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Follow the ATP: Tumor Energy Production: A Perspective

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Abstract: As early as the 1920s, the eminent physician and chemist, Otto Warburg, nominated for a second Nobel Prize for his work on fermentation, observed that the core metabolic signature of cancer cells is a high glycolytic flux. Warburg averred that the prime mover of cancer is defective mitochondrial respiration, which drives a switch to an alternative energy source, aerobic glycolysis in lieu of Oxidative Phosphorylation (OXPHOS), in an attempt to maintain cellular viability and support critical macromolecular needs. The cell, deprived of mitochondrial ATP production, must reprogram its metabolism as a secondary survival mechanism to maintain sufficient ATP and NADH levels for macromolecule production, membrane integrity and DNA synthesis as well as maintenance of membrane ionic gradients.

A time-tested method to identify and disrupt criminal activity is to "follow the money" since the illicit proceeds from crime are required to underwrite it. By analogy, strategies to target cancer involve following and disrupting the flow of ATP and NADH, the energetic and redox "currencies" of the cell, respectively, since the tumor requires high levels of ATP and NADH, not only for metastasis and proliferation, but also, on a more basic level, for survival. Accordingly, four broad ATP reduction strategies to impact and potentially derail cancer energy production are highlighted herein: 1) small molecule energy-restriction mimetic agents (ERMAs) that target various aspects of energy metabolism, 2) reduction of energy 'subsidization' with autophagy inhibitors, 3) acceleration of ATP turnover to increase energy inefficiency, and 4) dietary energy restriction to limit the energy supply.

Keywords: ATP, energy restriction, glycolysis, reactive oxygen species, warburg effect.

INTRODUCTION

"Cancer, above all other diseases, has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar. All normal body cells are thus obligate aerobes, whereas all cancer cells are partial anaerobes". Otto Warburg, at a 1966 meeting of Nobel laureates, described his theory on the origin of cancer as a metabolic disease.

Current anticancer drug development is dominated by a "personalized" archetype involving targeted inhibition of specific molecular drivers integral to tumor cell proliferation. The drive to the personalization of cancer has occurred directly as a consequence of the sequencing of the human genome and the identification of genetic predisposition to certain types cancer, leading to drug discovery efforts that targeted those specific mutated genes and/or the gene products. As specific gene signatures or gene mutations are not necessarily the driving mutations in all tumors, [1, 2] the outcome is a proliferation of different therapies that require specialized biomarker assays to identify the sometimes somewhat small proportion of patients that could benefit from the treatment [3].

However, if malignancy as Warburg [4] postulated is the result of dysfunctional mitochondria that cease to synthesize ATP leading to a compensatory increase in fermentation [5] then cancer is a homogenous, and not a heterogeneous, disease, driven by a simple energy imbalance. This metabolic perspective is contrary to the current consensus, which causally implicates the acquisition of multiple complex driver mutations resulting in distinct disease subtypes.

In the Warburg scenario, genetic mutations are the bystanders [6] at the scene of the crime, wrongly implicated based on circumstantial evidence, while the real criminal, fermentation, often escapes notice, despite Warburg's clear attestations to its centrality. In short, irreversible mitochondrial failure sets off and propagates a chain reaction of genetic abnormalities to support 'second-best' glycolytic ATP production. Indeed, the genetic mutations that are often seen as the primary origin of cancer may arise as a consequence of or as a compensation to the metabolic and energetic imbalances.

By extension, although never expressly stated by Warburg, mutated and/or overexpressed genes like HIF1-α, c-myc, ras, IGF-1, and PI3K/Akt/mTOR directly or indirectly enhance and support glycolytic metabolism and could therefore be viewed as secondary actors rather than the actual *agent provocateurs* of cancer since their function, ultimately, is to directly or indirectly enhance and support glycolytic metabolism. With hypoxia driving a switch to glycolysis and promoting expression of angiogenic factors and ultimately tumorigenesis [7] genetic instability [8] and metastases, a vicious cycle is formed potentiating reliance on sources of ATP other than oxidative phosphorylation. Like tumors which exist in a pseudo-hypoxic state, reliance on glycolysis for ATP is further amplified, driving an aggressive tumor phenotype [9] (Fig. **1**).

From a therapeutic point of view, with the exception of imatinib, [10] which has off target activity as an inhibitor of GLUT-1 and G6PD, and therefore also affects energy metabolism, the personalized genetic medicine revolution [11] has, to date, failed to deliver on its promise of safer and more effective anti-cancer agents. Nevertheless, in the opinion of authors such as Seyfried, it has become the predominant scientific paradigm, eclipsing and monopolizing all others; under its procrustean aegis any 'contrarian' theory such as the Warburg Effect, which dissents from the current gene-centric framework is banished to the margins [12]. The fervor to tackle and identify the bewildering complexity of druggable (and sometimes barely druggable! [13]) pathways has, for all intents and purposes, devolved into a molecular 'witch hunt' [2] that, largely devalues the role of energy status in tumor proliferation *in vivo* [14].

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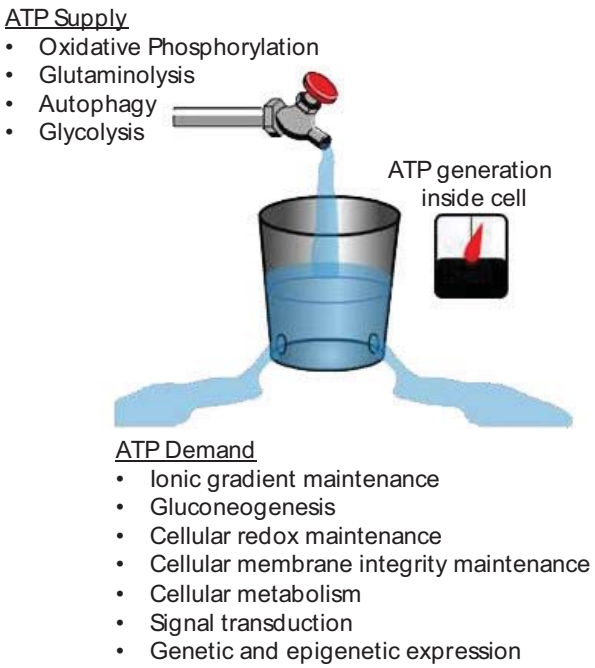


Fig. (1). ATP supply and demand.

Cancer is a many-headed Hydra and in response to signaling pathway 'decapitation' (i.e. inhibition) new pathways are activated in replacement. As long as there is sufficient energy available, a shift to a new pathway is likely to occur, even if it is energetically costly. Taken to its logical extreme, personalized medicine is akin to a game of pharmacogenomic "Whac-A-Mole", where the object is to knock down oncogenic driver mutations as they pop up, thereby forcing the tumor down different pathways. The assumption that underpins this personalized medicine strategy is that genetic mutations are at the heart of the neoplastic process. At the other end of the spectrum is the perspective that the network of overexpressed or aberrantly-functioning genes including HIF1-α, c-myc, ras, PI3K/Akt/mTOR, and IGF-1 [15] exist directly or indirectly to subserve the cellular energetic infrastructure, suggesting that genetic abnormalities are a consequence rather than a cause of cancer [14].

