumors in mice and can

induce tumor regression when combined with nicotinamide phosphoribosyltransferase inhibitors (NAMPT)[53], supporting LDHA as a promising therapeutic target.

Targeting NAD^+ metabolism

Cells possess a limited pool of NAD^+/NADH, yet these molecules exist as important cofactors in metabolic reactions involving oxidation/reduction. They are also substrates for enzymes such as sirtuins and poly-ADP-ribose polymerases 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 involved in regenerating NAD^+ via a salvage pathway from nicotinamide and phosphoribosyl-pyrophosphate, was identified as the target of a molecule identified in a screen to find novel cytotoxic compounds[120]. Cells treated with NAMPT inhibitors die as a result of NAD^+ depletion, and NAMPT inhibition has shown activity as an anti-cancer agent in pre-clinical cancer models[119]. Because NAD^+ is a required cofactor for the GAPDH step of glycolysis, cells must regenerate NAD^+ from NADH to allow continued flow of glucose carbon through glycolysis (Figure 3). Consistent with NAMPT inhibitors limiting glucose metabolism in cells with high activity of NAD^+ consuming enzymes, NAMPT inhibition in cells has an effect primarily on the cytosolic (rather than the mitochondrial) NAD^+ pool[121]. NAMPT inhibition can also be toxic to lymphocytes[122], suggesting that use of NAMPT inhibitors in patients might be limited by

immunosuppression. Mild lymphopenia was observed in early trials of NAMPT inhibitors, but thrombocytopenia was the dose limiting toxicity[123]. Limited clinical efficacy has been observed thus far, although work is ongoing to develop more potent compounds and define those patients 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 dictated by isocitrate dehydrogenase (IDH) mutations. Point mutations in IDH1 and IDH2 found in cancer always involve a residue in the active site of only one allele[125-127], and lead to production of 2-hydroxyglutarate (2HG) - a metabolite found only at very low levels in normal cells[128-130]. IDH mutations define a clinically distinct subset of both glioma and leukemia suggesting that these mutations contribute to a unique biology within each tumor type[125, 127, 131, 132]. It is not clear how IDH mutation and 2HG production 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 stabilization of the HIF transcription factor that controls expression of many genes important for cancer and metabolic regulation[10, 11]. αKG-dependent dioxygenases are also involved in demethylation reactions that affect chromatin structure having plieotropic effects on global transcription and cellular differentiation[136, 137], and this methylation

pattern is altered in cells with IDH1 mutations[133, 134]. Thus, when developed, small molecules that inhibit 2HG production by mutant IDH may restore normal αKG-dependent dioxygenase function and normalize both HIF levels and chromatin structure. In addition, because these dioxygenases are influenced by the αKG/succinate ratio, delivery of αKG-analogs may be another way to restore normal dioxygenase activity. These cell permeable esters of αKG can both raise αKG levels and increase dioxygenase activity[138]. This latter strategy has seen some success in models of human cancer with abnormal αKG/succinate ratios caused by loss of function mutations in succinate dehydrogenase or fumarate hydratase[138].

Additional strategies to target 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 plays an important role in replenishing TCA cycle intermediates depleted by biosynthetic reactions (Figure 3)[139]. The enzyme glutaminase catalyzes glutamine to glutamate conversion on the pathway to αKG production. Glutaminase has two major isoforms in mammals, GLS1 and GLS2, and expression of these enzymes can have opposite effects on cell proliferation[140]. GLS1 is an important downstream effector of MYC and promotes glutamine entry into the TCA cycle[49, 50], while GLS2 is regulated by p53 and influences the cellular redox state[141]. These different functions of GLS1 and GLS2 likely play key roles in cancer metabolism, and targeting glutaminase activity can selectively inhibit the growth of transformed

cells[142, 143]. Blocking GLS1 activity can prevent glutamine entry into cells as a source of 2HG production by mutant IDH1, and slow the growth of these cells[143]. GLS1 was also identified as the target of a molecule that blocks cell transformation by Rho GTPases, and the molecule can slow growth of Rho GTPase transformed fibroblasts and breast cancer cells[142]. However, lymphocytes are also dependent on glutamine metabolism[144], suggesting that immune suppression may be a side effect of drugs targeting glutamine metabolism for cancer therapy.

