Dysregulation of glutaminase and glutamine synthetase in cancer

José M. Matés*, José A. Campos-Sandoval, Juan de los Santos-Jiménez, Javier Márquez

Department of Molecular Biology and Biochemistry, Canceromics Lab, Faculty of Sciences, Campus de Teatinos, University of Málaga, 29071 Málaga, Spain and Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain.

* Corresponding author (J.M. Matés)

E-mail address: jmates@uma.es

Abbreviations: AKG, α-ketoglutarate; ALDH1L2, aldehyde dehydrogenase 1 family member L2; ALT, alanine transaminase; AMPK, adenosine monophosphate-activated protein kinase; AST, aspartate transaminase; ASCT2, alanine/serine/cysteine transporter 2; BSO, L-buthionine-(S,R)-sulfoximine; CDK, cyclin-dependent kinase; CQ, chloroquine; CRC, colorectal cancer; CTH, cystathionine gamma-lyase; DBZ, dibenzazepine; DHA, dihydroartemisinin; DON, 6-diazo-5-oxo-L-norleucine; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; ESCC, esophageal squamous cell carcinoma; 5-FU, 5-fluorouracil; FH, fumarate hydratase; GA, glutaminase; GAB, long glutaminase 2 isoform; GAC, short glutaminase isoform; GBM, glioblastoma; GCLC, γ-glutamate-cysteine ligase catalytic subunit; GCLM, γ-glutamate-cysteine ligase regulatory subunit; GCS, γ-glutamylcysteine synthetase; GLUD, glutamate dehydrogenase; Glc, glucose; Gln, glutamine; GLS, glutaminase isoenzyme; GLS2, glutaminase 2 isoenzyme; Glu, glutamate; GLUL, γ-glutamyl:ammonia ligase; GOT, glutamate-oxaloacetate transaminase; GS, glutamine synthetase; GSH, glutathione; GSI, γ-secretase inhibitor; GST, glutathione S-transferase; G-TPP, gamitrinib triphenylphosphonium; HCC, hepatocellular carcinoma; 2HG, 2-hydroxyglutarate; HIF-1α, hypoxia-inducible factor-1α; IDH1/2, isocitrate dehydrogenase isoenzymes; KGA, long glutaminase isoform; KGDH, α-ketoglutarate dehydrogenase; KRAS, V-Ki-ras2 Kirsten rat sarcoma; LAT2, L-type amino acid transporter 2; LGA, short glutaminase 2 isoform; MAPK, mitogen-activated protein kinase; ME1/2, malic isoenzymes; mTORC1, mammalian target of rapamycin complex 1; MTX, methotrexate; MSO, L-methionine sulfoximine; NADH, nicotinamide adenine dinucleotide (reduced form); NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); NSCLC, non-small-cell lung cancer; OAA, oxaloacetate; OXPHOS, oxidative phosphorylation; PC, pyruvate carboxylase; PDA, pancreatic ductal adenocarcinoma; PET, positron emission tomography; PI3K, phosphatidylinositide 3-kinase; PKM2, pyruvate kinase M2 isoform; PPP, pentose phosphate pathway; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SLC, solute carrier; SCC, squamous cell carcinoma; SDH, succinate dehydrogenase; SNAT2, sodium-coupled neutral amino acid transporter 2; T-ALL, T-cell acute lymphoblastic leukemia; TCA, tricarboxylic acid; TAMs, tumor-associated macrophages; TNBC, triple negative breast cancer; TXNIP, thioredoxin-interacting protein; YAP, Yes-associated protein.

ABSTRACT

Besides fast glucose catabolism, many types of cancers are characterized by elevated glutamine consumption. Medical oncology pursuits to block specific pathways, mainly glycolysis and glutaminolysis, in tumor cells to arrest cancer development. This strategy frequently induces adaptive metabolic resistance that must be countered. Combination therapy is an anticancer synergistic tool to overcome both cancer growth and resistance mechanisms. Dysregulation of glutaminase and glutamine synthetase are key events that allow anabolic adaptation of tumors. Several specific drugs that inhibit metabolic enzymes dealing with glutamine metabolism have

been able to eliminate some neoplasms. Targeting the tumor microenvironment can be also another essential factor to be taken into account when single or combined cancer metabolic therapy fails.