Just as law enforcement follows the money that finances the underworld operations of criminal and terrorist organizations [16] scientific investigation needs to follow the flow of ATP because the tumor economy is inextricably linked to a precarious single-source energy pipeline. This pipeline is potentially vulnerable to any demand or supply-side shocks, and significant ATP disruption will translate to a loss of ion homeostasis from Na+-K+-ATPase pump inhibition [17] with edema and cell death. The overreliance of otherwise heterogeneous cancer cells on glucose and glycolytic metabolism can be evidenced by understanding that increased glucose uptake is the basis for tumor detection and monitoring with ^{18}F-FDG PET/CT scans.

In addition to the dependency on glycolysis, cancer cells require glutamine and react poorly to glutamine [18] deprivation. Like glucose, glutamine is anaplerotic, meaning that it provides energetic precursors, such as oxaloacetate for the Krebs Cycle. As the carbon flux through glycolysis and glutaminolysis is increased, precursor intermediates accumulate, which are shunted to the Pentose Phosphate Pathway [19] resulting in the generation of NADPH, which reduces glutathione, enhancing amelioration of oxidative stress and producing ribose-5-phosphate, which is essential for the biosynthesis of nucleic acids (Fig. **2**).

In the face of genotoxic stress from chemo and radiotherapeutic treatments, the tumor must adapt to changing conditions and

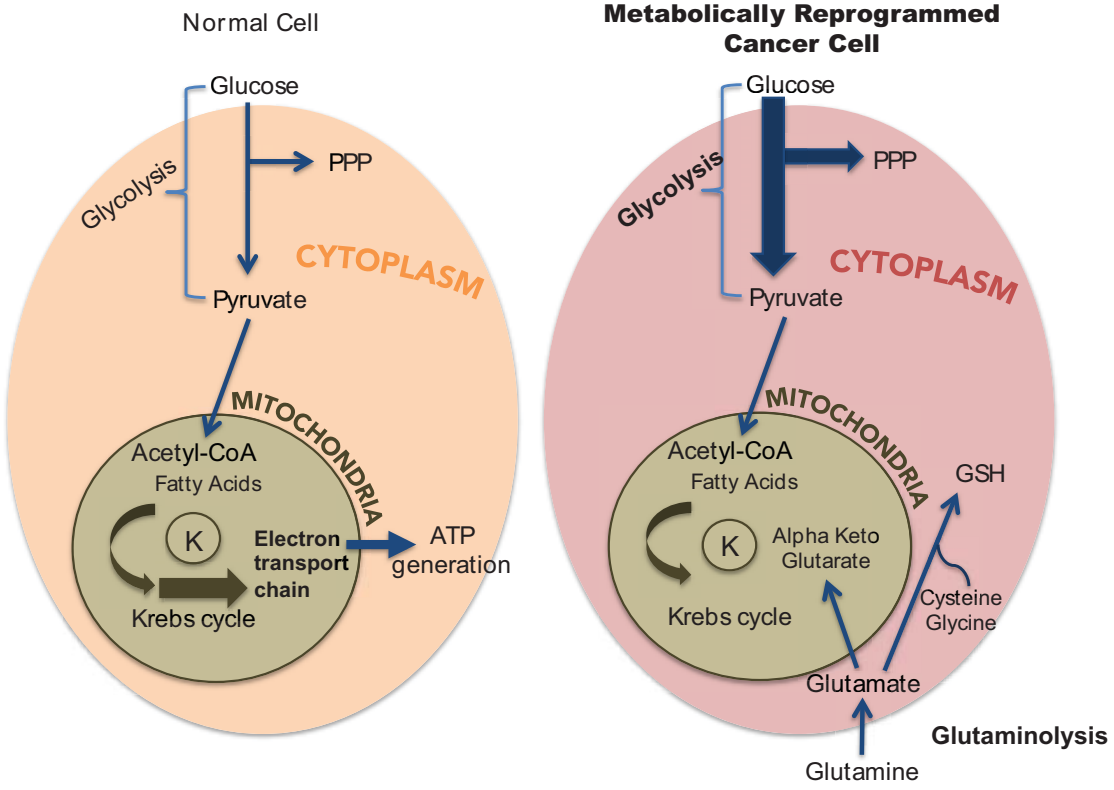


Fig. (2). Comparison between metabolism in normal cells relying on oxidative phosphorylation vs tumor cells utilizing glycoloysis and glutaminolysis.

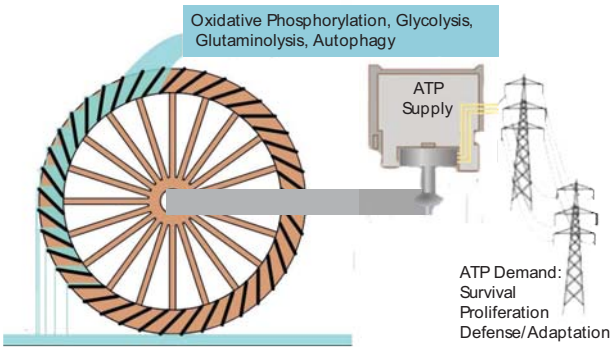


Fig. (3). Sources of ATP and energy in tumors.

mobilize self-defense mechanisms including drug efflux, DNA damage repair, upregulation of survival-related genes, including anti-apoptotic and intracellular survival signaling, all of which fuel the constant need for ATP [20] (Fig. **1**). The energetic price for each adaptation/defensive strategy must be covered by those pathways that are available to tumors for the production of ATP, notably the aerobic glycolysis/glutaminolysis and/or the Pentose Phosphate Pathway. However, these pathways are less efficient than oxidative phosphorylation and, if the demand for ATP overwhelms supply, leading to an 'energy deficit', the tumor has three strategic alternatives: increase ATP production, 'subsidize' ATP production with autophagy or curtail ATP consumption [21] (Fig. **3**).

With an abatement or limitation in ATP supply, energy expenditure is reduced to the detriment of various ancillary activities such as drug efflux mediated through the ATP-dependent multidrug resistance pumps and epigenetic silencing of tumor suppressor genes such as p53. Chen *et al* have reported that the energy restricting mimetic agents, 2-deoxyglucose and 3-bromopryuvate, epigenetically reactivate the silenced KLF6 tumor suppressor gene [22] Zhou *et al* have demonstrated [20] that ATP depletion in drug-resistant cells results in chemosensitivity while direct delivery of ATP into drug-sensitive cancer cells leads to chemoresistance.

Cancer cells require higher levels of ATP than normal tissue while lacking the flexibility to alternate between fuel sources [14]. Therefore ATP deprivation via direct inhibition of glycolysis will be selectively deleterious to tumor tissue.

This results in an emphasis on survival over growth and the maintenance of acquired resistance. Although this strategy has some similarity to the Warburg Effect as a unifying tenet of cancer and the basis and rationale for a shift in treatment strategies as promoted by multiple authors from Seyfried to Cairns, we propose that research on treatment strategies should consider not metabolism *per se* but overall ATP and NADH production.

