REVIEW

Clinical development of cancer therapeutics that target metabolism

B.F. Clem¹, J. O’Neal², A.C. Klarer³, S. Telang³ and J. Chesney³

From the ¹Department of Biochemistry and Molecular Genetics, University of Louisville, 319 Abraham Flexner Way, Louisville, KY 40292, ²Department of Medicine, Washington University, 660 South Euclid Ave, St. Louis, MO 63110 and ³Division of Medical Oncology and Hematology, Department of Medicine, University of Louisville and Molecular Targets Program, James Graham Brown Cancer Center, 505 South Hancock Street, Louisville, KY 40202, USA

Address correspondence to B.F. Clem, Department of Biochemistry and Molecular Genetics, University of Louisville, 505 South Hancock Street, Louisville, KY 40202 or J. Chesney, Division of Medical Oncology and Hematology, Department of Medicine, University of Louisville and Molecular Targets Program, James Graham Brown Cancer Center, 505 South Hancock Street, Louisville, KY 40202, USA. email: brian.clem@louisville.edu or jason.chesney@louisville.edu

Summary

Glucose and glutamine metabolism in cancer cells are markedly elevated relative to non-transformed normal cells. This metabolic reprogramming enables the production of adenosine triphosphate and the anabolic precursors needed for survival, growth and motility. The recent observations that mutant oncogenic proteins and the loss of tumor suppressors activate key metabolic enzymes suggest that selective inhibition of these enzymes may yield effective cancer therapeutics with acceptable toxicities. In support of this concept, pre-clinical studies of small molecule antagonists of several metabolic enzymes in tumor-bearing mice have demonstrated reasonable therapeutic indices. We will review the rationale for targeting metabolic enzymes as a strategy to treat cancer and will detail the results of several recent clinical trials of metabolic inhibitors in advanced cancer patients.

Rationale to target metabolic enzymes for the treatment of cancer

Metabolic reprogramming in cancer cells was first observed by Otto Warburg a century ago and has recently been recognized as a common and essential characteristic of human cancers.¹ As evidence, glucose uptake by tumors using positron emission tomography (PET) correlates directly with tumor aggressiveness and patient prognosis.² Furthermore, several highly effective small molecule antagonists of signaling oncoproteins such as B-Raf in melanoma and the estrogen receptor in breast cancer acutely suppress glucose metabolism suggesting that the glycolytic pathway is a key downstream target of these agents.^{3,4}

Increased glycolytic flux permits the rapid production of adenosine triphosphate and anabolic precursors needed for cancer cell growth, while simultaneously promoting invasiveness by reducing the extracellular pH and inducing apoptosis in surrounding normal cells. Although alterations in glycolytic metabolism drive a substantial number of anabolic pathways that support neoplastic growth, the majority of cancer types also require other nutrient sources such as glutamine to drive mitochondrial metabolism.⁵ Not surprisingly, several metabolic proteins required for glucose and glutamine metabolism are under the direct control of oncogenes and tumor suppressors, including RAS, MYC, p53, PTEN and pRb, suggesting that activation of these metabolic pathways may be a prerequisite for

tumorigenesis. Given the recent advances in our molecular understanding of how glucose and glutamine metabolism mediate resistance to cancer,^{6,7} the development of small molecules that target these metabolic pathways may yield synergistic improvements in the clinical activities of multiple cytotoxic and targeted agents. (Figure 1)

Targeting hexokinase 2: 2-deoxy-D-glucose

The transcription factor, hypoxia inducible factor-1 α (HIF-1 α), is stabilized in human cancers and cooperates with the oncogene MYC to promote glucose metabolism via induction of the enzyme hexokinase 2,⁸ which phosphorylates carbon 6 of glucose resulting in intracellular trapping. 2-deoxy-D-glucose (2DG) is a glucose mimetic, which is readily transported into cells by glucose transporters and then phosphorylated by hexokinase 2, resulting in accumulation and allosteric inhibition of hexokinase 2. In 1958, Landau *et al.*⁹ first reported that a single intravenous infusion of (2DG) caused hyperglycemia and a reduction in white blood cell counts in leukemic patients. Stein *et al.*¹⁰ then demonstrated in a phase 1 trial that oral administration of 2DG (45 mg/kg; daily x 2 weeks every 3 weeks) to castrate-resistant prostate, lung and cervical cancer patients resulted in reduced