Keywords: Cancer metabolism, Combination therapy, Glutaminase isoenzymes, Glutamine, Glutamine synthetase, Synergistic inhibitors

Introduction

Glutamine (Gln) is a key molecule in cancer beyond its role as a critical amino acid for biosynthetic purposes [1]. Gln has essential functions to provide amino acids, lipids, nucleotides, hexosamines, and polyamines, but also to render metabolic energy (ATP), to be used as a pleiotropic cell signaling molecule, or to highlight several cancer tracking pathways [2] (Fig. 1). Besides, Gln is indispensable to generate glutathione (GSH), the most important intracellular antioxidant molecule [3]. Cancer cells frequently increase oxidative damage in response to changes of the metabolic circuits [2]. Oxidative damage and induction of the apoptotic pathway in cancer cells are activated by reactive oxygen species (ROS), that trigger changes in mitochondrial membrane function, and act as mediators between mitochondria and apoptosis [1]. In fact, the inner mitochondrial membrane potential is severely altered by both Gln and GSH levels [4]. While glutamate (Glu) provides precursors for GSH production (Fig. 1), which contribute to modulate the adequate oxidative status of cells [5], partial oxidation of Gln through glutaminolysis evades oxidative phosphorylation (OXPHOS) and attenuates excess of ROS that otherwise are toxic to cells [6]. Simultaneously, Gln can be essential for the protection of metabolic enzyme α-ketoglutarate dehydrogenase (KGDH) from inactivation by ROS [1].

Mitochondria is the physical network where Gln carries out most of its substantive and regulatory capacities (Fig. 1). Gln/Glu ratios are controlled by glutaminase (GA) and glutamine synthetase (GS), also called γ-glutamyl:ammonia ligase (GLUL) [7]. Gln can fuel tricarboxylic acid (TCA) cycle through its conversion to Glu by GA, and subsequently to α-ketoglutarate (AKG) by aspartate transaminase (AST) and alanine transaminase (ALT), as well as by glutamate dehydrogenase (GLUD) [2,7]. AKG enters the TCA cycle, generating reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$), which are used to produce an important source of ATP via electron transport and OXPHOS [8]. OXPHOS refers to the formation of ATP from ADP and phosphate by complex V (ATP synthase) using the proton gradient generated across the inner mitochondrial membrane [9]. Thus, mitochondrial OXPHOS is a key pathway for ATP generation in many cancer sets [10].

Gln has a key role as precursor of other anabolic processes such as pentose phosphate pathway (PPP), generating reduced nicotinamide adenine dinucleotide phosphate (NADPH) [9]. Besides, Gln is involved in facilitating cell building biomolecules (Fig. 1). In addition, several cancer types, like pancreatic cancer and non-small-cell lung cancer (NSCLC), can utilize proteins from outside or inside the cell (i.e.: autophagy) to generate Gln and other amino acids to feed the TCA cycle for cell progression [11]. Usually, cancer cells appear to enhance both glycolysis and OXPHOS simultaneously, relative to surrounding normal cells [3]. Besides, TCA cycle can also be fueled by pyruvate carboxylation, which generates oxaloacetate (OAA) from glucose (Glc) [11]. The discovery of mutations in genes that encode succinate dehydrogenase (SDH), fumarate hydratase (FH) or isocitrate dehydrogenase (IDH1 and IDH2) in some types of cancer points out the key role of these genes as tumor suppressors (SDH, FH) or oncogenic factors

(IDH1/2) [12]. Of note, such mutations in IDH1/2 produce the oncometabolite 2-hydroxyglutarate (2HG), which impairs histone demethylation and blocks the cell differentiation [13]. However, GAs y GS, which are not mutant oncoproteins, are the main enzymes that regulate glutaminolysis and mitochondrial function in cancer [1,7,14]. In this mini-review, we will discuss both the dysregulation of GAs and GS in cancer, as well as the targeting of these metabolic enzymes as a valuable tool to fight against cancer.

Glutaminases and cancer

Glutamine metabolism

Increased metabolism of Gln is a hallmark of cancer [15]. Fifty years ago, the Gln analog 6-diazo-5-oxo-L-norleucine (DON) was tested as an antineoplastic agent [16]. Unfortunately, promising results became useless because of its high toxicity. However, later experiments proved the effects of DON (and another Gln analog, acivicin, Fig. 2) as synergistic molecules inhibiting GA and cancer growth [17]. Nowadays, many and less toxic agents have been described as valuable drugs to inhibit GA [18]. Cancer metabolism might be described as a large city underground map, and when a pathway is blocked another can be activated to reach the energetic and biosynthetic requirements of tumor cells. The only hope is to block several related pathways to synergistically arrest cancer development. Among synergistic tools against cancer, GA has become a key target in the metabolic therapy of cancer.