Hence, a mismatch between energy supply and demand may trigger 'ATP anorexia' and contribute to a downward spiral, resulting in the ultimate failure of the tumor.

Rather than a Darwinian advantage, the dependence on fermentation may be a metabolic liability for the energetically boxed-in tumor, putting it at a competitive *disadvantage* compared to normal tissue. This disadvantage is therapeutically exploitable, in particular to increase chemosensitivity, as discussed at length below.

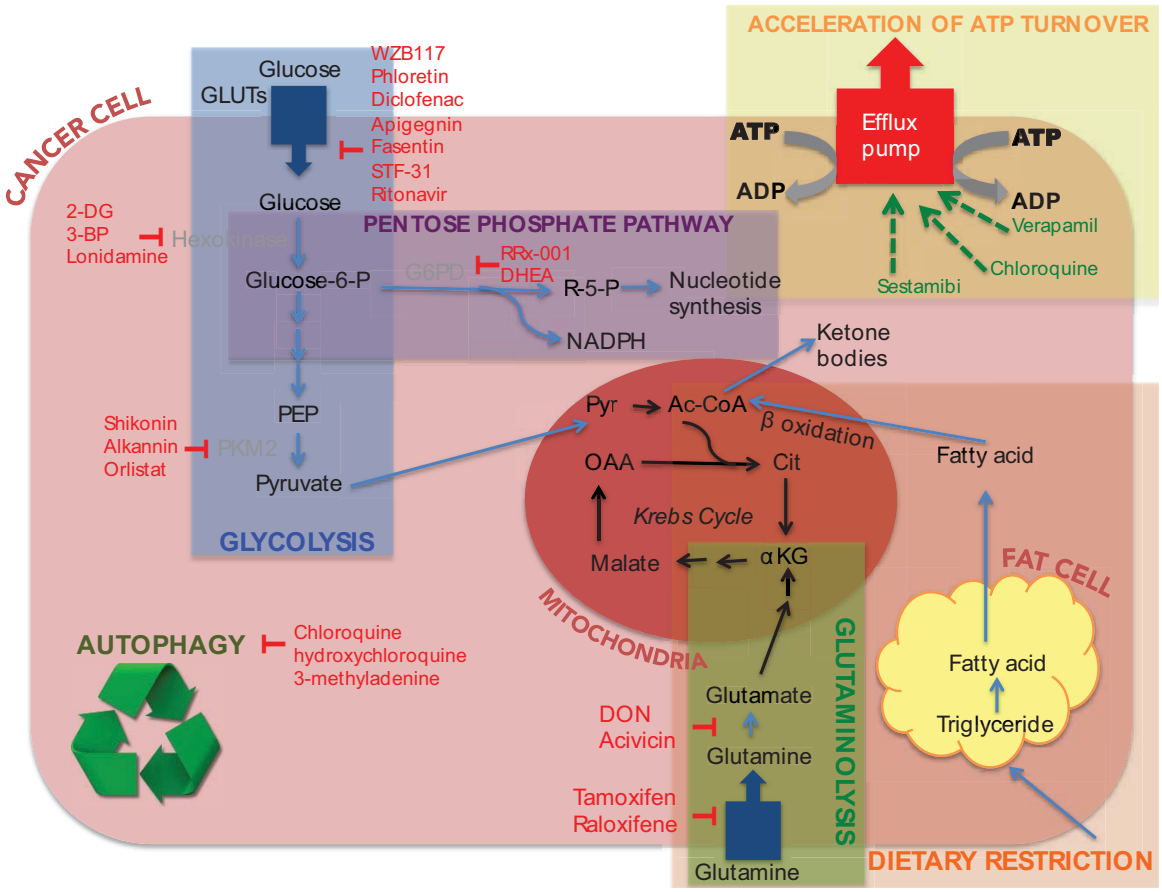


Fig. (4). Tumor energy production and disruption.

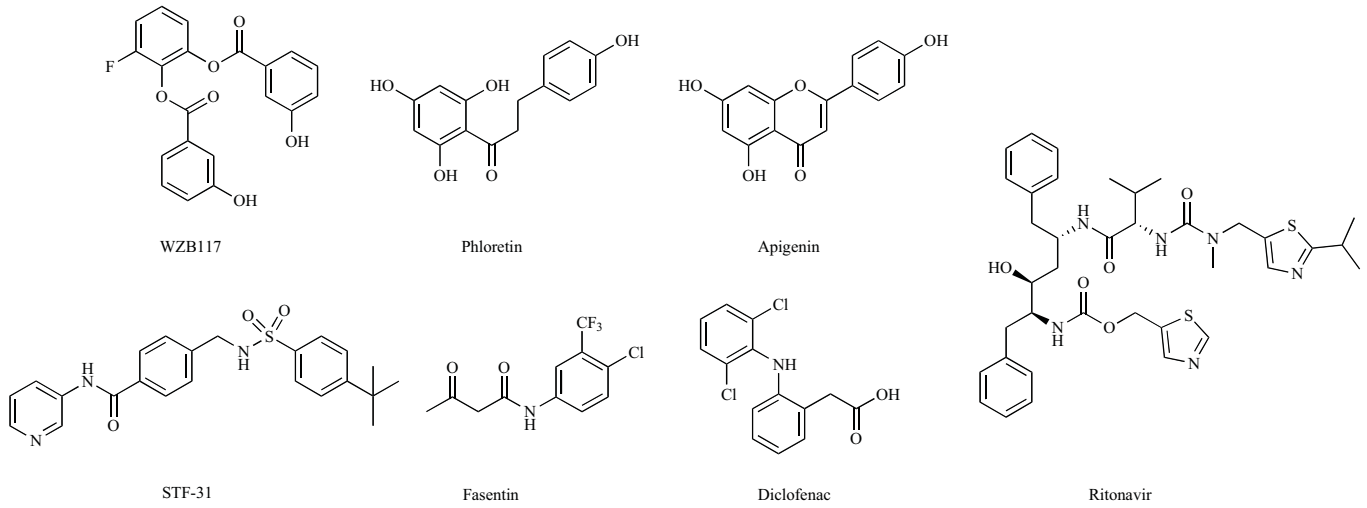


Fig. (5). Glucose transport inhibitors.

Renewed chemosensitivity to formerly failed chemotherapy, which has the potential to make cancer a chronic but treatable disease, is also a potentially imageable parameter in real time via a technetium 99m (99mTc) sestamibi scan. Since sestamibi is a substrate for the ATP dependent p-glycoprotein pump (Pgp) [23], encoded by the MDR-1 gene, this radionuclide imaging agent measures inhibition of Pgp, and therefore the relative degree of chemosensitivity compared to baseline. In this way, with imaging, it may be possible to "follow the ATP" (Fig. **4**).