glucose uptake assessed by fluorodeoxyglucose (FDG)-PET imaging. However, no objective response or survival data were presented in this publication and no phase 2 trial has been completed. Recently, Raez *et al.*¹¹ conducted a phase 1 dose-escalation trial of 2DG alone or combined with docetaxel in solid tumor patients and, at the maximum tolerated dose of 63 mg/kg daily for 7 days every other week, noted that 32% of patients had stabilization of their tumors and 3% had a partial response. Unfortunately, the ability of 2DG to act as a competitive inhibitor of glucose metabolism has major limitations since the normal concentration of glucose in the body ranges from 7 to 10 mg/ml and the maximal tolerated dose of 2DG (63 mg/kg) results in a median maximum plasma concentration of only 0.116 mg/ml. However, there does appear to be some clinical benefit of 2DG and rational combination strategies of 2DG with approved cancer therapies in which resistance is mediated by enhanced glucose metabolism may improve clinical outcomes.

Targeting 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3): 3PO/PFK158

The PFKFB family of glycolytic regulators control the steady-state concentration of fructose 2,6-bisphosphate (F2,6BP) which

Figure 1. Metabolic inhibitors in or entering clinical trials. Ras, HIF-1 α and c-Myc increase the expression of metabolic transporters and enzymes which in turn cause a reprogramming of metabolic utilization that supports the enhanced energetic and anabolic requirements of cancer cells. Several metabolic inhibitors are under study in clinical trials, including the following inhibitors (targets in parantheses): (i) 2DG (hexokinase); (ii) 3PO/PFK158 (PFKFB3); (iii) AT-101 (LDH-A); (iv) AZD3965 (MCT1); BPTES and CB839 (GLS); (vi) AG-120 and AG-221 (mutant IDH) and (vii) DCA/CPI-613 (PDK). Black lines indicate suppression and red lines indicate stimulation of activity and/or expression. Key regulators, transporters and enzymes are highlighted in red. GLUT1: glucose transporter 1; HK2: hexokinase 2; PFK1: 6-phosphofructo-1-kinase; PK-M2: pyruvate kinase M2; PDH: pyruvate dehydrogenase; LDH-A: lactate dehydrogenase A; PFKFB3: 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PTEN: phosphatase and tensin homolog; pRb: retinoblastoma protein and c-Myc: avian myelocytomatosis virus oncogene.

regulates the activity of 6-phosphofructo-1-kinase (PFK-1), an irreversible, rate-limiting step of glycolysis. In particular, the PFKFB3 family member is upregulated by multiple oncoproteins including HIF-1 α , the estrogen receptor and Ras and is downregulated by the PTEN tumor suppressor (reviewed in Ref. 12). Since activation of PFK-1 is a key regulatory step in glycolysis, the modulation of PFKFB3 activity and F2,6BP directly affects flux through the entire glycolytic pathway. The requirement of PFKFB3 for neoplastic transformation was recently demonstrated by the observations that heterozygous genomic deletion of the *Pfkfb3* gene reduced the concentration of F2,6BP, glucose uptake, glycolytic flux and growth of tumors in syngeneic mice.¹²

In silico screening identified a small molecule antagonist of PFKFB3, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), that suppresses glucose uptake, cellular F2,6BP production, glycolytic flux to lactate and growth of cancer cell lines and tumor growth in multiple mouse tumor models.¹³ The development of 3PO derivatives by Advanced Cancer Therapeutics has resulted in the identification of a second generation compound, PFK15 with improved potency against the recombinant enzyme activity.¹⁴ PFK15 provided a synthetic platform for the synthesis of third generation derivatives, which led to the pharmaceutical-grade agent, PFK158, that exhibits increased potency and improved pharmacokinetic properties. PFK158 is currently being tested in a dose escalation phase 1 trial at MD Anderson Cancer Center, the University of Louisville, the University of Texas-San Antonio and Georgetown University with no reported drug-related serious adverse events and multiple examples of disease stabilization reported thus far (clinicaltrials.gov no. NCT02044861).