Targeting other metabolic dependencies in cancer cells

Therapies targeting cancer metabolism should attack those metabolic pathways that meet critical needs specific to cancer cells. This approach is analogous to targeting nucleic acid metabolism with anti-metabolites, 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 involved directly in fatty acid synthesis have been suggested as cancer targets[145, 146]. Lipids also play important signaling functions for cells, and chemical genetic screens have identified lipases that release fatty acyl chains from glycerol as therapeutic targets for some cancers[147]. It remains to be determined if 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 fuel 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 pentose phosphate pathway NADPH production is characteristic of patients with glucose-6-phosphate dehydrogenase (G6PD)-deficiency, and G6PD-deficiency has not been found to be protective against cancer[149]. Furthermore, at least some cancer types do not have a large pentose phosphate pathway flux[150]. Cells can generate NADPH via other pathways and conversion of glutamine to lactate via malic enzyme has been suggested both as a therapeutic target and a major source of NADPH in glioblastoma cells[139]. Whether other targets important for NADPH generation exist remains to be determined.

Conclusions and future directions

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

enzymes in a pathway.

Despite the success of targeting enzymes involved in nucleotide synthesis, efforts to target other enzymes and pathways in cellular metabolism are in their infancy. As targets become better defined, targeting these enzymes could deliver effective therapies that spare normal tissues and have an impact across a variety of cancers. 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 determine the best targets for intervention. Development of new methods to study tumor metabolism *in vivo* will be critical. Ultimately these efforts will determine if a sufficient therapeutic window exists to spare normal tissues and allow new drugs targeting these enzymes to make a difference in patients' lives.

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Boxes

Box 1. The discovery of anti-folates as effective anti-cancer agents.

Targeting metabolism has figured prominently in some of the first efforts to treat cancer with drugs. Shortly after the discovery of folic acid as a nutrient needed to prevent anemia in pregnancy, Sidney Farber noted that administration of folic acid conjugates appeared to stimulate leukemic cell proliferation[56, 152]. 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 analog 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 leukemia[153]. Another folate analog, methotrexate (amethopterin), replaced aminopterin as a cancer chemotherapy and produced the first cures of a solid tumor by chemotherapy (choriocarcinoma) 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.

Box 2. Development of L-asparaginase to treat ALL.

The potential use of L-asparaginase to treat cancer was discovered when it was noted that guinea pig serum, but not that of other animals, had an inhibitory effect on the proliferation of lymphoma cells in mice[156]. Guinea pigs are relatively unique among mammals in having serum aspariginase activity[157], which was found to be responsible for the anti-lymphoma effect in mice[55, 158]. Asparaginase was found to

be a particularly effective agent in ALL and associated high-grade lymphomas, inducing remission as a single agent in greater than 50% of children with the disease[159]. These remissions were not durable; however, when used as part of a combination chemotherapy regimen, aspariginase has contributed to a greater than 80% cure rate for children with ALL and its inclusion in adult regimens has contributed to improved clinical outcomes[160].

Figure Legends

Figure 1. 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 a significant 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 holds the key to successfully targeting enzymes for therapy.

b. Not all tumors exhibit the same metabolic phenotype. Tracer uptake studies in patients and cancer model systems have demonstrated that cancers show differential uptake of nutrients. This variety is seen even among different tumors

arising from the same normal tissue. This heterogeneity should be considered when stratifying patients for trials using novel therapies that target cancer metabolism.

Figure 2. Some existing chemotherapies target specific metabolic enzymes.
5-fluorouracil (5-FU) inhibits thymidylate synthase (TS), an enzyme required to generate thymidine for DNA synthesis. Methotrexate targets the dihydrofolate reductase (DHFR) step in folate metabolism. Interrupting folate metabolism compromises thymidine synthesis, but also interferes with purine synthesis and other reactions involving one-carbon transfers. Folinic acid can enter the folate pool downstream of dihydrofolate reductase and rescue the effects of inhibiting this enzyme in some cells. Glycine can also be used to convert tetrahydrofolate (THF) to 5,10-methylene-THF.

Figure 3. Targeting metabolic enzymes as a strategy to block biosynthesis or induce energy stress.
A graphical representation of central carbon metabolism is presented, with some metabolic enzymes currently being considered as therapeutic targets for cancer marked with a target. Five drugs that influence metabolism and have been tested in humans are shown in red. How these enzyme targets relate to the synthesis of important macromolecules needed for cell growth is shown in yellow, and their relationship to mitochondrial ATP production is shown in green. Enzyme abbreviations are described in the text. Metabolic intermediates are abbreviated as follows: G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; FBP, fructose-

1,6-bisphosphate; F-2,6-BP, fructose-2,6-bisphosphate; PEP, phosphoenolpyruvate; αKG, α–ketoglutarate; OAA, oxaloacetate.