GLUCOSE METABOLISM

Glucose Transporter Inhibitors

Due to its polar hydrophilicity, glucose is impermeable to the hydrophobic plasma membrane, requiring a family of specialized membrane-spanning transport proteins, Glucose Transporters or GLUT (GLUT1-14) to facilitate cell entry [24]. GLUT expression is highly upregulated in malignant cells, [25] reflecting the importance of glucose as a tumor ATP source, promoting increased glucose consumption, which, in turn, increases the rate of glucose metabolism [26] and ATP production [27]. The overexpression of GLUTs in various cancers has been associated with more aggressive tumor progression, greater invasiveness and poorer prognosis [28]. Below we discuss several GLUT-1 inhibitors including irreversible inhibitors (WZB117 and Phloretin), diclofenac, apigegnin, fasentin, STF-31 as well as the approved product ritonavir (Fig. **5**).

WZB117 and Phloretin are irreversible inhibitors of GLUT1 that decrease glucose uptake and intracellular ATP levels, resulting in a lowered rate of glycolysis and cellular growth [29]. Exogenously administered ATP rescued cancer cells from WZB117-induced cytotoxicity, suggesting that WZB117 and, by extension, other GLUT inhibitors, induce a loss of viability through depletion of ATP [29].

Diclofenac, in addition to its anti-inflammatory and anti-cancer activity, both of which are reported to be mediated through COX-1 and -2, is also a GLUT-1 inhibitor. Recent studies have demonstrated inhibition of glucose uptake in melanoma cells by this mechanism [30].

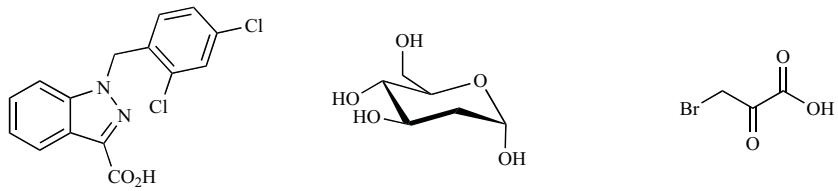
Similarly to Diclofenac, the naturally occurring flavonoid, apigenin, is also a GLUT-1 inhibitor. While other mechanisms, such as cell-cycle arrest have been postulated to rationalize its anti-tumor activity [31]. Apigenin was found to decrease glucose uptake in human pancreatic cell lines, through an inhibition of the expression of the GLUT-1 transporter [32]. A clinical trial investigating apigenin for the prevention of neoplasia recurrence in colorectal cancer is listed on clinicaltrials.gov (NCT00609310), but is currently not recruiting.

The observation that some patients being treated with the anti-HIV cocktail, HAART, had a lower incidence of some cancers prompted an investigation into the anti-tumor effects of the HIV protease inhibitors. Ritonavir was found to induce apoptosis in multiple myeloma [33] and ovarian cells [34]. Although the effect in ovarian cells was attributed to inhibition of the PI3K/Akt pathways, overexpression of glucose transporters in ovarian cancer (GLUT 1 and 3 rather than GLUT4) is linked with poor prognosis, ritonavir is known to inhibit the insulin-dependent glucose transporter GLUT4 [35], whose activation is indirectly linked to the presence of hypoxia [36]. An analysis of glucose transport in multiple myeloma cells revealed the importance of GLUT8 and GLUT11 for proliferation and cell viability and GLUT4 for basal glucose consumption, hence ATP generation [33]. Inhibition of glucose transporters also appears to result in chemosensitization *in vitro* possibly via downregulation of the P-glycoprotein ATPase pump. Ritonavir increased doxorubicin sensitivity in multiple myeloma cell lines while the GLUT-1 inhibitor, phloretin, sensitized P-glycoprotein overexpressed doxorubicin-resistant cells to daunorubicin [37].

In contrast to the molecules above which either exhibit off-target activity against GLUT-1 or are promiscuously active natural products, the selective small molecule GLUT-1 inhibitor, fasentin, was specifically discovered by a high throughput screening campaign [38] for its anti-GLUT-1 activity. Fasentin was found to sensitize cells to FAS and tumor necrosis factor apoptosis-inducing ligand (TRAIL) through glucose and nutrient restriction, in particular inhibition of glucose transport by interaction with a unique binding site in the intracellular channel of the transporter [39]. Although fasentin itself did not induce cell death, it represented a good starting point to a search for more potent non-natural product inhibitors of glucose transport such as the molecule STF-31 which, exhibits synthetic lethality towards cells that depend on GLUT1 for aerobic glycolysis [40].

GLUT-1 AND KIDNEY CANCER

The importance of metabolic pathways in cancer is exemplified by renal cell carcinoma. These typically hypoxic tumors rely heavily on glycolysis as a source of ATP. For example, inhibition or absence of function of the tumor suppressors succinate dehydrogenase (SDH) and fumarate hydratase (FH) allow the accumulation of their substrates (succinate and fumerate). These



Lonidamine 2-Deoxy-D-glucose (2-DG) 3-Bromopyruvate (3-BP)

Fig. (6). Hexokinase inhibitors.

function as metabolic signaling agents, inhibiting the action of prolyl hydroxylases (PHD). The action of these hydroxylases is critical to prevent the accumulation HIF-1α. Accumulation of HIF-1α leads to overexpression of the gene products VEGF, TGFβ, PDGF, GLUT1 and EPO promoting angiogenesis, differentiation, migration and proliferation. Overexpression of GLUT1 in particular, results in an addiction to glucose [41].

Renal Cell Carcinoma (RCC) is the most common form of kidney cancer in adults, responsible for 90-95% of renal cancer and is mostly resistant to chemotherapy and radiotherapy, including immunotherapy [42]. Recently, newer, targeted therapies, especially sunitinib (Sutent) and sorafenib (Nexavar), have provided some improvement in treatment outcomes [43, 44].

In most cases of RCC, the von Hippel–Lindau (VHL) tumor suppressor is inactivated [45-47] resulting in an accumulation of HIF-1α and a reliance on GLUT1 as a source of glucose and hence ATP, opening the possibility of designing molecules that exploit the synthetic lethality effect of inhibiting GLUT1 in VHL-inactive RCC.

The small molecule GLUT1 inhibitor, STF-31, was identified as the most potent of library of compounds by synthetic lethality screening with respect to inactivity of *VHL*. STF-31 inhibited glucose uptake and production of ATP in VHL-dependent manner leading to cell death in demonstrated tumor growth delay in a RCC tumor xenograft mouse model [40].

The ubiquitous expression of GLUT receptors in normal tissues may result in systemic toxicity at higher doses however, at lower doses, these agents have the potential to chemosensitize via ATP depletion and downregulation of MDR transporters without excessive toxicity. Therefore, at lower doses, these agents could be combined with cytotoxic drugs to both prevent and overcome chemoresistance.

HEXOKINASE INHIBITORS

Following transport across the cell membrane, glucose is phosphorylated to form glucose 6-phosphate (G6P).

This reaction, the first committed step of glycolysis, is catalyzed by the isoenzyme, hexokinase [48]. The hexokinase reaction phosphorylates nonionic glucose into the charged glucose-6-phosphate that is not a substrate for the glucose transporters and remains trapped in the cell [49].