Glutaminase isoenzymes

GA (EC 3.5.1.2) is the enzyme responsible for catalyzing the conversion of Gln to Glu, and represents the first step in Gln metabolism (glutaminolysis). This process endows cancer cells with high levels of energy to cover their accelerated growth and proliferation, and also with biosynthetic precursors for synthesis of protein and nucleic acids [19]. To date, intense focus is placed on inhibiting GA as an alternative or parallel target for potential cancer treatment regimens, as this strategy might help to tackle the heterogeneity among cancer cells [18]. Human GA proteins are encoded by two paralogous genes named *GLS* and *GLS2*. Two isoforms derived from each GA gene have been so far identified in humans. The transcripts known as KGA and GAC arise by alternative splicing of the *GLS* gene, whereas two GLS2 transcripts were identified from the *GLS2* gene: the canonical long transcript termed GAB isolated from human breast cancer cells, and the short transcript LGA, which was originally identified in rat liver [19].

GLS has been shown to be associated with Gln addiction in tumors and has oncogenic properties whereas GLS2 has been described as a context-dependent tumor suppressor factor [18]. The heterogeneity of tumor-induced changes in the expression of key metabolic genes—like *GLS* and *GLS2*—suggests that different tumors might have differential requirements of glutaminolysis. These results suggest that the metabolic profiles of tumors are likely to depend on both the genotype and tissue of origin and have implications regarding the design of therapies targeting tumor metabolism [14]. Among chemicals targeting GLS, BPTES (Fig. 2) has been extensively used against several types of tumor cells [2]. Some promising results have been obtained using both chemical (BPTES) and genetic (*Gls* gene silencing) inhibition of GLS in HCC and xenografts

[20]. Further, specific GLS inhibition has triggered synergistic effects in the therapy of different cancers, as described below.

Rewiring glutaminolysis by combination therapy

Metabolic reprogramming of cancer cells rests on overexpression of key metabolic enzymes in order to upregulate determinant pathways such as glycolysis, glutaminolysis and fatty acid synthesis. Combined inhibitory treatment with lodinamine, DON and orlistat targeted hexokinase-2, GLS and fatty acid synthase, respectively. This synergistic therapy exhibits a significant degree of cell viability inhibition in the human colon cancer SW480 cell line, and in mice *in vivo*, showing good tolerance and overcoming the mechanisms of resistance to standard treatment [21].

