Glucose addiction in cancer therapy: advances and drawbacks

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Abbreviations

OXPHOS, oxidative phosphorylation; FDG-PET, fluoro-deoxy-glucose-positron emission tomography; RTK, Receptor Tyrosine Kinase; GLUT, glucose transporter; HKII, hexokinase II; PFK1, phosphofructokinase 1, PKM pyruvate kinase; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter, PDK, pyruvate dehydrogenase kinase; PPP, pentose phosphate pathway; VDAC, voltage-dependent anion channel; 2-DG, 2-Deoxy-D-glucose; 3-BP, 3-Bromopyruvate; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; ALL, acute lymphoblastic leukemia; Fru-2,6-P_2 2,6-bisphosphate; HIF-1α, hypoxia inducible factor 1, alpha subunit; ROS, reactive oxygen species; CHC, α-cyano-4-hydroxycinnamate; DIDS, 4,40-di-isothiocyanostilbene-2,20-disulfonate; PDH, pyruvate dehydrogenase; DCA, Dichloroacetate acid; GIST, Gastrointestinal Stromal Tumors; CML, chronic myeloid leukemia CML; TCA, tricarboxylic acid cycle; CLL, chronic lymphocytic leukemia; ECAR, extracellular acidification rate; NSCLC, non-small-cell lung carcinoma; HNSCC, head and neck squamous cell carcinoma;

Abstract

In contrast to differentiated normal cells, which primarily use mitochondrial respiration to generate the energy needed for cellular processes, most cancer cells rely on glycolysis, even in sufficient oxygen conditions, a phenomenon known as the "Warburg effect" or aerobic glycolysis. In the last years, much attention to the metabolic reprogramming of cancer cells has been paid by many research groups and, as a result, this altered energy metabolism was recognized in 2011 as one of the "hallmarks of cancer". Aerobic glycolysis allows a rapid growth of tumor cells, with high rates of glucose consumption and lactic acid production, leading to cellular acidosis.

Metabolic reprogramming renders cancer cells dependent on specific metabolic enzymes or pathways that could be exploited in cancer therapy. The development of treatments that target tumor glucose metabolism is receiving renewed attention, with several drugs targeting metabolic pathways currently in clinical trials. However, the search for suitable targets may be limited by the high plasticity of the metabolic network that can induce compensatory routes. Moreover, deregulated glucose metabolism has been also shown as a prominent feature associated with resistance to either classical chemotherapy or oncogene-targeted therapies, strengthening the clinical potential of combining these therapies with glycolysis inhibitors.

The aim of this review is to compare the advances of the different therapeutic strategies targeting the glucose "addiction" of tumor cells, highlighting the potential of these findings to be translated into effective weapons against cancer. Further, we will also present and discuss recent evidence for the involvement of glucose metabolism as a compensatory response to the use of drugs that target different signaling pathways, in which combination with glycolysis inhibitors are potentially useful.

Keywords: Glucose, cancer metabolism, Warburg effect, targeted therapy, drug resistance

Introduction

Reprograming energetic metabolism of cancer cells has been recently recognized by Hanahan and Weinberg in their review paper, as one of the "hallmarks of cancer" [1]. Altered cancer metabolism was first described by Otto Warburg in the 1920's, who observed that cancer cells exhibited high rates of glycolysis, independently of the levels of O_2 [2]. This phenomenon is known as "aerobic glycolysis" or "Warburg effect" and has been described in many cancer cells [3-5]. Warburg proposed that cancer cells adopt the glycolytic phenotype as a result of damages in mitochondria at the level of oxidative phosphorylation (OXPHOS) [2]. Actually, mitochondrial alterations can be found at different levels, including decreased expression of mitochondrial protein complexes required for OXPHOS [6, 7], and mitochondrial mutations, leading to malfunction of OXPHOS [4]. Additionally, inactivation of p53, one of the most commonly mutated genes in cancer, may also trigger the Warburg effect by interfering with the activity of cytochrome c oxidase, a protein also involved in OXPHOS [8]. However, Warburg's hypothesis has been recently refuted. In fact, altered energy metabolism is an oncogene-driven cell adaptation to support cancer cell proliferation and survival [9, 10]. It was demonstrated that OXPHOS is still functional in many cancer cells, since inhibition of glycolysis can enhance OXPHOS activity [11].

Even though the metabolic phenotype adopted by cancer cells can vary within the of cancer type and even among subtypes of the same cancer, addiction to glucose metabolism appears to be a widespread characteristic of cancer cells. Despite not being a very efficient pathway (glycolysis only produces 2 ATP molecules per glucose molecule consumed in contrast to the 30 ATPs produced by mitochondrial activity), glycolysis provides rapid production of energy, metabolites for anabolic reactions and even confers higher aggressiveness to cancer cells by the metabolites produced, mainly lactic acid [12]. Cancer cells overcome this lower energetic efficiency, by increasing the rates of glycolysis, partly by upregulation of glucose transporters, especially GLUT1-4 and some key glycolytic enzymes [13].

Glycolytic metabolism has recently drawn more attention to cancer research scientists and even to the pharmaceutical industry, either in the diagnosis or therapeutic area. The high capacity to promote the uptake of glucose has been already explored as a diagnostic tool, in which a radioactive non-metabolizable glucose analogue is used to identify the tumoural areas in the body, in the non-invasive imaging technique, FDG-PET (fluoro-deoxy-glucose-positron emission tomography) scan. Considering the vital role of metabolic reprogramming for tumor growth, during the last years, many studies have demonstrated that targeting cancer bioenergetics is a very promising approach for anti-cancer therapy development. In fact, many compounds have been developed to selectively and effectively inhibit metabolic enzymes that are upregulated in several tumors. These inhibitors are currently at various stages of the clinical trial process and we will expose during this review the success and the failures of targeting glucose metabolism.

Further, and not less important, the use of FDG-PET scan has also shown the first evidence that alterations in glucose metabolism can occur upon patients' treatment with oncogene-targeted therapies [14-20]. That evidence has suggested that, on one hand, glycolytic alterations could be involved in tumors' response to targeted therapies and, on the other hand, glucose metabolism is

regulated by protein kinases. In fact, the fast increase of research on the metabolic alterations of tumors has also improved our understanding of how oncogenes, specifically RTK pathways, are linked to altered cancer cell metabolism [9, 10]. Prolonged glucose deprivation induces cellular stress, which has been known to contribute to oncogenic mutations [21] and activation of survival pathways, including the PI3K/AKT pathway [22]. Thus, unlike the growth factor dependence of normal cells, it is known that cancer cells can maintain growth factor–independent glycolysis and survival through expression of oncogenic kinases [23-25]. Knowing that, very recent publications have demonstrated, in preclinical models, that metabolic rewiring of cancer cells can potentially drive resistance to targeted therapies, which will be discussed in detail in this review.

1. Glucose metabolism as target for cancer therapy

Different steps of the glucose metabolic pathway have been explored in diverse cancer models and some drugs against this pathway already entered clinical trials. The main targets include the glucose transporters 1.3 and 4 (GLUT1/3/4), hexokinase II (HKII), phosphofructokinase 1 (PFKF3B), pyruvate kinase 1 and 2 (PKM1/2), lactate dehydrogenase 5 (LDH5), monocarboxylate transporters (MCTs) and pyruvate dehydrogenase kinase (PDK) (see Figure 1).

1.1 Glucose transporters (GLUT1, GLUT3 and GLUT4)

The uptake of extracellular glucose is facilitated by the glucose transporter (GLUT) family proteins and, as a result, some of these family members have been described as commonly upregulated in cancer. The family comprises 14 members, being GLUT1 isoform the most well studied, showing overexpression in a variety of tumors, as well as a close association with tumor development and poor prognosis. Additional evidence indicates overexpression of other GLUT isoforms in cancer, especially GLUT3 and GLUT4 (reviewed in [13]). Thus, due to the fundamental role of glucose uptake in the glycolytic pathway, targeting glucose transporters is an attractive approach to inhibit tumor growth. Until recently, GLUT inhibitors showed no specificity, however, new compounds are now emerging.

1.1.1 GLUT inhibitors

Flavonoids (phloretin and silybin)

Phloretin is a natural phenol and has been shown to inhibit growth and trigger apoptosis in *in vitro* and *in vivo* models of a variety of tumor types, as a result of glucose transmembrane transport inhibition [26-30]. However, although frequently used as a specific GLUT inhibitor, phloretin has also shown activity as an inhibitor of the Na^+/glucose cotransporters SGLT1 and SGLT2 [31]. Importantly, phloretin was shown to sensitize colon and leukemia cancer cells to daunorubicin treatment, overcoming drug resistance under hypoxia [32], and to potentiate paclitaxel anticancer activity in both *in vitro* an *in vivo* models of liver cancer [33]. More recently, phloretin was also described as an

enhancer of γδ T cells' killing effect on SW116 colon cancer cells [34], providing an additional anticancer mechanism for phloretin.