The anti-tumor activity of the indazole-derived, orally bioavailable hexokinase inactivator, and anti-trypanosomal agent, Lonidamine, (Fig. **6**) acting through disassociation of HK from mitochondria, has been extensively studied both preclinically and in clinical trials. A small, single-agent, 12 patient Phase 2 trial, in malignant glioma produced only moderate activity with 2 responders and 3 stable disease of 10 evaluable patients [50], suggesting a limited role for dosing Lonidamine alone. However, more promising results were obtained in combination with chemotherapy. For example the combination of Lonidamine with epirubicin in breast cancer resulted in the reversal of resistance to epirubicin. Similar chemosensitization studies [51-54] were carried out in ovarian and lung cancer that have resulted in promising, but not conclusive, results, suggesting that further studies are required [55].

The identification of Mitochondria-bound hexokinase as target for therapy of malignant gliomas, [56] a tumor particularly dependent on glycolysis, renewed interest in lonidamine. The molecule showed promise as a radiosensitizer or chemo-radiosensitizer (with XRT and temizolamide [57]) in the treatment of malignant gliomas and resulted in a moderate increase in survival in a clinical study. Most recently, Lonidamine was studied in two clinical trials in BPH, however these were halted due to drug-related liver toxicity [58, 59]. A search of clinicaltrials.gov on Lonidamine, shows no current active clinical trials. Nevertheless, interest in lonidamine persists, with the nano-particle formulation approaches designed to overcome the toxicity issues [60].

2-deoxy-D-glucose (2-DG) and 3-bromopyruvate (3-BP) are small molecule inhibitors of hexokinase-II that deplete ATP and lead to chemosensitization and/or cell death.

2-DG is a glucose analog that is imported into cells and phosphorylated to 2-DG-P. However 2-DG-P is a terminal or dead-end product, incapable of further metabolism, resulting in an inhibition of glycolysis. While its efficacy as monotherapy is limited *in vivo* due to the high doses required as a result of an intrinsic competition with the endogenous glucose for the active site of hexokinase [49], in combination it significantly enhanced the anticancer activity of adriamycin and paclitaxel in mice bearing models of human osteosarcoma and non-small-cell lung cancer, [49] presumably through ATP depletion and inhibition of the multidrug resistance pumps, since to extrude drugs cancer cells must consume ATP [61].

Trastuzumab, a humanized monoclonal antibody against ErbB2, has shown efficacy treating ErbB2-positive breast cancer patients, however acquired resistance to this agent occurs in most patients and is a major drawback to the use of this drug. Tumors that are resistant to trastuzumab have been shown to have increased glucose uptake and lactate production, indicating a potential role for an adjuvant agent targeting glucose utilization. 2-DG was found to resensitize trastuzumab-resistant breast cancer cells to trastuzumab [62], both *in vivo* and *in vitro* suggesting a potential clinical strategy in breast cancer [63]. 2-DG has entered multiple Phase 1 clinical trials both alone and in combination with taxotere and radiation in which in all cases it was well tolerated and relatively non-toxic [64].

3-bromopyruvate (3-BP), an analog of pyruvic acid, is an inhibitor of hexokinase and 3-glyceraldehyde phosphate dehydrogenase, that also plays a part in glycolysis. In preclinical studies, 3-BP had significant *in vivo* therapeutic activity [65], having "eradicated" intra and extrahepatic tumors without apparent toxicity when administered *via* hepatic artery infusion. As a demonstration of the importance of ATP generation by tumors, Cardaci [66] showed that the cytotoxic effects of 3-BP could be enhanced by the inhibition of glutaminolysis, another non-mitochondrial source of ATP utilized by tumors. Since glutamine complements glucose in tumor cells, the cytotoxicity of 3-BP was significantly enhanced *in vitro* in the presence of glutamine-deprivation.

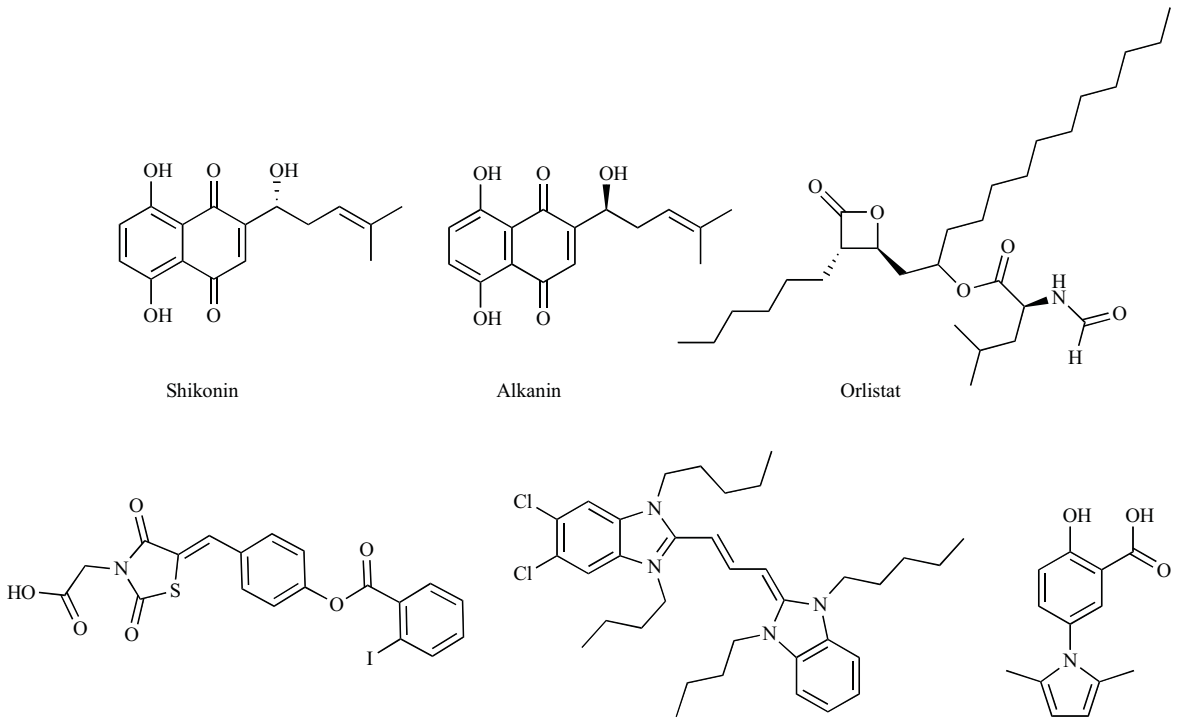


Fig. (7). PKM2 inhibitors.

PKM2 INHIBITION

In the penultimate step of glycolysis, phosphoenolpyruvate (PEP) is converted to pyruvate prior to conversion to lactate. Pyruvate formation is catalyzed by pyruvate kinase, which is expressed in four isoforms: L, R, M1 and M2 of which M2 is the predominant isoform in cancer cells. Paradoxically, the activity of PKM2 [67] is reduced compared to the other isoforms, which leads to an accumulation of upstream glycolytic intermediates for macromolecule biosynthesis such as Ribose-5-phosphate through the pentose phosphate pathway. While a direct tumor-suppressing role for PKM2 inhibition may seem counterintuitive, the PKM2-mediated conversion of PEP to pyruvate is responsible for ATP production and, therefore, indispensible for energetic homeostasis [68].