Targeting LDH-A: AT-101

Generation of lactate from pyruvate by lactate dehydrogenase A (LDH-A) replenishes NAD⁺ required for enhanced flux through the glyceraldehyde-3-phosphate dehydrogenase step of glycolysis and may provide a carbon source to adjacent cells. Increased expression of LDH-A has been observed in several tumor types, LDH-A is regulated by MYC and inhibition of LDH-A in tumor cells with siRNA reduces tumor growth in mice suggesting that inhibition of LDH-A activity may be an effective anti-tumor therapy.¹⁵

An LDH-A inhibitor, Gossypol, is naturally derived from the cotton plant and was originally evaluated in China as a male contraceptive. AT-101 (Ascenta Therapeutics) is an orally bioavailable form of the R-(-) enantiomer of gossypol with improved potency against LDH-A. However, AT-101 also is reported to antagonize the anti-apoptotic BCL2 family of proteins by acting as a BH3 domain mimetic¹⁶ and thus the mechanism of action is likely to be secondary to suppression of both targets. Unfortunately, three clinical trials of AT-101 in non-small and small cell lung cancer revealed either limited or no efficacy but that the drug was well tolerated. A phase I study conducted in 24 patients with a variety of refractory solid tumors treated with AT-101, paclitaxel and carboplatin resulted in four prostate cancer patients with stable disease and multiple partial responses (two prostate, one non-small cell lung cancer and one esophageal adenocarcinoma), but it is unclear whether AT-101 contributed to these objective responses.¹⁷ In a phase 2 trial, patients with recurrent, locally advanced head and neck squamous cell carcinoma were randomized to receive docetaxel, docetaxel with pulse-dose AT-101 or docetaxel with metronomic AT-101—no differences in overall response rate,

progression-free survival or overall survival were observed¹⁸. Last, a phase 2 trial of AT-101 in glioblastoma multiforme (GBM, 56 patients) reported stabilization of tumors in 16% of patients as well as a single patient with a partial response¹⁹. While there is some clinical activity with AT-101, a caveat is that the concurrent antagonism of the anti-apoptotic BCL2 proteins by AT-101 makes it difficult to draw conclusions pertaining to the utility of targeting LDH-A as a therapeutic strategy against cancer.

Targeting monocarboxylate transporter 1: AZD3965

Highly glycolytic tumor cells excrete lactate into the tumor microenvironment through a family of monocarboxylate transporters (MCTs) and the simultaneous movement of protons with lactate acidifies the tumor microenvironment, which promotes tumor progression and invasion.²⁰ Although MCT4 serves to export lactate from the cell, MCT1 imports lactate from the extracellular environment and enables lactate excreted by stromal cells within the microenvironment to be imported by tumor cells and be used for oxidative metabolism (reviewed in Ref. 21). Importantly, inhibition of MCT1 has been found to decrease growth and metastatic potential of multiple tumor types.

The selective MCT1 small molecule inhibitor, AZD3965, can alter lactate transport and sensitize small cell lung cancer xenografts to radiation.²² Notably, AZD3965 is currently undergoing phase 1 clinical trials in the United Kingdom for advanced solid cancers and diffuse large B-cell lymphoma (NCT01791595) with the expected completion date of May 2017. However, MCT1 inhibitors failed to substantially affect hypoxic regions within solid tumors, primarily as a consequence of HIF-1 α -mediated increase in MCT4 expression,²³ suggesting that combinatorial treatment of AZD3965 with MCT-4 antagonists or the use of global MCT inhibitors may be a more effective therapeutic strategy.

Targeting glutaminase: BPTES and CB-839

The requirement for enhanced anaerobic glycolysis in tumors results in diminished glucose oxidation and an 'addiction' to other nutrient sources to support mitochondrial function.²⁴ Glutamine is the most abundant amino acid in the blood and may provide a ready source of both carbon and nitrogen not only for bioenergetics but also for the synthesis of macromolecules needed for rapid proliferation. In addition to supplying anaplerotic carbon for entry into the tricarboxylic acid (TCA) cycle, glutamine plays a significant role in maintenance of redox homeostasis by facilitating glutathione production from glutamate as well as providing significant production of NADPH from metabolism through malic enzyme. While the activity of many transporters/enzymes facilitate glutamine metabolism, glutaminase (GLS) initiates glutaminolysis via the deamidation of glutamine to glutamate, which can then serve as a direct precursor for glutathione biosynthesis or as a mitochondrial substrate through conversion to the TCA intermediate, α -ketoglutarate. Not surprisingly, as a consequence of the requirement of tumor cells for glutamine metabolism and glutamate production, several oncogenes and tumor suppressors have been found to regulate glutaminase 1 (GLS1). Specifically, c-Myc can increase GLS1 expression²⁵ and loss of pRb family of tumor suppressors results in increased GLS1 and glutamine metabolism.²⁶ Multiple RNAi studies also have highlighted the requirement for GLS1 in the proliferation of different cancer cell

models both *in vitro* and *in vivo*, including c-Myc driven B-cell lymphoma and prostate cancer.²⁴