Another natural compound, silybin/silibinin, has gained much attention in the last years, and was recently shown to be a GLUT inhibitor [35]. Preclinical studies, including both *in vitro* and *in vivo* studies, showed very promising results (reviewed in [36]), and phase I and II clinical trials using this flavonoid have been concluded in prostate cancer (NCT00487721) [37, 38] and advanced hepatocellular carcinoma (NCT01129570) [39]. In prostate cancer, although the phase I trial showed no grade 4 toxicity, liver toxicity was the most common adverse effect [38]. In the phase II trial, 1 out of 6 patients showed grade 4 toxicity and low tissue penetration was observed, notwithstanding high blood levels, warranting additional studies, such as longer treatment durations, sustained-release formulations to increase silybin half-time or combination with chemotherapy [37]. In fact, preclinical studies have shown that silybin reverses drug resistance and shows synergism with chemotherapeutic drugs [40-48], and a phase II clinical trial to assess the efficacy of combined erlotinib and silybin in patients with EGFR mutant lung adenocarcinomas is currently recruiting patients (NCT02146118). Although described as an inhibitor of GLUT [35], many other antitumoral activities have been attributed to silybin, including inhibition of telomerase expression [49, 50] and activity [51], inhibition of Notch signaling [52, 53], suppression of nuclear factor kappa B activation [54], decrease of angiogenic modulators [55], among others (reviewed in [36]).

As mentioned, these natural compounds have several biological properties besides GLUT inhibition, rendering objective conclusions regarding the therapeutic value of glucose uptake inhibition difficult. For that, studies using specific GLUT inhibitors are warranted.

STF-31

Recently, a promising specific inhibitor of GLUT1 aroused from a high-troughput chemical synthetic lethal screen in renal cell carcinomas. In this study, a class of compounds, exemplified by STF-31, that targets loss of von *Hippel-Lindau* tumor suppressor gene by direct binding to GLUT1 was identified. These compounds specifically target glucose uptake, showing promising results both *in vitro* and *in vivo*, without *in vivo* toxicity to normal tissues [56]. Additionally, STF-31 was shown to selective eliminate human pluripotent stem cells (hPSCs), which show similarities with cancer cells, from mixed cultures, in an attempted to investigate if surface expression of GLUT1 is required for hPSC biology [57]. However, a very recent publication demonstrated that nicotinamide phosphoribosyltransferase (NAMPT) is the actual target of STF-31, while providing data that suggests that GLUT1 does not mediate the cytotoxic effects of STF-31-like compounds [58]. Therefore, further studies are warranted to define the activity of these compouds.

WZB117

WZB117 was also described as a specific inhibitor of GLUT1. This compound was shown to induce a dose-dependent decrease in glucose transport, a decrease in extracellular lactate levels as well as in intracellular ATP in cancer cells, accompanied by a reduction of GLUT1 and glycolytic enzyme levels. Additionally, WZB117 induced cell cycle arrest *in vitro* and inhibited tumor growth *in vivo*,

with relatively low toxicity. Importantly, exogenous ATP rescued *in vitro* cancer cell growth, indicating that intracellular ATP reduction plays an important role in WZB117-induced growth inhibition. Also, WZB117 showed synergistic effects when combined with cisplatin or paclitaxel [59]. Similarly to STF-31, WZB117 was also shown to selectively eliminate hPSCs from mixed cultures, effect attributed to GLUT1 specific inhibition [57]. However, also similarly to STF-31, further studies are warranted to validate the activity of WZB117 as a specific inhibitor of GLUT1.

HIV protease inhibitor therapy

Studies addressing the mechanism involved in HIV protease inhibitors-induced insulin resistance demonstrated that these inhibitors, especially ritonavir, inhibit GLUT4, but not GLUT1, activity, both *in vitro* an *in vivo* [60, 61]. This off-target activity of ritonavir has been explored in multiple myeloma cell lines, where ritonavir treatment decreased both glucose transport and proliferation in a dose-dependent manner. Also, ritonavir treatment sensitized cancer cells to doxorubicin treatment, showing a promising therapeutic potential of ritonavir-mediated GLUT4 inhibition in multiple myeloma [62].

From all the above, as well as additional data showing upregulation of GLUT3 in temozolomide-resistant glioblastoma cells [63], the use of combined therapies using glucose uptake inhibitors and standard chemotherapeutic agents seems the most promising approach to potentiate cancer therapy as well as overcome drug resistance.

1.2 Hexokinase II (HKII)

The first rate-limiting step in the glycolytic pathway consists in the phosphorylation of glucose to form glucose 6-phosphate, trapping glucose inside the cell to fuel both glycolysis and the pentose phosphate pathway (PPP). This reaction is provided by hexokinases (HK), a family of 4 members (I-IV); HKI and HKII are usually found in the outer membrane of mitochondria, HKIII is found in a perinuclear compartment and HKIV is found in the cytoplasm. Is has been described that mitochondrial location of HKI and HKII is largely dependent on interaction with the outer membrane voltage-dependent anion channel (VDAC) and that this location is a strategy for preferential access to ATP as well as insensitivity to negative regulation by glucose-6-phosphate. Importantly, the association of HK with VDAC interferes with the interaction of VDAC with the apoptotic inhibitor Bcl-X_L, contributing to apoptosis evasion (reviewed in [64]). HKII is the predominant isoform overexpressed in malignant tumors and the properties of this isoform that are on the basis for its upregulation over the other isoforms in cancer is reviewed elsewhere [65]. Importantly, since most normal mammalian tissues express very little HKII, with muscle, adipocytes and lung expressing low but significant levels [65], a therapeutic window to inhibit HKII without important on-target side-effects is antecipated [66].

1.2.1 HK inhibitors

2-Deoxy-D-glucose (2-DG)

The glucose analogue 2-deoxy-D-glucose (2-DG) is the most widely used glycolysis inhibitor [67], being one of the most advanced clinical agent inhibiting cancer metabolism. It is phosphorylated to 2-DG-6-phosphate by HK but cannot be further metabolized, therefore inhibiting HK activity by noncompetitive inhibition. Although 2-DG is mainly described as a HK inhibitor, 2-DG-6-phosphate is a competitive inhibitor of enzymes that metabolize glucose-6-phosphate and 2-DG may also compete with glucose for GLUT, further inhibiting the glycolytic rate, NADH and lactate production, as well as decreasing ATP levels. Importantly, besides the expected anticancer effects of 2-DG, including *in vitro* inhibition of proliferation and colony formation as well as *in vivo* tumor growth inhibition, 2-DG induces apoptosis, autophagy, proteolytic events and endoplasmic reticulum stress (reviewed in [68]). As a competitive inhibitor, the single use of 2-DG is limited by the high concentrations necessary to compete with glycolytic pathway products [69], however, *in vitro* studies using combination of 2-DG and standard therapeutic agents such as cisplatin and docetaxel show that 2-DG may potentiate the effect of standard therapy [64, 67]. The first use of 2-DG in humans as an anticancer drug dates back to 1958 [70] and there is no clinical trial currently is ongoing. Previous studies showed an improvement of radiotherapy effect if combined with 2-DG in brain tumors, with acceptable toxicity (reviewed in [71]), however, there are doubts if these studies used clinically relevant doses of 2-DG [72]. From the three 2-DG therapeutic clinical trials listed in the U.S. National Institutes of Health's database (NCT00096707, NCT00247403 and NCT00633087; clinicaltrials.gov), only one has been completed (NCT00096707). In this phase I clinical trial, the safety, tolerability, pharmacokinetics, and biologic effect of daily oral doses of 2-DG in combination with weekly docetaxel were evaluated in subjects with advanced solid tumors. After determining the 2-DG clinically tolerable dose, the most significant adverse effects were reversible hyperglycemia, gastrointestinal bleeding and reversible 3 QTc prolongation [73]. The other two clinical trials were not successful, as one was withdrawn prior to enrollment since the pharmaceutical company ceased drug manufacturing (NCT00247403) and the other was suspended due to slow accrual (NCT00633087), but was able to define a dose for phase II trials [74]. Therefore, 2-DG phase II/III clinical trials are warranted to progress beyond the current state.

Lonidamine

Lonidamine is a derivative of indazole-3-carboxylic acid and was firstly designed and synthesized to act as an antispermatogenic drug [75]; however, currently, this compound is vastly known thanks to its anticancer activity. In the early 80s, lonidamine was described as a specific inhibitor of mitochondrially bound hexokinase [76], but, more recently, other effects have been described for lonidamine, including inhibition of lactate transport and modification of membranes' permeability (reviewed in [77]). In fact, preclinical studies showed that lonidamine, besides inhibiting cancer cell energy metabolism [76], also induces apoptosis [78, 79], enhances the activity of standard anticancer drugs [80-83] and reverses chemoresistance [84, 85] (reviewed in [77]). A large number of

clinical studies using lonidamine, alone or in combination with standard chemotherapeutic agents, were performed during the 80s and 90s in various tumor types, mainly lung and breast cancer (reviewed in [77]), and additional studies were performed more recently in breast cancer [86, 87], glioblastoma multiforme [88] and ovarian cancer [89]. Also, until recently, this compound was in phase II/III clinical studies for the treatment of benign prostatic hyperplasia (NCT00237536 and NCT00435448), however, the trials have been suspended due to hepatotoxicity [90].