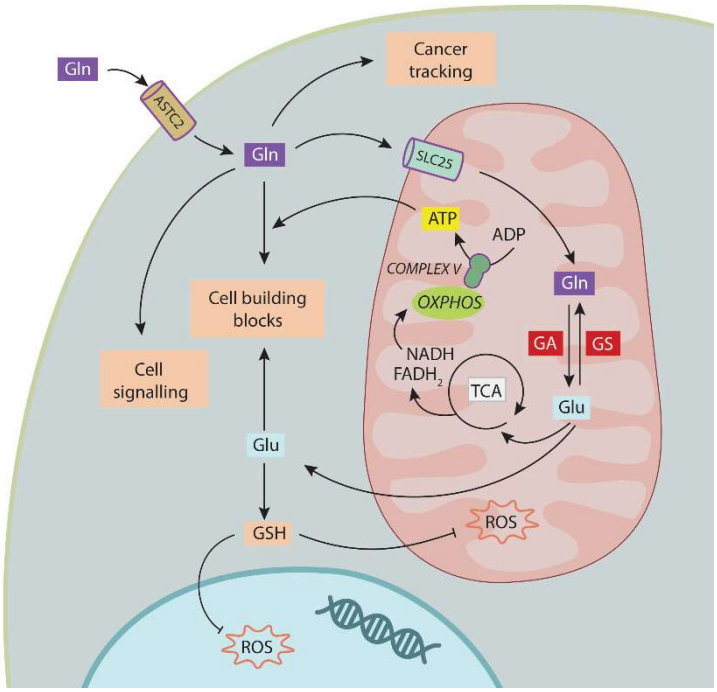

Fig. 1. Glutamine (Gln) as a pleiotropic molecule in cancer. Gln has key roles generating cell building blocks (i.e.: amino acids, proteins, fats, carbohydrates, and nucleotides), energy (ATP), buy also can work as a cell signaling molecule, and like a cancer tracking agent. Gln is equally essential because it is used for the synthesis of GSH, the most important non enzymatic antioxidant in cells, that fights against oxidative damage countering ROS in mitochondria and nucleus. GA (GLS and GLS2) converts Gln into Glu, and GS catalyzes the opposite process. Both metabolic enzymes regulate Gln/Glu ratio. Glu is transformed into AKG, that enters TCA cycle to produce NADH, $FADH_2$ and ATP. The human complex V, or mitochondrial ATP synthase, is the 5th multi subunit of OXPHOS complex used to generate ATP in the inner mitochondrial membrane. AKG, α-ketoglutarate; ASCT2, alanine/serine/cysteine transporter 2, human Gln/neutral amino acid transporter (also called SLC1A5); FADH2, reduced flavin adenine dinucleotide; GA, glutaminase; Gln, glutamine; GLS, glutaminase isoenzyme; GLS2, glutaminase isoenzyme 2; Glu, glutamate; GS, glutamine synthetase; GSH, glutathione; NADH, reduced nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species, SLC25, solute carrier 25, a mitocondrial transporter for Gln; TCA, tricarboxylic acid cycle.

Noteworthy, the pleiotropic effect of c-Myc in cancer growth and Glc uptake is dependent on the increased rate of glutaminolysis. Thus, GLS inhibition by GLS inhibitor CB-839 (Fig. 2) led to a reduction of Glc uptake in PC3 prostate cancer cells. Consequently, synergistic effect of repression of thioredoxin-interacting protein (TXNIP) to block Glc uptake and the inhibition of GLS through CB-839 can be used as therapeutic tools against prostate cancer [22]. In highly Gln-dependent triple negative breast cancer (TNBC) basal subtype cell lines, treatment with compound CB-839 leads to an activation of the integrated stress response pathway, given that GLS inhibition mimics a nutrient-starvation situation. Furthermore, CB-839 also leads to a decreased mammalian target of rapamycin complex 1 (mTORC1) activity, since it depends on nutrient availability. Combined treatment of CB-839 and mTORC1 inhibitor AZD8055 aims to exploit the connection between Gln metabolism and mTORC1 activity pathways, being especially promising in highly glutaminolysis-dependent tumor cells and resulting in a synergistic inhibition of *in vitro* cell growth [23]. In fact, in lung squamous cell carcinomas (SCCs) a similar pattern has been recently found. The mTORC1 inhibitor MLN128 suppresses glycolysis by targeting mTORC1, but cancer maintains its high rate of anabolic growth by adaptive Gln metabolism through GSK3α/β pathway upregulating c-Myc and c-Jun. GLS inhibition by compound CB-839 allowed to overcome that acquired metabolic resistance to MLN128. Interestingly, this combined and synergistic treatment promises not only to reduce growth of human lung SCC, but also in other tumor types that share a similar metabolic signature as head and neck squamous cell carcinoma and osteosarcoma [24]. Very recently, THZ1, a covalent inhibitor of cyclin-dependent kinase 7 (CDK7), suppressed the proliferation and inhibited the migration of several human NSCLC cell lines, blocking the glycolysis pathway. Noteworthy, combined treatment of THZ1 with compound CB-839 had a great synergistic effect [25].