Stemming from these genetic studies, several distinct small molecule inhibitors of GLS are currently in development. Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide 3 (BPTES) is an allosteric antagonist that targets GLS1 and administration of BPTES suppresses tumor growth in mice.²⁷ Unfortunately, attempts to identify more potent second-generation molecules of BPTES have, as yet, yielded few results. More recently, researchers from Calithera Biosciences identified a potent antagonist of both GLS1 splice variants that is orally bioavailable and can suppress the growth of triple negative breast cancer cells at low nanomolar concentrations. CB-839 was found to suppress the outgrowth of patient-derived breast xenografts as a single agent and block the growth of established tumors in combination with paclitaxel.²⁸ CB-839 is currently being examined in three separate phase I clinical trials for solid tumors (clinicaltrials.gov no. NCT02071862), hematological tumors (NCT02071888) and leukemias (NCT02071927) although no clinical data have yet been presented.

Targeting mutant isocitrate dehydrogenase: AG-120 and AG-221

The majority of metabolic proteins that are being targeted for the development of cancer therapeutics are activated by oncogenic alterations but do not contain residue mutations. In contrast, open reading frame mutations within the genes encoding separate isoforms of NADP-dependent isocitrate dehydrogenase (IDH1 and IDH2), which catalyze the conversion of isocitrate to α -ketoglutarate within the cytoplasm or mitochondrial matrix, respectively, have been identified in several human cancers.²⁹ Missense mutations resulting in amino acid substitutions primarily at R132 within IDH1 and the corresponding R172 in IDH2 have been demonstrated. Unlike other enzyme mutations that result in either increased activity or loss of intrinsic function, these IDH mutations lead to the production of the 'oncometabolite', 2-hydroxyglutarate (2HG) and the consumption of NADPH. Notably, in patients harboring mutant IDH1 or IDH2, 2HG levels may accumulate to millimolar concentrations. Given that 2HG has no known biological function, the significance of 2HG production in promotion of tumorigenesis is presumed to lie in its ability to act as a competitive inhibitor for α -ketoglutarate-dependent enzymes, such as TET proteins, prolyl hydroxylases and lysyl hydroxylases. Accordingly, 2HG may alter major cellular processes, including epigenetic regulation, histone modification and HIF signaling, all of which are known to contribute to tumor initiation and growth (please see review Ref. 29).

Within the past 3 years, potent small molecule inhibitors of both mutant isocitrate dehydrogenase 1 (mtIDH1) and mtIDH2 have been developed, which were found to suppress 2HG production, and ultimately decrease glioma xenograft growth or induce terminal differentiation in patient-derived acute myeloid leukemia (AML), respectively.^{30,31} Because of the selective targeting of these agents, multiple clinical Phase I trials have been initiated to examine the toxicity and efficacy in both solid and hematological malignancies. AG-120 is an orally bioavailable selective mtIDH1 inhibitor developed by Agios Pharmaceuticals that is being tested against solid tumors (NCT02073994) or refractory AML or myelodysplastic syndrome (NCT02074839) in patients harboring IDH1 mutations. Novartis is currently assessing another mtIDH1 inhibitor (IDH305) in patients with advanced malignancies (NCT02381886). In addition to an

mtIDH1 inhibitor, Agios is examining an mtIDH2 antagonist (AG-221) in advanced solid tumors (NCT02273729) and hematological malignancies (NCT01915498). Recent data from these trials have demonstrated that both AG-120 and AG-221 exhibit significant clinical activity. Bone marrow assessment at day 28 in 14 refractory mtIDH1-positive AML patients revealed that dose-escalation treatment with AG-120 resulted in an overall response rate of 50% including four complete responses, two bone marrow complete responses and one partial response.³² In addition, administration of AG-221 led to an overall response rate of 56% (24/45: in patients with hematological malignancies with IDH2 mutations).³² Importantly, both AG-120 and AG-221 were found to induce blast differentiation in these patients and both agents were well tolerated as evident by the lack of an achievable maximum tolerated dose.³² Taken together, these results indicate that selective targeting of mutant metabolic enzymes may hold great promise for the treatment of cancer patients.