Overall, although some studies showed encouraging results, an unequivocal clinical benefit of lonidamine use was not found.

Interestingly, lonidamine has also been shown to interfere with angiogenesis-related endothelial cell functions including proliferation, migration, invasion and morphogenesis, which may contribute to the antitumor effect of lonidamine observed in *in vivo* experiments and patients [91]. Finally, recent studies using paclitaxel/lonidamine loaded EGFR-targeted nanoparticles in *in vitro* and *in vivo* models of multidrug resistant breast and ovarian cancer showed that the nanocarrier system enhanced the therapeutic index of both drugs and decreased toxicity, indicating that this new delivery approach may be a promising strategy for treatment of multidrug resistant tumors [92-94].

3-bromopyruvate (3-BP)

The halogenated analog of pyruvate 3-bromopyruvate (3-BP) is the current leading HKII inhibitor [90]. This compound, similarly to the other HKII inhibitors, depletes cellular ATP reserves, decreasing ATP-binding cassette transporters (ABC transporters) activity and restoring drug retention in malignant cells. This leads to 3-BP-induced enhanced cancer cell sensitivity to anticancer drugs such as daunorubicin and doxorubicin, and inhibition of *in vivo* tumor growth, alone or combined with standard anticancer agents [95-98]. Also, ATP depletion by 3-BP reverses colon cancer cells' resistance to oxaliplatin and 5-fluorouracil treatment [99]. Although initially described as a HKII inhibitor more effective than 2-DG [100], GAPDH rather than HKII is the major target of 3-BP. Other targets have also been described for 3-BP, including pyruvate kinase, lactate dehydrogenase, endoplasmic reticulum and lysosomes (reviewed in [101, 102]). Importantly, massive parallel sequencing identified monocarboxylate transporter 1 (MCT1), which is frequently upregulated in cancer (reviewed in [103]) as the main determinant for 3-BP sensitivity, most probably by mediating 3-BP uptake by cancer cells, being a potential biomarker for selection of 3-BP responsive tumors [104]. Following the promising results from both *in vitro* an *in vivo* studies (reviewed in [102]), recently, a 3-BP case study was performed in a young adult patient presenting fibrolamellar hepatocellular carcinoma, who survived a much longer period than expected with an improved quality of life, as a result of treatment with 3-BP [105]. Despite all these promising results, 3-BP has not yet entered clinical trials.

Additionally, the three above-mentioned HKII inhibitors have been shown to increase *in vitro* sensitivity of acute lymphoblastic leukemia (ALL) cells to glucocorticoids, while 2DG was able to revert glucocorticoid resistance in both ALL cell lines and primary leukemia cells isolated from pediatric ALL [106].

Alternative approaches to target HK include targeting HKII-VDAC complexes, the plant hormone methyl jasmonate, Casiopeina II-gly synthetic compound and small hairpin RNA (reviewed in [64]).

1.3 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3)

One of the most critically regulated enzymes of glycolysis is 6-phosphofructo-1-kinase (PFK-1), being fructose 2,6-bisphosphate (Fru-2,6-P_2) a powerful activator of this enzyme. The levels of Fru-2,6-P_2 are controlled by a family of bifunctional enzymes (6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase; PFK-2/FBPase-2 or PFKFB), which are responsible for both the synthesis of Fru-2,6-P_2 from fructose 6-phosphate (PFK-2 domain) and hydrolysis of Fru-2,6-P_2 to fructose 6-phosphate (FBPase-2 domain). Among the several isoenzimes that constitute PFKFB family, PFKFB3, due to its predominant kinase activity compared to phosphatase activity, is more likely to contribute to the high glycolytic activity of cancer cells (reviewed in [107]). In fact, several studies show upregulation of PFKFB3 in cancer cells [108-110], and silencing studies show a role of this enzyme in cell cycle, anchorage-independent growth and apoptosis suppression [111, 112]. Additionally, a role of PFKFB3 in vessel sprouting has been recently described [113].

1.3.1 PFKFB3 inhibitors

3PO and derivatives

Until very recently, the only specific inhibitor of PFKFB3 was 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one, commonly known as 3PO [114]. This inhibitor was shown to decrease the intracellular concentrations of Fru-2,6-P_2, leading to a decrease in glucose uptake, as well as in intracellular ATP and lactate. Also, *in vitro* use of 3PO inhibited the proliferation of several human cancer cells lines and showed selectivity for *ras*-transformed cells *versus* normal cells, while inhibition of tumor growth *in vivo* was also observed [114]. More recently, 3PO was shown to cause, besides a decrease in glucose consumption, an increase in autophagy [115]. The authors raised the hypothesis that 3PO-induced autophagy may protect cells from 3PO-induced apoptosis, defending the combined use of 3PO with inhibitors of autophagy for cancer treatment [115]. In the meantime, the 3PO derivative 1-(4-pyridinyl)-3-(2-quinolinyl)-2-propen-1-one (PFK15), which showed to be a more potent PFKFB3 inhibitor, was shown to rapidly induce apoptosis in transformed cells and suppresse tumor growth in Lewis lung carcinomas in syngeneic mice as well as in human xenograft tumors [116]. A phase I clinical trial using PFK15 was expected to initiate in 2013 [116]. Besides direct inhibition of cancer cell growth, additional effects of 3PO-induced inhibition of PFKFB3 include angiogenesis inhibition [117] and suppression of T cell activation [118], which may further contribute to the exploitation of PFKFB3 as a strategy for cancer therapy.

1.4 Pyruvate kinase isozyme M2 (PKM2)

The M1 isoform of PK (PKM1) is expressed in normal proliferating cells and tissues such as lung, retina, pancreatic islets, fat cells, while M2 isoform (PKM2) an alternatively spliced variant of M1, is expressed during embryonic development [119]. Moreover, this enzyme is overexpressed in many cancers and not in normal adult tissues making it a promising anti-cancer therapeutic target. PKM2 may be expressed either as an active tetramer or as a nearly inactive dimer PKM2 [120]. The balance between tetrameric and dimeric PKM2 conformation occurs in an oscillating form, subject to phosphorylation and allosteric regulation [121].

PKM2 has been reported to promote cell survival, cell migration and cell invasion in several types of cancer such as colon and gastric carcinoma cells. There is are also evidence suggesting that PKM2 could involved in cancer initiation and transformation (reviewed in [122]). Importantly, due to the higher expression of PKM2 in cancer, a clinical trial was created to determine in plasma and saliva if PKM2 could be a useful biomarker for pleural malignancies (NCT001130584). This study was already completed, unfortunately with no reported results. Given all these important functions, targeting PKM2 in cancer cells is an attractive approach and that, different inhibitors have been developed and tested with promising results.

1.4.1 PKM2 inhibitors

TLN-232/CAP-232

Thallion Pharmaceuticals initiated in 2007 the first clinical trial with the PKM2 inhibitor TLN-232/CAP-232, a seven amino-acid peptide. This phase II clinical trial (NCT00422786) was conducted in patients with refractory metastatic renal cell carcinoma and promising results were reported in 2008. Thus, the drug TLN-232 was generally safe and well tolerated and the outcome was that two out of the 3 patients complete the study with stable disease. Thallion's studies suggest that the anti-cancer activity of TLN-232 is mediated by a mechanism involving the translocation of PKM2 to the nucleus, resulting in cell death. This compound was also in phase II clinical trial for the treatment of metastatic melanoma (NCT00735332), however, the trial has been suspended due to an ongoing dispute with the licensor. Additionally, the inhibition of PKM2 has become controversial since some studies showed that inhibition of PKM2 by post-translational modifications could support cancer cell proliferation and different PKM2 activators had an inhibitory effect on tumorigenesis [123, 124]. PKM2 contains an inducible nuclear translocation signal in its C-domain, making its role complex [123, 125]. Furthermore, this enzyme is involved in a variety of pathways with potential to perform multiple non-glycolytic functions that could be the base to explain the controversial results. Thus, it is urgent to explore the multidimensional role of this protein to find new therapeutic approaches or to identify possible metabolic weaknesses that could be exploited using drug combination.