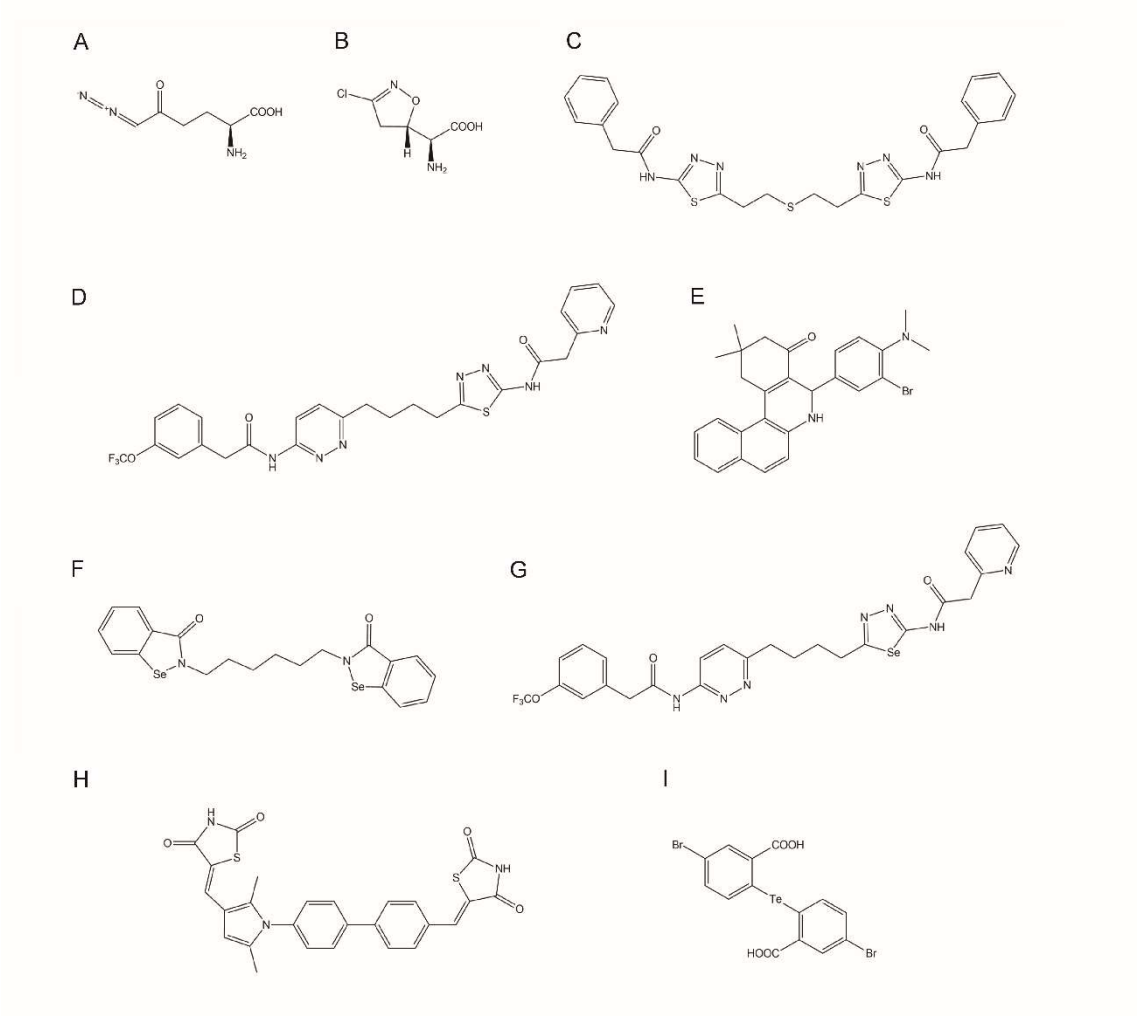

Fig. 2. GLS and GS inhibitors, with anticancer properties, described in literature. DON: (5S)-5-amino-1-diazonio-6-hydroxy-6-oxohex-1-en-2-olate. Acivicin: (2S)-amino[(5S)-3-chloro-4,5-dihydro-1,2-oxazol-5-yl]ethanoic acid. BPTES: bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide. CB-839: 2-(pyridin-2-yl)-N-(5-(4-(6-(2-(3-(trifluoromethoxy)phenyl)acetamido)pyridazin-3-yl)butyl)-1,3,4-thiadiazol-2-yl)acetamide. 968: 5-(3-bromo-4-(dimethylamino)phenyl)-2,2-dimethyl-2,3,5,6-tetrahydrobenzo[a]phenanthridin-4(1 H)-one. CPD-3B: 2,2'(hexyl)bis(benzo[d][1,2]selenazol 3(2H)-one. CPD-20: bis-2-(5-phenylacetamido-1,2,4-selenadiazol-2-yl)ethyl sulfide. CPD-5: 1-(4-bromophenyl)-2,5-diphenyl-1H-pyrrole-3-carbaldehyde. 3B: 4,4'-dibromo-2,2'-tellurodibenzoic acid. MSO: (2S)-2-amino-4-(S-methylsulfonimidoyl)butanoic acid. Phosphinothricin: 2-amino-4-methylphosphinobutyric acid. Alendronate: (4-amino-1-hydroxybutylidene)bisphosphonic acid. G-TPP: 6-[[(4E,6E,8S,9S,10E,12S,13R,14S,16R)-9-carbamoyloxy-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1]docosa-1(21),4,6,10,18-pentaen-19-yl]amino]hexyl-triphenylphosphanium. (R)-Etololac: 2-[(1R)-1,8-diethyl-4,9-dihydro-3H-pyrano[3,4-b]indol-1-yl]acetic acid. Sorafenib: 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-N-methylpyridine-2-carboxamide.