Table 1: Strategies to target metabolic enzymes for cancer therapy

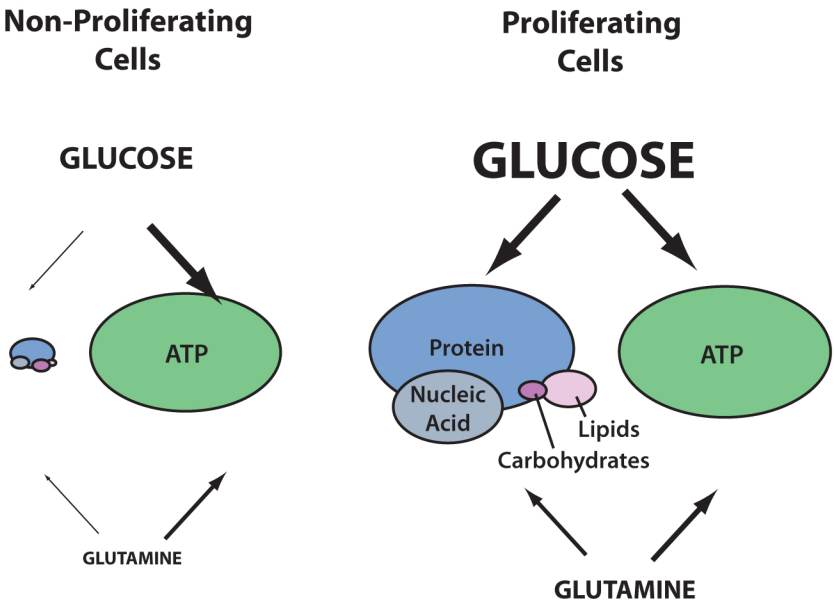
	AGENT(s)	DEVELOPMENT STAGE	INDICATION / KEY PRECLINICAL FINDINGS
BIOSYNTHESIS OF KEY MACROMOLECULES NECESSARY FOR CELL GROWTH			
Nucleic Acids			
Folate metabolism · DHFR	Methotrexate Pemetrexed	Approved agents	· Effective therapy for various cancers
Thymidine synthesis · TS	5-Fluorouracil	Approved agent	· Effective therapy for various cancers
Deoxynucleotide synthesis · RR	Hydroxyurea	Approved agent	· Effective therapy for leukemia
Nucleotide incorporation · DNA polymerase / RR	Gemcitibine Fludarabine	Approved agents	· Effective therapies for various cancers
Ribose synthesis · TKTL1	Preclinical Data Only		· TKTL1 allows non-oxidative ribose production, and expression correlates with poor prognosis and RNAi inhibits cell proliferation[161]
· G6PD			· G6PD is necessary for oxidative ribose production. High levels of G6PD seen in some cancers and expression can transform fibroblasts[148]
Amino Acid Metabolism/Protein Synthesis			
Asparagine availability	L-asparaginase	Approved agent	· Effective therapy for leukemia
Arginine availability	PEGylated arginine deiminase	Phase II	· Arginine auxotrophy thought to be related to low arginosuccinate synthase expression in some tumors[62]. · Clinical efficacy being explored in hepatocellular carcinoma, melanoma, small cell lung cancer and mesothelioma.
Glutamine availability · GLS1	Preclinical Data Only		· GLS1 converts glutamine to glutamate, likely is more important as a means of generating anapleurotic carbon for the TCA cycle[162].
PHGDH	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			
FAS	Preclinical Data Only		· FAS is a key enzyme in *de novo* lipogenesis. Growth of human xenograft tumors in mice is inhibited by tool compounds[165].
ACL	Preclinical Data Only		· ACL is necessary to export citrate from the mitochondria to the cytosol for *de novo* lipogenesis, is important for cell proliferation and growth of human xenograft tumors[71, 166].
ACC	Preclinical Data Only		· ACC is necessary for *de novo* lipogenesis and is required for growth of culture cancer cells in the absence of exogenous lipids.[161, 167]
CENTRAL METABOLIC PATHWAYS NECESSARY TO SUPPLY CARBON, ATP, AND/OR NAD(P)H			
Glycolysis			
Glucose transport	Preclinical Data Only		· Efforts to inhibit glucose transport ongoing.
HK	2-Deoxyglucose	Clinical Data Preclinical	· Unacceptable toxicity observed at high doses[88, 89], trials at lower doses currently on hold. · Inhibition of HK inhibits proliferation, and is a rationale for selective HK2 inhibitors[91, 92].
PFK2FB3	Preclinical Data Only		· Controls key regulatory step in glycolysis, tool compounds inhibit growth of xenograft tumors[51].
PGAM	Preclinical Data Only		· Identified in a screen as the target of molecule that kills cancer cells[168].
PKM2	Preclinical Data Only		· Both enzyme activation and inhibition being explored (see text). · Cancer cells expressing the PKM1 isoform do not grow as xenografts[99].
LDHA	Preclinical Data Only		· Enzyme responsible for lactate production, tool compounds inhibit xenograft tumor growth[53].
Lactate excretion			· Lactate is excreted from cells via monocarboxylate transporters.
· MCT4	Preclinical Data Only		· MCT4 is the transporter used by some cancer cells[111], and small molecule MCT inhibitors can block cell proliferation[114].
TCA cycle / mitochondrial metabolism			
PDK	Dichloroacetate	Phase II	· Compound available in the clinic for treating lactic acidosis related to inborn errors of metabolism. Can modulate mitochondrial metabolism in human gliomas, and clinical efficacy being studied[81].
IDH1 IDH2	Preclinical Data Only		· 2-hydroxyglutarate production by mutant enzymes linked to cancer pathogenesis[128, 129, 130]. · Decreased wildtype enzyme using RNAi can impair proliferation of wildtype IDH cancer cells[129].
Malic Enzyme	Preclinical Data Only		· Key enzyme involved in NADPH production[139, 1].
Mitochondrial Complex I	Metformin	Approved Agent	· Improved survival in diabetics with cancer[24], and increased response rate