1.5 Lactate dehydrogenase A (LDHA)

LDH belong to a family of tetrameric enzymes that are formed by two major subunits, M and H, which can assemble into five different combinations. Two of the resulting isoforms are

homotetramers – LDH-1 (H4) and LDH-5 (M4) also known as LDHB and LDHA, respectively [126, 127]. LDHB is ubiquitously expressed in normal tissue an it is responsible for the conversion of lactate into pyruvate, while, LDHA that is induced by the hypoxia inducible factor 1α (HIF-1α) is found in highly glycolytic tissues [128]. Therefore, LDHA plays a key role in regulating glycolysis by catalysing the final step of anaerobic glycolysis, converting pyruvate into lactate for the regeneration of NAD+ to accelerate glycolysis and facilitates the efficiency of these metabolic pathway in tumor cells reducing their dependency on oxygen [129]. The levels of LDHA are elevated in many malignant tumors and is associated with tumor proliferation and malignant growth with potential implications in cancer therapy. Moreover, monitoring serum LDH levels have been shown to be a useful prognostic tool [130]. Thereby, inhibition of LDHA expression could potentially interfere with cancer development and indeed there are already molecules that have been identified to inhibit LDH.

1.5.1 LDHA inhibitors

Gossypol/AT-101 and derivates

Gossypol (also known as AT-101), is a non-selective inhibitor of LDH that blocks binding to NADH, with a Ki of 1.9 μM for LDHA. This drug was initially developed as a therapy against malaria [131] and it was firstly used in humans as a male contraceptive. Previous clinical studies showed that gossypol is well-tolerated with the most common adverse events being gastrointestinal toxicities and fatigue. A phase I/II clinical oncology trial (NCT00773955) developed in patients with chemotherapy-sensitive relapsed small cell lung carcinomas revealed disappointing results [132]. Moreover, a phase I/II study (NCT00397293) of AT-101 in combination with topotecan in patients with the same type of cancer was also designed, but it was interrupted because since it did not meet its primary endpoints [133]. Currently, no clinical trial as monotherapy agent is ongoing, yet some new studies in combination with other drugs are starting to recruit participants diagnosed with lymphocytic leukemia and non-small cell lung cancer (NCT01003769 and NCT01977209, respectively). Unfortunately, due to the two aldehyde groups, gossypol has a chemical structure highly reactive and can chelate metal ions. This results in biological system toxicity and different side effects such as cardiac arrhythmias, hypokalemia, renal failure, muscle weakness and sometimes paralysis [126].

Additionally, 3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propylnaphthalene-1-carboxylic acid (FX11), has been identified through screening of a bank of compounds derived from gossypol. This compound has been shown to increase the activity of unhealthy mitochondria, decreasing ATP levels cell, proliferation, and increasing oxygen consumption, reactive oxygen species (ROS) production and cell death [134]. However, there are still no studies reporting of FX11 in the clinical setting, probably because of its highly reactive catechol portion and off-target effects [126, 135].

Other LDH inhibitors

Additionally, there are other references to other LDH inhibitors that have been shown promising *in vitro* results in targeting cancer. One of the most known and studied LDH inhibitor is so

far oxamic acid [126]. *In vitro* experiments have shown the potential of oxamic acid in tumor cells, however, since this compound has a poor cell permeability, the concentration used is high and cannot be reached *in vivo*. Likewise, the simple and small structure of oxamate confers several disadvantages, making it a nonspecific LDH-inhibitor.

Furthermore, Galloflavin, has been recently identified as a novel inhibitor of human LDH isoforms and has been shown to induce apoptosis of hepatocellular carcinoma cell lines, and inhibit breast cancer cell proliferation [127]. Galloflavin proved to be less cytotoxic to normal cells, and to be well tolerated by mice [126]. Clearly, these promising results encourage further studies with this molecule.

Recently, N-hydroxy-2-carboxy-substituted indole compounds have been identified as specific LDHA inhibitors, which can reduce lactate production by acting as competitors of NADH and pyruvate. However, these new natural compounds apparently have low stability [135], and further studies are needed to support their potential as anti-cancer drugs.

1.6 Monocarboxylate transporters

Monocarboxylate transporters (MCTs), constitute a family of plasma membrane transporters with 14 members already identified, albeit, only the first four isoforms are able to catalyse the proton-coupled transport of monocarboxylates, playing an important role in cell metabolism. The expression of each isoform depends on its function and with the metabolic requests of each tissue (reviewed in[136]).

MCT1 and MCT4 are commonly overexpressed by cancer cells to maintain lactate and pH homeostasis [137]. Taking into account the tumor microenvironmental scenario and molecular events that occur in carcinogenesis the importance of these lactate transporters makes them attractive targets in cancer therapy. Considering the cell-cell lactate shuttle in cancer cells described by Sonveaux and co-workers [138], where MCT1 has a crucial role as the gatekeeper of metabolic symbiosis of cancer cells, by importing lactate into oxidative cells, targeting of MCT1 will have important implications in cancer homeostasis. Also, inhibition of MCTs will have a direct effect in lactate transport, interfering with intracellular pH homeostasis and also with the acidic microenvironment.

1.6.1 MCT inhibitors

AZD3965 MCT1/2 specific inhibitors

A new class of specific and high-affinity inhibitors of MCT1 have recently been developed by AstraZeneca. The authors found it to be active against MCT1 and MCT2 but not MCT4 [139]. Specifically, AR-C155858 compound seems to exert its function by binding MCT1 from the cytosolic side. The use of chimeric transporters combining different domains of MCT1 and MCT4 revealed that the binding site for the inhibitor is contained within the C-terminal of MCT1, and involves the transmembrane domains 7–10 [139].

A specific inhibitor of MCT1/2, AZD3965 (NCT01791595), is now being tested in a phase I/II clinical trial in patients with advanced solid tumors (prostate and gastric) or lymphomas.

Classic inhibitors

There are numerous non-physiological competitive inhibitors described that are known to inhibit the function of MCTs [140] . Several studies have demonstrated the affinity and specificity of each inhibitor to each MCT isoform. These include aromatic compounds such as α-cyano-4-hydroxycinnamate (CHC) and phenylpyruvate, stilbene disulfonates such as 4,40-di-isothiocyanostilbene-2,20-disulfonate (DIDS) and 4,40-dibenzamidostilbene-2,20-disulfonate (DBDS) and bioflavonoids such as phloretin and quercetin. These inhibitors inhibit MCTs with higher affinity for MCT1 and 2, although, they have the ability to also inhibit other transporters at higher concentrations. Moreover, it has been already identified other targets for these inhibitors. CHC has been shown to be a potent inhibitor of the mitochondrial pyruvate transporter, whereas DIDS and DBDS inhibit the chloride/bicarbonate exchanger AE1. Phloretin and quercetin are also non-specific inhibitors [103], even thought, several studies describe how these inhibitors affect lactate transport. Recently, our group showed that CHC reduces lactate efflux, cell proliferation, invasion and migration and increase cell death in *in vitro* glioma [141] and breast tumor cells [142]. This effect was also corroborate using iRNA experiments [141, 142]. Cell death induced by CHC was also observed in colorectal and cervix carcinoma in *in vitro* and *in vivo* models [138]. Nevertheless, inhibition of one MCT isoform can be compensated by another MCT isoform, leading to resistance to treatment [143].

Previous results show that CD147 and MCTs are co-expressed in human cancer tissues [141, 143-149]. Moreover, results published by our group showed that the prognostic value of CD147 appears to be associated with its co-expression with MCT1 in breast cancer [150]. Therefore, targeting CD147 to inhibit MCTs appears to be a rational approach.

In this context, CD147 silencing has been reported to inhibit MCT1/MCT4 function decreasing lactate efflux [151] and consequently reducing pHi [143, 152, 153] and reducing the malignant potential of cancer cells *in vivo* [143, 153]. Moreover, CD147 expression is also associated with tumor progression, prognosis and chemoresistance [154], however, there are no clinical studies with respect to inhibition of CD147 in cancer patients, probably because effective and specific inhibitors are scarce. Recently exciting clinical progress has been made by the development of CD147-directed monoclonal antibodies [155].

1.7 Pyruvate dehydrogenase kinase 1 (PDK1)

PDK regulates the enzymatic activity of pyruvate dehydrogenase (PDH), the enzyme responsible for pyruvate conversion into acetyl-CoA to feed the tricarboxylic acid (TCA) cycle. Four isomeric forms of PDK (PDK1-4) exist having tissue specific expression, singular activities, and different phosphorylation rates (reviewed in [156]). Under hypoxia conditions, PDK1 phosphorylates the E1α subunit of PDH, inhibiting its catalytic activity and consequently oxidative phosphorylation

[157]. OXPHOS impairment in cancer is predominantly due to the inhibition of PDH by PDK being this enzyme that controls the fate of pyruvate. Thus, and following the idea that many cancer cells depend on glycolysis for their energy demands, inhibiting PDK and consequently this metabolic pathway could be the "Achilles' heel" for these cells.