GLS inhibition using BPTES potentiated anti-cancer effect when combined with DNA damage by 5-fluorouracil (5-FU), that blocked pyrimidine synthesis in NSCLC. Dual BPTES and 5-FU treatment demonstrated a great synergistic effect in cell death induction [26]. These authors propose that the synergistic mechanism of the dual therapy against GLS and thymidylate

synthetase (EC 2.1.1.45) is connected with the inactivation of carbamoyl phosphate synthetase II (EC 6.3.5.5). Moreover, BPTES pretreatment has been shown to have similar effects to Gln deprivation in basal-type TNBC cell line HCC1937. Inhibition of Gln metabolism via BPTES pretreatment also synergized with DNA targeting therapy by cisplatin, another DNA-damaging drug. Combined treatment sensitized HCC1937 cells to subtoxic dose of cisplatin and resulted in reduced proliferation and increased apoptosis. In contrast, in a claudin-low TNBC cell line, BT-549, combination of BPTES plus cisplatin showed almost no improvement compared to DNA-targeting therapy alone, which could be attributable to higher GLS expression levels in BT-549 cells, and therefore a higher dose of BPTES would be required. These results suggest that combination of BPTES plus DNA-damaging drugs could be a potential therapeutic strategy in TNBC, but efficacy and drug concentration needed will be dependent on cell intrinsic characteristics [27]. Mutated NOTCH1 is a common alteration in T-cell acute lymphoblastic leukemia (T-ALL), driving cell growth through regulation of multiple pathways, one of which is glutaminolysis. NOTCH1 signaling directs Gln metabolism towards TCA cycle, promoting Gln utilization as a source of carbon in NOTCH1-induced T-ALL. Dibenzazepine (DBZ) is a γ-secretase inhibitor (GSI) which effectively inhibits NOTCH1. However, DBZ treated cells overexpressing GLS increased their Gln utilization, which suggests GLS overexpression could be a potential mechanism of resistance upon NOTCH1 inhibition. Combined treatment of T-ALL cell lines with DBZ and BPTES showed strong synergistic effects impairing cell growth, thus sensitizing cells to NOTCH1 inhibitory treatment, with a dominant role for glutaminolysis over glycolysis. *In vivo* models of T-ALL xenografted mice administered with combined treatment of BPTES plus DBZ showed a deep tumor growth inhibition [28]. On the other hand, the tumor suppressor phosphatase and tensin homolog (PTEN, EC 3.1.3.67) phenotype also appeared as a determinant factor for combined DBZ plus BPTES treatment efficacy, since *Pten*-deleted T-ALL cells showed no response to GSI alone nor combined with GLS inhibition therapy. Hence, both GLS and PTEN phenotype could act as predictive factors of the efficacy of the combined inhibitory treatment in T-ALL [28].

Dihydroartemisinin (DHA), a semisynthetic derivative of artemisinin which has been shown to have potent anticancer activity by augmenting intracellular ROS has been assayed together with the GLS inhibitor compound 968 (Fig. 2) yielding a decreased antioxidant capacity via disrupting redox homeostasis. Compound 968 and DHA cooperatively induced excessive intracellular ROS resulting in severe synergistic apoptosis in hepatocellular carcinoma (HCC) cells, thus increasing antitumor efficacy while sparing normal cells [29]. Metformin, a biguanidine that interferes with Glc metabolism by activating adenosine monophosphate-activated protein kinase (AMPK) pathway, causes an inhibitory effect on mTOR pathway. Ribavirin (1-β-D-ribofuranosyl-1, 2, 4, -triazole-3-carboxamide) is a well-known agent against several RNA and DNA virus. One commonly used drug against colorectal cancer (CRC) is oxaliplatin. Of note, treatments using metformin and ribavirin enhanced the synergistic effect of oxaliplatin when combined with the anti-GLS specific compound 968 for CRC therapy [30]. CB-839 plus phenformin/metformin combined treatment synergistically reduced tumor burden *in vitro* and *in vivo*, also overcoming CDK4/6 inhibitor resistance in esophageal squamous cell carcinoma (ESCC) cells, which overexpress cyclin D1, supporting that cyclin D1-CDK4/6 drives Gln-addiction through a mechanism including Retinoblastoma protein (Rb) and mTORC1 [31]. Additionally, treatment with 968 greatly reduced tumor growth and migration of NSCLC cells, inhibiting epidermal growth factor receptor (EGFR) and phospho-extracellular signal-regulated kinases 1 and 2 (ERK1/2) expression levels. However, treatment with compound 968 also induced autophagy as a mechanism of resistance for NSCLC cells. Although 968 could inhibit cell growth alone, combined treatment with chloroquine (CQ), a classical antimalarial drug which is also an autophagy inhibitor, resulted in synergistic effects. Since CQ inhibited 968-induced autophagy and sensitized cells to 968, a much lower dose of 968 was necessary to significantly reduce GLS expression and activity levels and effectively inhibit cell growth [32]. Allosteric mTORC1 inhibitor