Shikonin (Fig. **7**), a naphthoquinone derivative of a Chinese herb with antitumor activities that are mediated, in part, through the generation of reactive oxygen species (ROS), inhibits PKM2 expression in cancer cell lines likely through oxidation of the critical cysteine 358 residue [69]. A Phase 2 trial has been opened with shikonin in bladder carcinoma, however no data has been reported to date.

Because of the prevalence of the M2 isoform in cancer compared to normal tissue, PKM2 has been an attractive target for drug discovery. High throughput screening of library of small drug-like molecules using an MTT-based assay generated an approximately 7% hit-rate for >50% inhibition [70]. Hit confirmation, carried out using secondary assays, resulted in the identification of three distinctive families of small molecule PKM2 inhibitors with IC_{50}'s ranging from 10-20 μM. Although inhibitors against PKM2 were identified, only moderate selectivity against the M1 isoform was achieved. For single agent activity, highly potent PKM2 inhibitors that fully shut down glycolysis and therefore ATP production would be required. Nevertheless, opportunities to exploit mildly active inhibitors that possess greater selectivity against other isoforms and that could be combined with other cytotoxic agents exist.

Shikonin and its analogues, including alkannin, a natural dye and food additive, have long been of interest due to their presence in Chinese herbal medicines and their wide ranging medicinal effects [71]. Both shikonin and alkannin were found to potently inhibit PKM2 at IC_{50}'s that did not inhibit PKM1 or pyruvate kinase-L (PKL), thereby lowering glycolysis and ATP production [72]. Chemopreventative activity in human skin cancer cell lines had also been attributed to these molecules [73]. No USA clinical trials for shikonin or the enantiomeric alkannin have been reported.

In an interesting example of drug repurposing, the gastrointestinal lipase and fatty acid synthase (FASN) inhibitor orlistat, that is indicated for the control of obesity, has been found to possess activity against tumor cell lines. Orlistat was recently found to inhibit expression of PKM2 in ovarian carcinoma cells, SKOV3, suggesting a potential application in the treatment of ovarian cancer [74].

PENTOSE PHOSPHATE PATHWAY

G6PD Inhibition

G6PD is a critical antioxidant enzyme for tumors, essential for maintenance of GSH levels to protect against ROS injury [75] and for the generation of NADPH via the Pentose Phosphate Pathway [76, 77]. Regeneration [78] of the cellular glutathione pool is contingent on the reducing power of NADPH, and tumors must maintain sufficient levels of reduced glutathione to protect against the deleterious effects of ROS. Therefore, inhibition of G6PD may result in a catastrophic build-up of RONS, promoting cell death.

Although the inhibition of G6PD by steroids has been reported as early as 1960 [79], the number of G6PD inhibitors under active development as anti-cancer agents is very low. To date, the

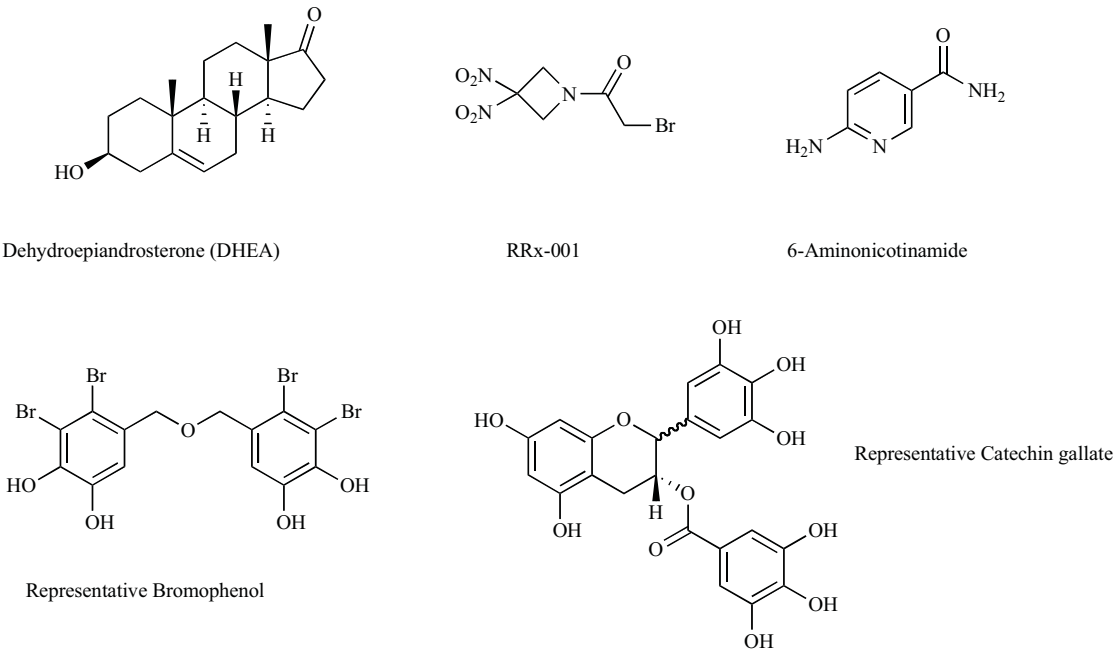


Fig. (8). G6PD inhibitors.

majority of G6PD inhibitors are limited mostly to steroids, including the endogenous dehydroepiandrosterone (DHEA) and to catechin gallates [80] (Fig. **8**). 6-Aminonicotinamide has also been reported as a selective and reversible inhibitor of G6PD together with naturally occurring bromophenols, isolated from marine algae [81]. With a rise in the importance of G6PD as a potential target for anticancer therapies [78, 82], approaches to the identification of more potent analogs of DHEA have become the focus of new work [83]. The development of multidrug resistance (MDR) in tumor cells has been linked to the activation of efflux pumps. Increased expression of the efflux pumps MRP1 and MRP2 in doxorubicin-resistant colorectal cancer cells (HT-29DX) was found to be associated with increased G6PD activity as well as the Pentose Phosphate Pathway, together with higher intracellular GSH levels. These data suggested that inhibition of G6PD, with or without intratumoral GSH depletion, may increase the sensitivity of the HT-29DX cells to doxorubicin through inactivation of efflux pumps [84].

The pan-epigenetic modulator, RRx-001 (Fig. **8**), which inhibits HDACs and DNA Methyltransferases, is the archetype of a structurally novel class of aerospace-derived compounds, designated dinitroazetidines [85], with evidence of antitumor activity in Phase 1 trials. As an electrophile [65], RRx-001, binds selectively to glutathione and a nucleophilic thiol on deoxyhemoglobin, beta Cys 93 [86]. The mechanism of anti-tumor activity of RRx-001 is related to increasing reactive oxygen and nitrogen species (RONS) [87, 88] in cancer cells, which are already under high constitutive oxidative stress [89, 90], leading to activation or inhibition of various cell targets. Cysteine dependent enzymes are particularly susceptible to oxidative modification and/or damage and with a cysteine residue at the active site required for catalysis, inhibition of glucose-6-phosphate dehydrogenase (G6PD) could be potentially mediated through the action of ROS. RRx-001 is a potent and selective inhibitor of G6PD in tumor cells [76], presumably rendering them more sensitive to the oxidative burst produced by RRx-001. Similarly, RRx-001 may have inhibitory effects on PKM2 through the induction of ROS.