1.7.1 PDK inhibitors

Dichloroacetate (DCA)

Dichloroacetate (DCA) has been known for a long time as a PDK inhibitor [158] and was already used in the late 80's in clinical trials. This pyruvate mimetic was shown to occupy the pyruvate-binding site of PDK2, which is largely conserved among PDKs, therefore inhibiting non-selectively but with different potencies all PDK isoforms [159]. By inhibiting PDK, this drug preserves the PDH active form, promotes pyruvate-to-acetyl-CoA flux and reduces pyruvate-to-lactate conversion, thereby facilitating OXPHOS. This will lead to increased mitochondria depolarisation, increased ROS and apoptosis is induced in cancer cells with both cytochrome c and apoptosis-inducing factor efflux from the mitochondria [160]. This results in a decrease in tumor growth both *in vitro* and *in vivo* in xenotransplant models [161]. Preclinical studies with DCA already provided evidence showing the effectiveness of DCA as a potent anticancer drug in several tumors. Interestingly, being a small molecule able to penetrate most tissues and readily crossing the blood–brain Barrier after oral administration [162], together with its low price make this compound a promising anticancer drug capable of being used in clinical settings. In fact, in 2010 an open label trial of oral DCA in five patients with recurrent glioblastoma, showed promising results in three subjects in terms of tumor biochemistry and progression [163]. Drug safety was limited initially by peripheral neuropathy that was reversible upon DCA dose reduction. Another phase I trial (NCT01111097) in adults with recurrent malignant brain tumors showed that oral DCA administration is safe, well-tolerated and feasible in these patients [157]. Moreover DCA therapy was associated with clinical and radiographic evidence of disease stabilization through the first 4 weeks of DCA administration. Biochemical studies performed in fresh samples prevenient from patient with glioblastoma treated with DCA showed that the drug depolarized the mitochondrial membrane potential, increased ROS, activated p53 and inhibited the expression of HIF-1α and VEGF [164]. Despite these positive results, DCA has a plasma half-life of approximately 1 h in drug-naïve individuals, which could be increase several-fold with chronic administration, yet at a risk of serious side effects with higher doses [157].

A phase II clinical trial was designed to determine the safety, tolerability and response rate of DCA in patients with refractory metastatic breast and non-small cell lung cancers (NCT01029925), however, after the admission of the first seven patients, the study was closed based on safety concerns [165]. Moreover, a phase I clinical trial for the treatment of recurrent head and neck cancers (NCT01163487) is ongoing and multiple clinical trials are recruiting or undergoing to test DCA in combination with other therapies in several types of cancer (please see clinicaltrial.org for further details).

2. Involvement of glucose metabolism in response to pharmacological therapy

2.1. Classical chemotherapy

For several years, anticancer therapy was based on the higher capacity of tumor cell proliferation compared to normal cells. Therefore, most chemotherapeutic agents currently used in the clinical context target rapidly dividing cells. The main groups include: DNA alkylating agents, platinum-based agents, anti-neoplastic antibiotics, antimetabolites, anti-mitotic agents and topoisomerase inhibitors, which main target is DNA replication. Thus, this type of therapy does not distinguish between tumor and normal cells but instead between rapid and slowly proliferating cells, leading to important side effects at the level of highly proliferative tissues, including bone marrow, gastro-intestinal tract, hair follicles and gonads.

Tumor cells that initially may respond to chemotherapy, frequently develop resistance to a variety of drugs, leading to treatment failure. There are several mechanisms by which cancer cells become resistant to cancer chemotherapy, including higher capacity to repair DNA damage, production of nucleophilic substances (e.g. glutathione), target alteration, activation, and overexpression of efflux pumps. Additionally, and as a result of extensive studies to better understand the cell alterations behind drug resistance, the energetic metabolic switch appears as an important factor [166]. In fact, it is described that the hypoxic microenvironment [167], is one of the major factors involved in drug resistance and so the reactivation of a "normal metabolic state" could revert drug resistance and increase the efficacy of some chemotherapeutic agents already used in the clinic.

Based on the highly glycolytic phenotype, numerous mechanisms are behind tumor resistance to chemotherapy. Firstly, as mentioned before, the increase of glucose consumption will consequently lead to the production of high amounts of lactate [12]. In order to avoid deleterious intracellular acidity, tumor cells need to export lactate to the extracellular milieu, leading to an advantageous acidic microenvironment. In fact, it is attested that the alteration of the tumor microenvironment by the variation of the pH gradient between the extracellular environment and cell cytoplasm is behind the resistance to many cytotoxic drugs. For example, weakly basic chemotherapeutic drugs have their effect reduced in tumors, due to the more acidic extracellular pH of solid tumors, which impairs their uptake [168]. In addition, a higher glycolytic metabolism will also lead to higher production of NADPH, which will enable cells to maintain the levels of reduced forms of glutathione (GSH). This non-enzymatic antioxidant agent as been related to the capacity of tumor cells to counteract some of the effects of chemotherapeutic agents by maintaining their redox *status* [169]. All this evidence indicates a biochemical link between drug resistance and glycolytic metabolism, and, therefore the design of new therapeutic approaches combining chemotherapeutic drugs with glycolytic inhibitors will be of great value. In fact, some preclinical studies already demonstrated the potential of glycolytic inhibitors as an additional option for combination therapy. Studies involving the use of inhibitors of glycolytic targets in the response to classical chemotherapy are summarized below.

2.1.1 Hexokinase II inhibitors

Many studies explored the combination of hexokinase inhibitors with chemotherapy. The use

of 2-DG significantly increased the effect of adriamycin (anti-neoplastic antibiotic) and paclitaxel (anti-mitotic agent) in mice with human osteosarcoma or non-small-cell lung cancer xenografts [170]. Also, Maschek and co-workers showed that adriamycin-resistant breast cancer cells have a 3-fold increase of glycolytic rates, which could be due to defective mitochondria. Another preclinical study with 2-DG also demonstrated to overcome tumor cell resistance to cisplatin [171]. The use of another HK II inhibitor, lonidamine, rescued the resistance phenotype of breast and glioblastoma cancer cells to adriamycin and nitrosurea (alkylating agent), respectively [172]. Importantly, these two inhibitors of HK II are already in clinical trials in combination with other agents in different solid tumors [173]. Regarding lonidamine, clinical studies showed that combination with different chemotherapeutic agents (cisplatin, platinum-based agent; epirubicin, anti-neoplastic antibiotic; vindesine, anti-mitotic agent) improved survival in patients with advanced lung cancer [174], ovarian cancer [175] and with advanced breast cancer, also showing reduction in the size of liver metastases [176]. Although there are no clinical studies on the efficacy of 3-BP as monotherapy, there is evidence showing that 3-BP can also overcome resistance to doxorubicin [177].

2.1.2 Lactate dehydrogenase inhibitors

Going downstream of the glycolytic pathway, there are also studies showing the involvement of LDHA in drug resistance. Zhou and coworkers showed that LDHA play a crucial role in palitaxel resistance in breast cancer cells. Moreover, treatment of breast cancer cells with the combination of palitaxel plus oxamate showed a synergistic effect on tumor cell death [178]. In addition, other studies showed that LDHA inhibition sensitizes chondrosarcoma cells to doxorubicin (antibiotic) [179] or resensitizes colon cancer cells to 5-fluorouracil (anti-metabolite) [180]. Interestingly, there are several clinical trials already complete or still recruiting, testing the combination of AT-101 with different standard chemotherapy in several tumors. (further details in: clinicaltrial.gov)

2.1.3 Pyruvate dehydrogenase kinase inhibitors

Beyond the promising effect of DCA as anticancer therapy, few studies have combined this drug with standard chemotherapeutic agents in an effort to overcome resistance. Preclinical studies have demonstrated that DCA increases the antitumor effects of several chemotherapeutic agents, for instance DCA sensitizes lung tumor cells to capecitabine (anti-metabolite) [181] and also to platinum-based drugs [165, 182] promoting apoptosis of these cells. Additionally, in gastric cancer, DCA is able to revert resistance to 5-fluorouracil induced by the hypoxic microenvironment. [183] Moreover, Kumar and co-workers showed that DCA chemosensitized lymphoma cells to cisplatin, and this was due to modulation of glucose metabolism, followed by restoration of tumor microenvironment pH [184]. Interestingly, there is already an open-label trial of oral DCA in combination with cisplatin in patients with head and neck carcinoma.

2.1.4 Lactate transport inhibitors

As already mentioned, high tumor lactate levels are reported to correlate directly with resistance to different therapies, and thus lactate transporters appear as potential targets for

sensitization of cancer cells to therapy. Indeed, our group demonstrated that inhibiting MCT1 with CHC exhibited anti-tumoral and anti-angiogenic activity in gliomas and, more importantly increased the effect of temozolomide [141]. In another study, we also showed that MCT1 and the chaperone CD147 are responsible for cisplatin resistance in bladder cancer [144].