rapamycin, and with higher effect the ATP competitive mTORC1 inhibitor PP242, evoked compensatory increase of Gln metabolism. Combination of PP242 and GLS inhibition by 968 administered to glioblastoma (GBM) patient–derived tumor cells, assayed in xenograft models, blocked tumor growth. Of note, there was no significant induction of cell death in brain, liver, and kidney among mice treated with PP242, compound 968 alone, or in combination. These results demonstrated that GLS inhibition can reverse mTORC1-targeted therapy resistance *in vivo* and that it acted synergistically with PP242 by regulating tumor bioenergetics. Importantly, the cytotoxic effect of the drug combination was enhanced in tumor tissue, rather than in normal tissue [33]. These findings may have important implications for combining mTORC1 kinase inhibitors with GLS inhibition for patients with GBM and possibly other mTORC1-activated cancers. Intriguingly, a dual GLS/GLUD inhibitor completely disrupted mitochondrial function, showing potent anticancer activity in several glioma cell lines with a minimum level of toxicity [34].

State of the art

New potent GLS inhibitors have been characterized very recently (Fig. 2): (i) the hexylselenium compound described above [34] that also inhibited GLUD, named CPD-3B, and significantly slowed the growth of HCT116 (IC_{50} = 1.20 μ M) and H22 (IC_{50} = 0.92 μ M) cell lines, as well as liver cancer xenograft models [35], (ii) a selenium derivative of CB-839 (called CPD-20) that inhibited the growth of HCT116 (IC_{50} = 0.009 μ M) and H22 (IC_{50} = 6.78 μ M) cell lines, as well as liver cancer xenograft models [36], (iii) thiazolidin-2-4-dione derivatives, like CPD-5, that inhibited GLS and GLS2 and slowed down the growth of AsPC-1 (IC_{50} = 34.5 μ M) and MDA-MB-231 (IC_{50} = 42 μ M) cell lines, as well as inhibited tumor growth in a preclinical mouse model [37], (iv) a tellurodibenzoic acid (named 3B) that strongly inhibited HCT116 cells with (IC_{50} = 2.4 μ M) [38]. Nevertheless, targeted therapy induced mechanisms of resistance that make necessary the development of new combined treatments aiming to achieve synergistic effects, reduce dose and beat resistance [18].

Perspectives

Recent strategies have been proposed to integrate Gln metabolism into the diagnosis, classification, treatment, and monitoring of some types of cancers [39]. In animal models, a subset of many tumors requires Gln metabolism [40]. This finding implies that approaches to image, quantify, or block Gln metabolism in human cancers could be incorporated into the diagnosis and management of the disease [39], including the use of Positron Emission Tomography (PET) technology [41]. In particular, it is essential to use *in vivo* perioperative administration of isotope-labeled biomarkers (Glc and Gln) to cancer patients to differentiate metabolic pathways between tumors and benign/healthy tissue [42]. Additionally, Gln metabolic studies may help predict which tumors would respond to therapies targeting its metabolism [39].

Further work is needed to investigate the metabolic consequences of GLS silencing and GLS2 inhibition/overexpression. Of interest, aldehyde dehydrogenase 1 family member L2 (ALDH1L2), cystathionine gamma-lyase (CTH), and glutathione S-transferase (GST), all proteins dealing with the oxidative stress response protein network, are upregulated in response to GLS inhibition [43]. Hence, although CB-839 treatment had antiproliferative activity in PDA cell lines, it had not

antitumor activity in a genetically engineered mouse model of PDA, due to resistance to GLS inhibition, explained by compensatory metabolic pathways. An understanding of genetic and epigenetic circuits dealing with GA isoenzymes will be useful in developing combined and synergistic therapies to augment the effects of Gln metabolism reprogramming, providing new class of anticancer medication [18].