In a Phase 1 dose escalation study in 25 heavily pretreated patients with advanced metastatic cancer, RRx-001 was escalated through six cohorts, and anticancer activity was observed at all dose levels in the absence of systemic toxicity, confirming preferential toxicity to malignant cells.

Consistent with the observation that ATP levels are a critical determinant of chemoresistance [20], RRx-001 was found to enhance the effect of subsequent chemotherapy in at least 5 patients, 4 with colorectal cancer and 1 with NSCLC. Two of the colorectal cancer patients had a robust response to FOLFIRI when administered after progression on RRx-001 – both had failed FOLFIRI previously [91]. This observation may subvert the assumption that chemoresistance is irreversible and suggest that resensitization may be a new paradigm for the treatment of cancer. As an ERMA with epigenetic p53 reactivating properties and a favorable toxicity profile, several Phase 2 clinical trials with RRx-001 are being started both as a single agent and in combination in multiple tumor types.

GLUTAMINOLYSIS

Glutamine Transporters

Cancer cells are addicted primarily to two nutrients—glucose and glutamine [18]. Through glutaminolysis, the conversion of glutamine to lactate, glutamine, the most abundant circulating amino acid, is metabolized to α-ketoglutarate, a key Krebs' cycle intermediate. Because the Krebs cycle provides ATP as well as a variety of important anabolic precursors including malate for gluconeogenesis, NADH for oxidative phosphorylation and Succinyl CoA for heme synthesis, "replenishing" or *anapleurotic* reactions are essential to offset the depletion of these carbon intermediates [92]. While normal cells metabolize glutamine only sparingly, tumors anapleurotically shunt glutamine-derived carbons into the Krebs' cycle. In addition, glutamine is the precursor (by way of glutamate) for reduced glutathione (GSH) [93]. Tamoxifen and Raloxifene (Fig. **9**) both block cellular uptake of glutamine through inhibition of the glutamine transporter, ASCT2, leading to decreased GSH production, accounting, in part for their cytotoxic effects. These can be reversed by increasing GSH through the addition of N-acetyl-L-cysteine and 17-beta-estradiol treatment [94].

Glutamine analogues acivicin [95, 96] and 6-diazo-5-oxo-L-norleucine (DON) [97] that inhibit glutamine catabolism and lead

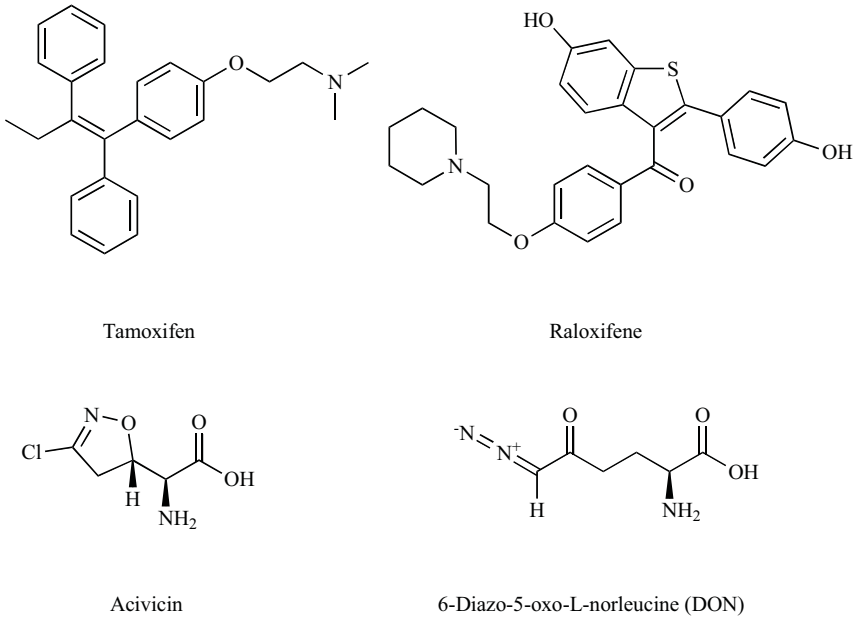


Fig. (9). Glutaminolysis inhibitors.

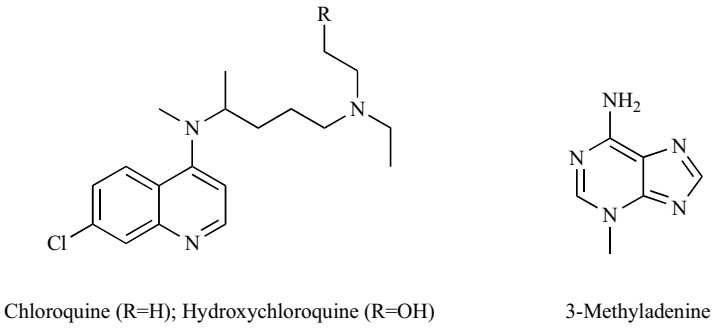


Fig. (10). Autophagy inhibitors.

to significant inhibition of cancer cell growth *in vitro* and in mouse xenograft studies, have been subjected to limited clinical investigation, because they lack specificity and therefore show a high rate of toxicity.

Autophagy inhibition

During resource deprivation, cancer cells scavenge and cannibalize critical molecules to fuel metabolism. Autophagy addiction is a form of self-subsidization to support ATP levels and thereby offset and minimize the drain on energy supplies. The most clinically studied autophagy inhibitors are the anti-malarial compounds, chloroquine (CQ) and hydroxychloroquine (HCQ) (Fig. **10**) that inhibit lysosomal proteases [98]. Like many of the ERMs highlighted above, chloroquine is associated with chemotherapeutic resensitization, for example in transtuzumab resistant Her2-positive breast cancer and also in other therapies [98]. Interestingly, chloroquine is also a known substrate for the P-gp pump MDR pump, which leads to ATP depletion (see below). Ongoing clinical trials in multiple tumor types involving CQ- and HCQ-mediated autophagy inhibition are listed at www.clinicaltrials.com. The flavinoid apigenin, that selectively induces tumor cell apoptosis, and inhibits GLUT-1 expression (described above), has also been found to strongly induce autophagy. Interestingly, the combination of apigenin and a potent autophagy inhibitor, 3-methyladenine, was found to significantly enhance tumor cell apoptosis induced by apigenin alone suggesting that a combination of apigenin and another autophagy inhibitor may be a promising anti-cancer therapy strategy [99].