Some explanations have been put forward for the success of the combination of anti-glycolytic agents with chemotherapy. For instance, cells treated with agents that cause DNA damage, such as cisplatin and alkylating agents, require high levels of ATP to rapidly repair these lesions, and so inhibiting ATP production using glycolytic inhibitors sensitizes tumor cells to these agents [185]. Moreover, inhibition of ATP production will also interfere with the effect of drugs that are substrates of p-glycoprotein effluxing pumps that require energy for their activity. Thus, with low ATP, the function of these pumps is compromised and drugs will no longer be exported outside the cells easily. In this line of evidence, combination of glycolytic inhibitors with anticancer agents should provide clinical benefit and maybe effectively kill cancer cells.

2.2 Oncogene-targeted therapies

The elucidation of deregulated kinase signaling pathways in cancer, including RTKs and their intracellular pathways (Figure 2) [186-189], along with the identification of kinases as targeted molecules, lead to a highly productive development of novel and effective antineoplastic drugs [190, 191]. Strategies include the development of selective components that can target the extracellular ligand-binding domain [monoclonal antibodies (mAb)], the intracellular tyrosine kinase or the substrate-binding region [small tyrosine kinase inhibitors (TKi)] (Figure 2).

Although the concept of oncogene addiction has been observed in many preclinical models and has led to some initially impressive clinical results, similarly to conventional chemotherapy, the main challenge of targeted therapy is drug resistance [192-195]. There are some hypothesis trying to explain the acquired resistance to oncogene-targeted therapies [194, 195], yet the molecular mechanisms underlying this resistant phenotype is far from being understood. Thus, a better knowledge of cancer cell biology and their response and adaptation to drug treatment is fundamental and urgent for rationally designed combination schemes and multi-targeted therapies required to achieve long-lasting effects [193, 194].

Recent reports have demonstrated that the metabolic rewiring of cancer cells can potentially drive resistance to oncogene-targeted therapies (summarized in Table 2). In this section we intend to give an overview of the actual knowledge on glucose metabolism alterations upon the use of targeted drugs, as well as the molecular mechanisms through which this glycolytic reprogramming can occur, and finally to discuss a way to overcome the resistance driven by the altered glucose consumption in cancer cells.

2.2.1 KIT, PDGFRA and BCR-ABL inhibitors

KIT and PDGFR belong to the type III subfamily of RTKs and due to the structural similarities of KIT and PDGFR kinase domains, the majority of TKi for KIT can also target PDGFR and vice-versa [191, 196]. Imatinib is a KIT and PDGFRA inhibitor that was first clinically used in chronic myeloid leukemia (CML) harboring BCR-ABL kinase fusion and gained posterior approval for gastrointestinal stromal tumors (GIST). Imatinib became a paradigm in solid tumor treatment, reversing an untreatable disease into a tumor entity in which up to 85% of patients that receive the drug achieve disease control [197]. However, since imatinib treatment was one of the first successful approved molecular targeted therapies, it was also one of the first to whom therapy resistance was reported, mainly in CML [191].

At clinically relevant concentrations, imatinib strongly induces p53-dependent cell death [198], suppressing cytosolic glycolysis then increases the TCA cycle intermediates evoking a compensatory activation of mitochondrial function in CML [199-201]. In contrast, imatinib-resistant CML cells maintain highly glycolysis irrespective of the treatment [202], and maintenance of glucose uptake inhibited p53 activation [198], suggesting that increased glucose metabolism participates in imatinib resistance. In accordance, it was found that BCR-ABL positive cells express the high-affinity GLUT-1 glucose transporter and have increased glucose uptake [203-206]. Activation of the PI3K/AKT/mTOR pathway by BCR-ABL contributes to this high glycolytic activity, through a PI3K-dependent translocation of glucose transporters to the plasma membrane [199, 206-208]. Furthermore, it has been shown that enhanced expression of BCR-ABL in imatinib-resistant cells correlated with a non-hypoxic induction of HIF-1α that was required for cells to enhance the rates of glycolysis but reduce glucose flux through both the TCA cycle and the oxidative arm of the PPP [201, 209, 210]. Additionally, mitochondrial dysfunction may also play a role in imatinib resistance via the production of mitochondrial ROS [201].

Due to the inherent resistance associated with imatinib treatment, novel BCR-ABL and KIT inhibitors have been developed, as is the case of multi-kinase targeted drugs dasatinib, sorafenib or axitinib, however they seem to share the same adaptations to glucose metabolism such as imatinib.

In chronic lymphocytic leukemia (CLL), it was shown that dasatinib induced glucose use, while reducing lactate production, suggesting that this tyrosine kinase inhibitor decreases aerobic glycolysis and shifts glucose use for OXPHOS [211]. In addition, dasatinib sensitive samples are more sensitive to both inhibition of OXPHOS (Metformin) and glycolysis (2-DG) than dasatinib resistant samples and that this difference might be associated with a higher capacity in this later subset of cases to adapt to energetic stress [211].

Sorafenib promotes an early perturbation of mitochondrial function in breast cancer cells, associated with a drop of intracellular ATP levels and increase of ROS generation, inducing the activation of AMP-activated protein kinase (AMPK) [212]. As a response to these alterations, AMPK enhanced glucose uptake by up-regulating the expression of GLUT-1, and increased lactate production [212].

Finally, Hudson et al. found that the resistant pancreatic adenocarcinoma cells to treatment with axitinib have a 2-fold increase in ^{14}C-DG uptake, followed by a translocation of GLUT-1 to the

cell surface membrane, a 2-fold increase in glycolysis rates measured by the extracellular acidification rate (ECAR) and increased levels of MCT-4 protein expression and AKT phosphorylation [213].

Expectantly, considering the resistance that has been described for this class of inhibitors, it has also been shown that inhibition of glycolysis with 2-DG enhanced imatinib sensitivity in BCR-ABL–expressing CML cells with wild-type p53 [198], as the GLUT-1 inhibitor fasentin blocked sorafenib-induced glucose uptake and potentiated its cytotoxic activity breast cancer cell lines [212] or by blocking AKT activation it is possible to reverse the GLUT-1 translocation and restored the sensitivity of pancreatic cells to axitinib treatment [213].

2.2.2 ErbB family Inhibitors

The HER family (or ErBb) of receptors comprises the family I of RTKs that includes EGFR, HER2, HER3 and HER4 [186]. Anti-EGFR and HER2 therapies are the most widely used and explored due to the high number of tumors harboring alterations in these two receptors [194].

In relation to glucose metabolism, it was recently shown that EGFR signaling up-regulate aerobic glycolysis in *EGFR*-mutated lung adenocarcinoma cells, through the regulation of GLUT3 and pentose phosphate pathways [214]. Besides, it was reported that hypoxia drives expression of pyruvate dehydrogenase kinase (PDK1) and EGFR along with the HIF-1α, initiating a feed-forward loop that can sustain malignant progression in human glioblastoma cells [215]. In accordance, inhibition of EGFR signaling with erlotinib or gefitinib abrogated the Warburg effect by inhibiting multiple steps including MYC-driven transcription, phosphorylation of PKM2 and hexokinase activity to regulate glycolysis in *EGFR* mutant lung adenocarcinoma [214, 216].

EGFR wild-type tumors are usually treated with the monoclonal antibody cetuximab. It has been shown that cetuximab treatment downregulates glycolysis through HIF-1α inhibition, which is dependent on effective inhibition of PI3K/AKT pathway in non-small-cell lung carcinoma (NSCLC) [217, 218], and in LDHA downregulation in head and neck squamous cell carcinoma (HNSCC) cells [219]. In contrast, it was also described that colorectal cancer models with acquired resistance to cetuximab have a significantly higher production of lactate [220], and in the HNSCC models the resistant cells overexpressed HIF-1α and are highly glycolytic [219]. Moreover, overexpression of HIF-1α conferred cellular resistance to cetuximab-induced apoptosis in sensitive lung adenocarcinoma cells [217, 218].

Komurov and colleagues also found that lapatinib (EGFR and HER2 inhibitor) induction of toxicity in HER2-positive breast cancer cells is associated with glucose deprivation, and that prolonged lapatinib treatment can lead to acquired resistance that is characterized by increased expression of networks involved in glucose deprivation or hypoglycemic response [221].

Thus, there is compiling amount of data that provide evidence linking the ErbB inhibitors response to the regulation of energetic metabolism in ErbB-dependent tumors. Therefore, combination therapies of ErbB inhibitors and drugs that block glycolysis pathway would be expected to be much more effective circumvent acquired resistance. In fact, Kim et al. showed that targeting of glycolysis with 2-DG was an effective therapeutic option to overcome the limited efficacy of afatinib (irreversible inhibitor of EGFR and HER2) in lung adenocarcinoma cells with *EGFR* resistant mutation [222].

Moreover, inhibiting LDHA activity with oxamate enhanced the response of HNSCC cells to cetuximab [219].