		(non-cancer)	observed in breast cancer patients taking metformin[30]. · Prospective trials planned to explore efficacy in cancer.
Glutamine availability			
· GLS1 · GDH	Preclinical Data Only		· GLS1 converts glutamine to glutamate, and GDH converts glutamate to αKG as a source of anapleurotic carbon for the TCA cycle[162]. · GDH required for proliferation of some cells[169]. · Inhibition of GLS1 impairs proliferation of some cells[142].
PC	Preclinical Data Only		· PC provides an alternative route to replenish the TCA cycle when GLS is inhibited, suggesting PC inhibition could synergize with GLS inhibition in glutamine addicted cells[170].
Fatty Acid Metabolism			
MAGL	Preclinical Data Only		· MAGL inhibition impairs the growth of xenograft tumors[147].
CPT1C	Preclinical Data Only		· Tool compounds inhibit growth of xenograft tumors[171].
NAD Metabolism			
NAMPT	Various	Phase II	· FK866 had a dose limiting toxicity of thrombocytopenia in phase I trials[123], and NAMPT inhibitors are being considered for further development as a cancer therapy[119, 124].

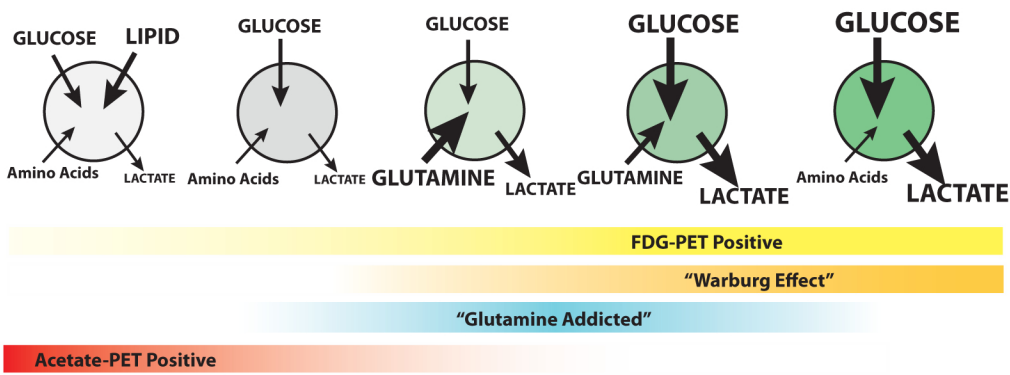
Abbreviations: DHFR, dihydrofolate reductase; TS, thymidylate synthase; RR, ribonucleotide reductase; TKTL1, transketolase-like protein 1; G6PD, glucose-6-phosphate dehydrogenase; GLS1, glutaminase 1; PHGDH, phosphoglycerate dehydrogenase; FAS, fatty acid synthase; ACL, ATP-citrate lyase; ACC, acetyl-coA carboxylase; HK, hexokinase; PFK2FB3, phosphofructokinase isoform FB3; PGAM1, phosphoglycerate mutase isoform 1; PKM2, pyruvate kinase isoform M2; LDHA, lactate dehydrogenase A; MCT4, monocarboxylate transporter 4; PDK, pyruvate dehydrogenase kinase; IDH, isocitrate dehydrogenase; GDH, glutamate dehydrogenase; PC, pyruvate carboxylase; MAGL, monoacylglycerol lipase; CPT1C, carnitine palmitoyl transferase 1C; NAMPT, nicotinamide phosphoribosyltransferase.