Acceleration of ATP turnover

In response to an environmental 'shock' such as treatment with chemo or radiotherapy cancer cells must scale up ATP production to meet the demands of complex and costly phenotypic [100] changes that substantially reduce its viability. For example, many cancers upregulate membrane efflux pumps, which are associated with high energy costs. Gatenby *et al* have tested a novel strategy to decrease the supply of ATP with 2-deoxyglucose while simultaneously increasing the demand for it with verapamil, a non-chemotherapeutic substrate of the P-gp pump to stimulate ATPase activity and create a 'consumption crisis'. At sub-cytotoxic levels, verapamil [101] is known to revert the MDR phenotype in P-gp over-expressing cell lines. This 'decreased supply and increased demand' strategy of Gatenby *et al* should in theory lead to chemoresensitization and increased survival in *in vivo* models.

Dietary Restriction

The basis of dietary restriction in cancer is that it lowers glucose and induces a ketotic state [102]. Ketones, which are produced from the metabolism of fatty acids during calorie restriction or intense physical activity, are converted into acetyl-CoA which then enters the citric acid cycle and electron transport chain for energy. However because oxidative phosphorylation is dysfunctional in cancer, ketones are metabolizable only by normal and not malignant cells. Thus dietary restriction puts tumor cells in a double bind: 1) glycolysis is decreased and 2) starvation of

malignant tissue only ensues due to the inability to substitute ketone bodies for glucose.

Seyfried *et al.* have [102] demonstrated that growth retardation of gliomas in orthotopic mouse models is directly correlated with reduced levels of glucose and elevated levels of ketone bodies. Maurer *et al.* demonstrated [103] that glucose-deprived glioma cells are not able to adapt their metabolism to use of the ketone, β-hydroxybutyrate, unlike benign neuronal cells.

While a randomized dietary restriction clinical trial has, to date, not been conducted, the results of two case reports are encouraging. In 1995, Nebeling *et al* reported [104] a disease-free progression interval of 12 months in one of two female pediatric patients with advanced stage malignant Astrocytoma tumors treated with a long-term ketogenic i.e. high fat diet *in the absence of calorie restriction*. Seyfried *et al* treated [105] a 65-year old woman with glioblastoma multiforme with a strict 600 kcal/day ketogenic diet, in combination with standard therapy. Although a weight loss of 20% and complete regression of the tumor after two months of treatment was observed, the tumor recurred 10 weeks after suspension of strict diet therapy.

Despite these positive empirical findings, and strong theoretical underpinnings, the lack of a standardized protocol and concerns about weight loss in cachetic cancer patients may hinder implementation of calorie restricted, low glycemic or ketogenic diets. However, a search of clinicaltrials.gov revealed one randomized clinical trial entitled "Calorie-restricted, Ketogenic Diet and Transient Fasting During Reirradiation for Patients With Recurrent Glioblastoma (ERGO2)" and at least 4 actively recruiting Phase 1 trials involving ketogenic diets in different tumor types. More data should be available in the near future to determine the relative efficacy of this type of treatment in cancer either alone or, more likely, in combination as an adjunctive therapy.

Imaging Considerations

Two biomarkers for ATP production in the tumor, which are available for correlation, are 2-[^{18}F] fluoro-2-deoxy-d-glucose (FDG) and Tc-Sestamibi (Tc-MIBI). PET scans are already well established in the management of tumor malignancies. Tc-MIBI, which is a substrate for the P-glycoprotein pump system (P-gp) and the multidrug resistance protein (MRP) not only indicates the status of the drug efflux pumps (faster Tc-MIBI clearance equates with a higher concentration of pumps and vice versa) but, as a substrate, also serves to increase the ATP turnover rate of the pumps, making it a therapeutic as well as a diagnostic. This strategy will be tested in Phase 2 studies with RRx-001 and, in theory, the ATP status of the tumor can be assessed via cross-correlation between PET and Tc-MIBI.

CONCLUSIONS

Criminal or terrorist [106] organizations are heavily dependent on income streams to finance and foster their illegal activities, a point of vulnerability that has been exploited as a law enforcement strategy to destroy or downgrade their organizational and operational capabilities. Likewise, ATP and NADH, the energy and redox currency of the cell, respectively, are the two *sine qua nons* for tumor survival, growth, and expansion, which make it an Achilles' Heel for therapeutic exploitation.

Unlike criminal organizations that have the capability to diversify their portfolios and circumvent a freeze in their assets through a switch to alternative income sources, tumors are metabolically restricted to relatively inefficient ATP generators, namely glycolysis/glutaminolysis; moreover, the same inhospitable hypoxic and acidic microenvironmental conditions that favor tumor cells over normal cells and confer chemoresistance also force them to increase their glycolytic metabolism to compensate for the loss of oxidative phosphorylation.

Tumors, therefore, suffer from a resource "curse" and are metabolically fragile as a result. The persistence of acquired resistance requires conditions of matched supply and demand. When the supply of ATP is reduced, the lower energy efficiency of the tumor is a liability, as it exacerbates and intensifies the magnitude of the deprivation.

While treating cancer as many different diseases on the basis of particular "driver" mutations unique to each individual under the personalized medicine paradigm, it is important to consider the role of energy restriction. Teleologically, the *raison d'etre* of the cancer cell is survival, which depends critically on rapid ATP generation to fulfill bioenergetic and biosynthetic demands and the maintenance of cellular redox status. Canonical oncogenic signaling pathways such as PI3K/AKT and mTOR are integrated with and subserve metabolic and energetic processes, directly or indirectly to support increased biosynthesis and cell proliferation [107] leading to new concepts on combination strategies that link signaling pathway inhibition with energy restriction.

Simple microeconomics dictates that if aggregate demand for a particular critical resource(s) outstrips the supply, shortages ensue, with knock-on consequences throughout the whole economic system, if the shortages are pervasive and chronic. In the same way, if ATP levels are 'squeezed' due to pharmacologic or dietary-mediated restriction, the resulting energy 'crunch' exerts a downward pressure on ATP use and a reallocation of scarce resources to where they are most needed i.e. survival. This reallocation leads to a downregulation of energy-intensive ancillary activities such as the ATP-driven drug efflux pumps or epigenetic silencing of tumor suppressor genes through histone deacetylases or DNA methyltransferases. Inhibition of these tumor-specific activities can be exploited resulting in chemo- and radio-sensitization.

For this reason, it is the contention of this review that energy restriction is a therapeutically feasible strategy, particularly when used judiciously in combination strategies. Resensitization to formerly failed chemotherapies with epigenetic agents (eg. RRx-001) may trigger a *geometric* change in cancer care, from a linear approach across successive lines of therapy to a circular or pendular one involving reintroduction of radio/chemotherapies with a significant improvement in overall survival, turning cancer into a chronic disease.

Ideally, combined treatment with non-overlapping therapies, which might include pharmacologic inhibition of glycolysis, the Pentose Phosphate Pathway, glutaminolysis, autophagy and the addition of non-chemotherapeutics like verapamil which accelerate energy intensive MDR-mediated turnover combined with dietary restriction, all directed at ATP production, the single phenomenon that ultimately drives the progression of the disease, will yield the most efficacious results. Using agents that are not typically considered as part of the anti-cancer armamentum as adjuvants will become progressively more important.

ATP is the pivot, around which the whole tumor 'economy' revolves, and with anti-energetic strategies as a fulcrum it may be possible to move the world in the direction of a common, unified treatment for cancer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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