2.2.3 BRAF inhibitors

The development of therapies targeting BRAF in melanoma is a clear example of successful targeting of an oncogene for the treatment of cancer [223]. Catalytic BRAF inhibitors (such as vemurafenib), have profound efficacy in tumors carrying activating mutations, particularly to the V600 amino acid substitution [223]. Importantly, activating *BRAF* mutations, have been associated with increased glycolytic activity and cell surface GLUT1 expression in colorectal and thyroid cancer cells [21, 224], indicating that glucose metabolism could be important for BRAF-driven tumorigenesis.

In melanoma cell lines it was shown that BRAF inhibition with vemurafenib potently suppressed glycolysis via suppression of hexokinase II and GLUT1/3 expression [225]. However, it was also found that glucose metabolism is restored upon development of vemurafenib resistance due to alterations in a network of transcriptional regulators of glycolysis, composed of HIF-1α, MYC, and MONDOA induced by BRAF inhibitor treatment [225, 226]. Notably, DCA potentiated the antitumor effects of the vemurafenib in resistant *BRAF* V600E-mutant melanoma cells [225, 227].

Other studies have reported that mitochondrial respiration and oxidative phosphorylation are decreased in metastatic melanomas, even under normoxic conditions due to the persistence of a high nuclear expression of HIF-1α [227]. Thus, acquired resistance to BRAF inhibitor could be in part attributed to increased mitochondrial biogenesis and enhanced stress tolerance in melanoma [228], that are sustained by autophagy, a survival mechanism exploited by tumor cells to meet their elevated metabolic demands and to tolerate stress [228]. Therefore, it has been suggested that combined inhibition of autophagy and BRAF may overcome BRAF inhibitor resistance [228].

2.2.4 VEGF/anti-angiogenic inhibitors

The anti-tumor activity of angiogenic inhibitors is often limited by the development of resistance to these drugs [229]. *In vivo* studies suggest that anti-VEGF therapy, such as bevacizumab, can alter the tumor's phenotype, by generating a hypoxic-stressed tumor microenvironment inducing invasion and tumoral aggressiveness [230].

In glioblastoma xenografts, bevacizumab decreased microvessel-density and increased intratumor-hypoxia, but did not induce apoptosis [231]. Moreover, bevacizumab alone caused a significant increase of HIF-1α-dependent gene expression in glioblastoma tissues [231]. Additionally, microarray analysis of resistant glioblastoma xenografts tumors revealed coordinated changes at the level of metabolic genes, in particular, uncoupling glycolysis from oxidative phosphorylation [232, 233], which has been confirmed by increased expression of glycolytic enzymes including PDK in the treated tumors [233]. Upregulation of HIF-1α appears to be also significant mechanism of resistance to antiangiogenic therapies in neuroblastoma [234].

In colorectal *in vivo* models, metabolic assays, revealed a significantly impaired mitochondrial function and hyperactive glycolysis, which were concomitant with the upregulation of HIF-1α in bevacizumab treated tumors [235]. In accordance, in colorectal cancer cell lines, bevacizumab caused a

significant increase in cellular senescence associated with upregulation of p16 [236]. Similarly, hypoxia-mediated autophagy promotes tumor cell survival in glioblastomas resistant to bevacizumab [237]. Importantly, glycogen metabolism is upregulated in tumors *in vivo* and in cancer cells *in vitro* in response to hypoxia, inducing autophagy and senescence mechanisms in glioblastoma [238]. Thus, glycogen metabolism seems to be a key pathway necessary for optimal glucose utilization in hypoxic-stressed tumor microenvironment induced by anti-angiogenic therapies [238], representing a targetable mechanism to overcome resistance to those therapies.

Actually, it is already known that suppressing HIF-1α with low-dose topotecan potentiates the effects of the antiangiogenic drugs in mouse models of neuroblastoma and glioblastoma [231, 234]. In colorectal cancer xenograft models, treatment of bevacizumab-resistant cells with the glycolysis inhibitor 3-BP resulted in smaller tumor volume and longer survival [235], while bevacizumab and DCA together dramatically blocked tumor growth compared to either drug alone [232], suggesting that glycolysis blockade may also potentiate the therapeutic effect of anti-angiogenic treatment [232, 233, 235].

As a summary of this part, in general, HIF-1α is pointed as the crucial player in the modulation of glucose metabolism, inducing cellular survival mechanisms such as glycogen storage, senescence and autophagy (summarized in Table 2), which culminates in tumor resistance to molecular targeted therapies.

It is apparent from experimental studies and also clinical experience with molecular targeted agents that cancers often "escape" their "oncogene addicted" state, which can be mainly attributed to deregulation of signaling pathways that can sometimes alleviate or bypass the "addiction" to another pathway, causing relapse of the tumor even after pronounced initial responses [192-195]. Also, it is of general knowledge that activation of some RTKs, mainly EGFR and VEGF signaling, may stimulate rise in HIF-1α in a cell type-specific manner [239-241]. Thus, is not clear whether the HIF-1α-induced glycolysis is the cause or just a consequence of development of therapy resistance, but it seems clear that HIF-1α up-regulation could be a consequence of a signaling bypass in the resistant tumors, as cited above. The work of Nilsson *et al*, support at least in part this hypothesis, since they found that multiple RTKs may regulate the HIF-1α axis in normoxia and hypoxia and suggest that multi-kinase inhibitors (such as sunitinib, in contrast to imatinib) may exert anti-angiogenic effects not only by direct effects on endothelial cells, but also by blocking compensatory hypoxia- and ligand-induced changes in HIF-1α and HIF-2α in neuroblastoma cells [242].

Actually, in preclinical models, the best strategies tested to overcome glycolysis induction of therapy resistance included HIF-1α inhibition, AKT phosphorylation blockage, glucose uptake impairment, LDHA and PDK inhibition, as well as autophagy reduction. In addition to glycolysis inhibition to revert resistance, as protein kinase inhibitors exert their action by binding to the ATP binding site on the targeted kinases, it has been also hypothesized that a decrease in ATP levels in neoplastic cells could reduce the competition for the same enzymatic site, thus increasing the efficacy of kinase inhibitors [243].

General Conclusions

The development of treatments that target tumor metabolism is receiving renewed attention, with several potential drugs currently in clinical trials. As discussed above, metabolic reprogramming renders cancer cells dependent on specific metabolic pathways that could be exploited in cancer therapy. Nevertheless, the rationale for targeting tumor glycolysis should be clear and precise and the success of selecting an anti-cancer therapeutic strategy should be based on the ability to choose a very specific agent for the molecular target. However, the success of such agents may be complicated by the high plasticity of the metabolic network that can induce compensatory biosynthetic routes [244], such as increased glutaminolysis or oxidative phosphorylation for energy production [245]. Thus, a new line of attacking cancer could be the combination of anti-glycolytic agents with other therapies. In fact, combination of glycolytic inhibitors with chemotherapeutic drugs, has already revealed to be a promising strategy to overcome drug resistance.

Moreover, glycolytic phenotype has a significant role in cancer chemoresistance, by acidification of the extracellular milieu, and thus inhibition of glycolytic metabolism could be a good strategy to sensitize tumor cells and to improve the outcome of chemotherapy. In fact, preclinical studies have been demonstrating the success of combining classical chemotherapy with glycolytic inhibitors. Additionally, cells with higher basal glycolytic rates are potentially more resistant to molecular targeted therapies. In this line of evidence, several studies suggest that a tumor is more responsive to a kinase inhibitor if it has a more inhibitory effect on glycolysis blockage, since glycolysis gives an important growth advantage to most transformed cells. Further, continuous treatment of tumors with kinase inhibitors elicits a restoration of the glucose metabolism rates, leading to acquired resistance. Thus, the upregulation of glucose metabolism as a compensatory response to chemotherapy elicits the potential of using anti-glycolytic drugs to overcome resistance.

In conclusion, it appears wise to assess tumour glucose uptake or glycolytic-related protein activity in the patient clinical management to predict therapy response, and importantly, to use therapeutic strategies to block abnormal glucose metabolism as suitable approaches to overcome or prevent therapy resistance to oncogene-targeted therapy.