Vander Heiden; Figure 1

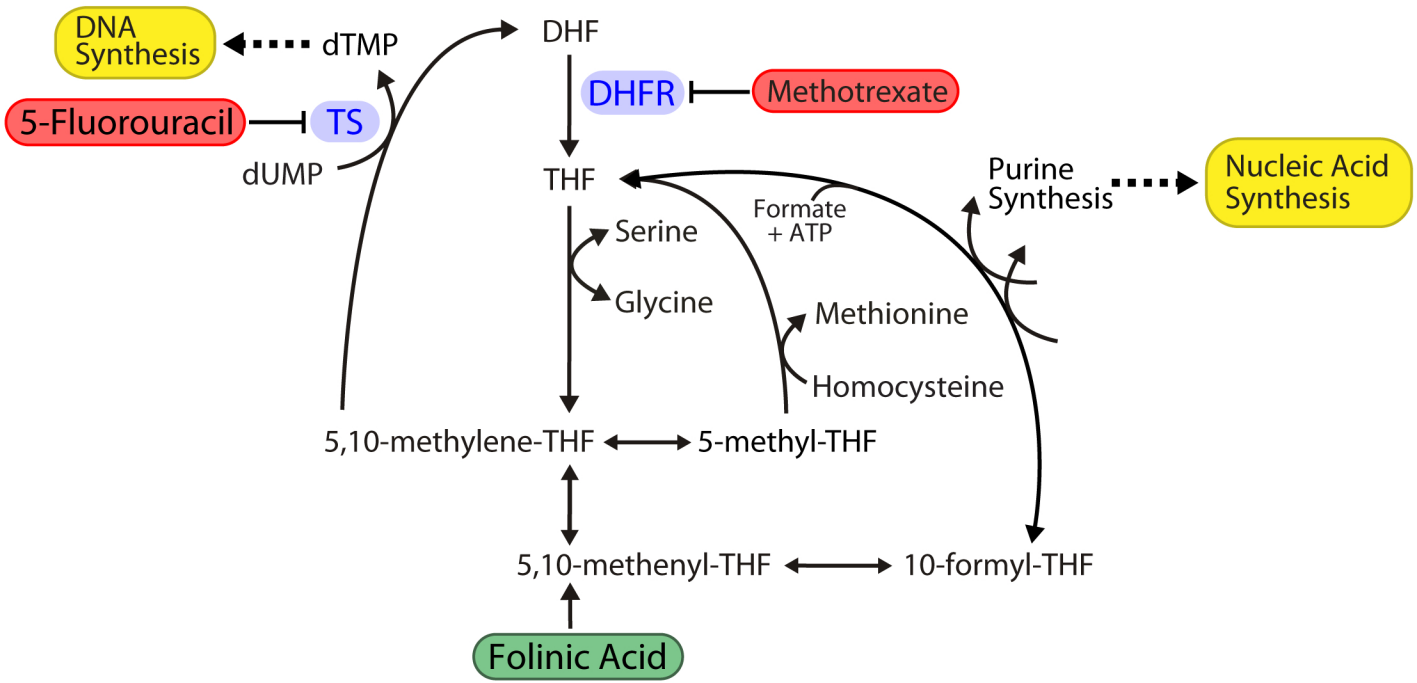
a.



b.



Vander Heiden; Figure 2



Vander Heiden; Figure 3