Targeting pyruvate dehydrogenase complex: CPI-613 and dichloroacetate

The pyruvate dehydrogenase complex (PDH) catalyzes the conversion of pyruvate to acetyl CoA for subsequent oxidation in the TCA cycle. PDH is positively regulated by pyruvate dehydrogenase phosphatase (PDP) and negatively regulated by phosphorylation of the E1 α subunit by the serine/threonine pyruvate dehydrogenase kinase (PDK). Glycolysis and the TCA cycle are connected by PDH which directs carbons away from lactate production and into the TCA cycle. Since the TCA cycle is important for the production of anabolic precursors, inhibition of PDH is potentially an effective anti-tumor therapy. There are two promising drugs that target PDH: CPI-613 that activates PDK (and thus inhibits PDH) and dichloroacetate (DCA) that inhibits PDK (and thus activates PDH).

A lipoamide mimic, CPI-613 activates PDK and thus causes phosphorylation and inactivation of PDH.³³ Preclinical studies demonstrated that CPI-613 reduced PDH activity and effectively killed tumor cells and was relatively non-toxic to primary normal cell counterparts. Results from a phase trial of CPI-613 with gemcitabine found that 50% of patients with breast and colon cancer experienced stable disease (4–16 weeks) with reductions in glucose uptake (4–42%) by FDG-PET imaging and initial results from the phase II study showed that increased CPI-613 dosing correlated with prolonged survival.³⁴ Finally, three phase 1 trials of CPI-613 in hematological malignancies reported at ASCO annual meetings (2010-2013) revealed significant signs of clinical activity with limited toxicity including a complete response in an AML patient and partial responses in a patient with Burkitt's lymphoma patient and another with cutaneous T-cell lymphoma.

As opposed to CPI-613, which activates PDK, and therefore inhibits PDH, DCA inhibits PDK, leading to activation of PDH. This causes a decrease in glycolytic flux to lactate and increase in oxidative metabolism that may create a hypoxic environment that disrupts oxidative phosphorylation in supporting host cells. The validation of DCA as an anti-cancer agent came from an initial report that demonstrated dramatic reductions in tumor size after DCA treatment in a nude rat A549 xenograft tumor model.³⁵ Unfortunately, DCA was not found to be effective in reducing tumor size or improving survival in a xenograft mouse model of breast cancer³⁶ suggesting that DCA may be effective in only certain types of tumors. Since GBM tumors are very glycolytic, and DCA can cross the blood brain barrier, DCA

was tested in a small clinical trial that included five GBM patients. After 15 months of DCA treatment, four patients had stable disease by computed tomography imaging and all were still alive at 18 months.³⁷ A phase 1 trial of DCA in 15 adults with recurrent malignant brain tumors (dosing based on haplotype variation of glutathione transferase zeta1/maleylcetoacetate isomerase) reported clinical stability in all patients and an average of 75.5 days of treatment.³⁸ A phase 2 trial of DCA in combination with chemoradiation (cisplatin with 70 Gy) in squamous cell carcinoma head and neck patients demonstrated increased myelosuppression but did not report any improvement in objective responses.³⁹ In summary, while there appears to be rationale to both inhibit and activate PDH in human cancers due to this enzyme's complex role at the crossroads between glycolysis and the TCA cycle, the clinical results thus far have been limited.

Conclusions

We have reviewed preliminary clinical data on multiple agents that target essential metabolic proteins and conclude that these agents have acceptable toxicity profiles and early signs of clinical activity. Several targeted agents that improve the survival of advanced cancer patients have been observed to reduce glucose uptake and to disrupt the TCA cycle and both high glucose and mitochondrial metabolism correlate with intrinsic and acquired resistance to cytotoxic and targeted agents. Although the pharmaceutical development and testing of these metabolic inhibitors is only just now beginning, we predict that combinations of metabolic inhibitors with cytotoxic and targeted agents will result in improved survival for cancer patients.

Acknowledgements

We thank Otto Grubraw and Brooke DeGroote for many discussions related to this manuscript. This work was supported by the National Cancer Institute [grant number CA149438 to J.C.; grant number CA166327 to B.F.C.] and by the American Cancer Society [grant number RSG 13-139-01-CNE to B.F.C.].

Conflict of interest: None declared.

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