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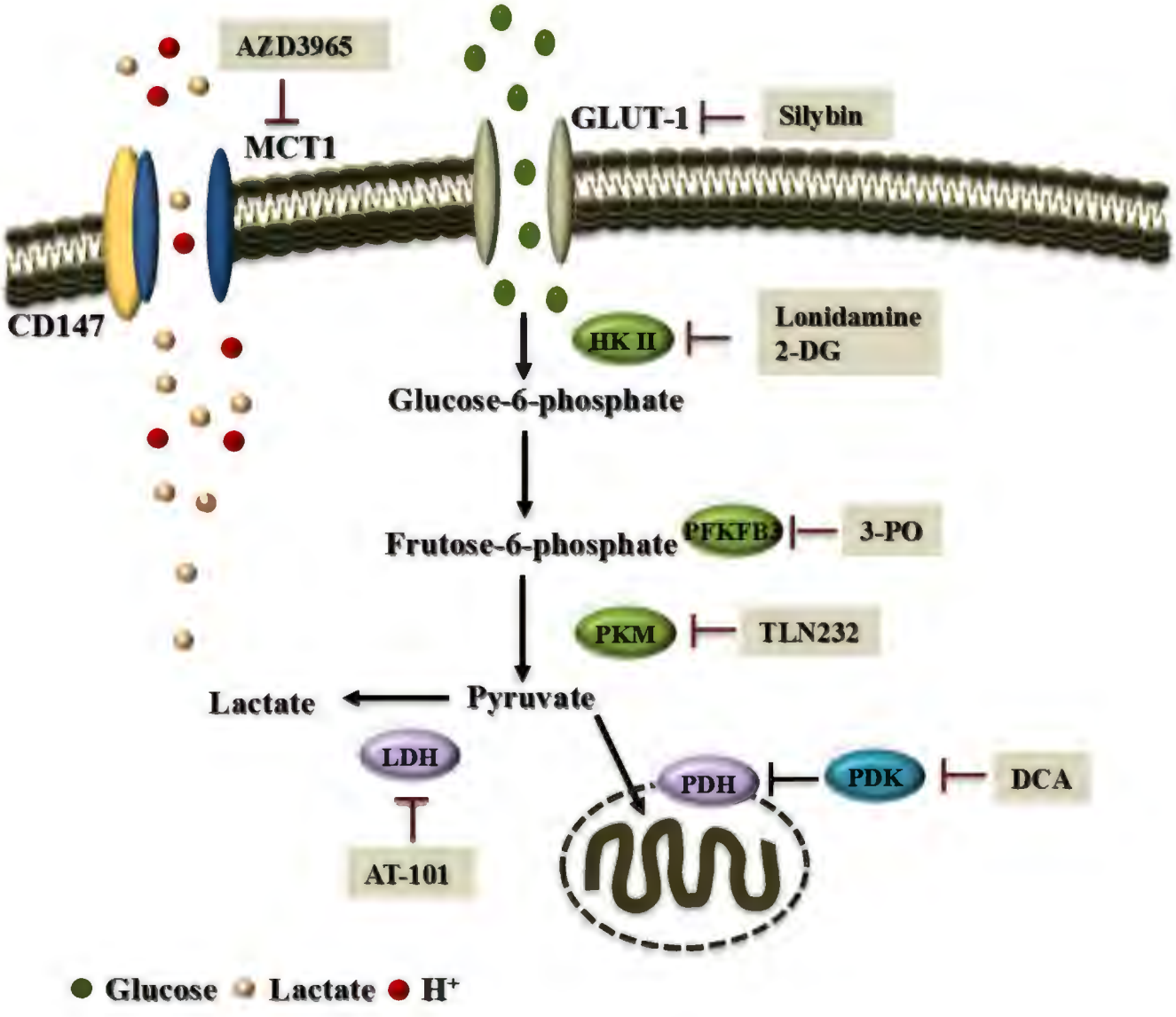
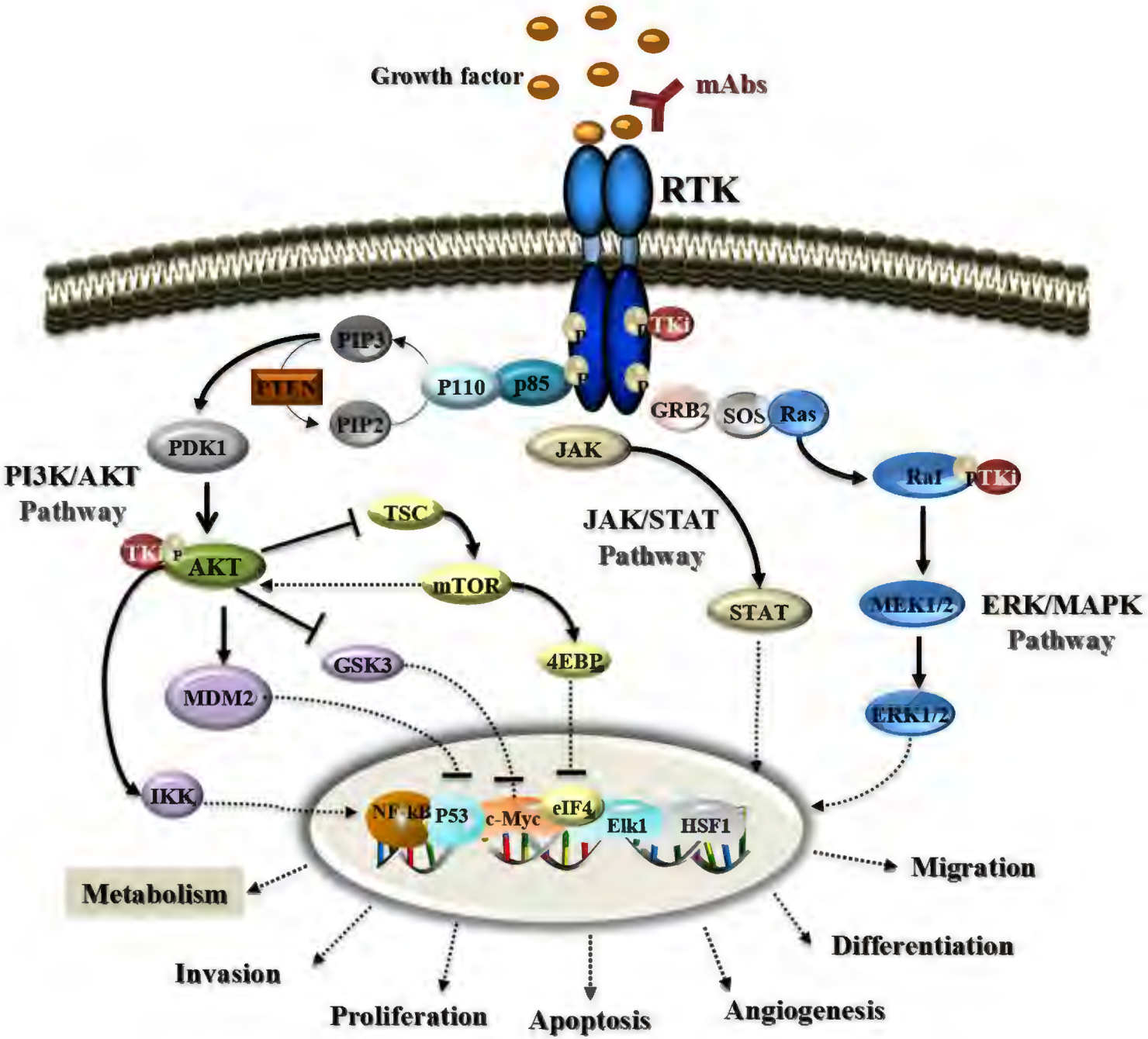
Figure legends:

Figure 1: Main proteins of glucose metabolism explored as therapeutic targets. GLUT-1, glucose transporter 1; HK II, hexokinase II; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PKM, Pyruvate kinase M2, PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; LDH, lactate dehydrogenase; MCT1, monocarboxylate transporter 1, 2-DG, 2-Deoxy-D-glucose; DCA, Dichloroacetate.

Figure 2: Receptor tyrosine kinases (RTK) signaling pathways. Broadly, binding of a ligand activates a RTK by inducing receptor dimerization, resulting in autophosphorylation (P) of its cytoplasmic domains, with consequent phosphorylation/activation of 3 main intracellular signaling pathways. The two main strategies to block activation of RTK-mediated signaling are also represented, which include the use of selective molecules that target the extracellular ligand-binding domain [monoclonal antibodies (mAbs)], the intracellular tyrosine kinase activity or the substrate-binding region [small tyrosine kinase inhibitors (TKi)].


AZD3965
MCT1
GLUT-1
Silybin
CD147
HK II
Lonidamine
2-DG
Glucose-6-phosphate
Frutose-6-phosphate
PFKFB3
3-PO
PKM
TLN232
Lactate
Pyruvate
LDH
AT-101
PDH
PDK
DCA
Glucose
Lactate
H+


Growth factor
mAbs
RTK
PIP3
PTEN
PIP2
P110
p85
PDK1
PI3K/AKT
Pathway
GRB2
SOS
Ras
JAK
Raf
JAK/STAT
Pathway
TSC
AKT
mTOR
STAT
MEK1/2
ERK/MAPK
Pathway
GSK3
4EBP
MDM2
ERK1/2
IKK
NF-kB
P53
c-Myc
eIF4
Elk1
HSF1
Metabolism
Migration
Differentiation
Invasion
Proliferation
Apoptosis
Angiogenesis