Indeed, recent analyses yielded several interesting combinatorial approaches that showed efficacy. In the above described PDA model an adaptive response to oxidative stress compensated CB-839 effect. Because of the lack of potent inhibitors of CTH, L-buthionine-(S,R)-sulfoximine (BSO) was elected to inhibit γ-glutamate-cysteine ligase catalytic subunit (GCLC), and γ-glutamate-cysteine ligase regulatory subunit (GCLM), which are downstream of CTH and essential for GSH synthesis [43]. Interestingly, combinatorial BSO and CB-839 treatment in CB-839-resistant cells decreased proliferation. Also methotrexate (MTX), albendazole and MG-132 showed synergistic effect against cancer growth with CB-839 in PDA cells. Similarly, GLS inhibition by BPTES sensitizes PDA by lowering anti-oxidant defenses and increasing NQO1-induced ROS damage [44]. In fact, PDAs upregulate GLS, mitochondrial glutamate-oxaloacetate transaminase 2 (GOT2), and cytoplasmic glutamate-oxaloacetate transaminase 1 (GOT1) to support redox balance following rapid proliferation and growth, as a consequence of metabolic rewiring driven by mutant V-Ki-ras2 Kirsten rat sarcoma (KRAS) [44]. On the other hand, combination of a pyruvate kinase M2 (PKM2) inhibitor (shikonin) plus BPTES decreased the proliferation of several hypoxia-resistant gastric cancer cell lines, as well as in xenografted tumor *in vivo* [45]. This fact suggests that PKM2 inhibitor and GLS inhibitor might be useful for several subtypes of aggressive gastric cancers. Inhibiting GLS and PKM2 was also a successful strategy against oxaliplatin-resistant CRC *in vitro* and *in vivo* [46].

Glutamine synthetase

Glutamine synthetase history and tumor microenviroment

GS (EC 6.3.1.2, also known as GLUL) is a ATP-dependent metalloenzyme containing three divalent cations (Mg^{2+} or Mn^{2+}) that combines ammonium and Glu into Gln [47]. In mammals, GSII (from now on GS) is the most common type of GS, forming a homodecamer structure (monomer is around 350 amino acid) organized in two pentameric rings, coordinated through divalent cations [48]. As usually referred, GLUL gene codes for GS protein, that is also critical for endothelial cells motility and migration, contributing to angiogenesis through Rho GTPases, in development and disease [49].

Dysregulation of metabolic enzymes in cancer depends on multiple factors, including some elements modulating tumor microenvironment (TME) [50]. TME consists in different cell types surrounding tumor cells, such as immune cells, fibroblasts, endothelial cells, and adipocytes [51]. In addition, TME is a target for therapeutics to overcome immunotherapy resistance [52]. TME also includes lowered pH, differential oxygenation (hypoxia), higher levels of immune-modulatory metabolites, as well as stress by Glc and Gln [2]. Besides, heterogeneity among tumors is a main characteristic of each type of cancer [18,53]. Accordingly, increased Gln catabolism in mouse liver tumors was associated with decreased levels of GS and the switch from GLS2 to GLS. In sharp contrast, MYC-induced NSCLC tumors display increased expression of both *GLUL* and *GLS* and accumulate Gln [14]. Although immersed in this very complicated scenario, GS is a critical metabolic activity because it can influence cancer circuits and cell fate [51]. Main discoveries of GS as an essential metabolic enzyme in cancer are depicted at Fig. 3

[54-67]. In pioneer works at Souba laboratory, rats implanted subcutaneously with fibrosarcoma showed increased GS in liver [58], as well as in skeletal muscle and kidney [59] to support tumor avidity by Gln. GLUL overexpression was later detected also in liver cancer from human patients [60]. Because GS can synthesize Gln from Glu and thus allow cells to survive in Gln-depleted conditions, GS supports another anaplerotic flux for Gln, that feeds TCA cycle [68]. Significantly, Yang et al. (2016) induced tumor regression in an orthotopic mouse model for ovarian carcinoma by co-targeting GLUL in the mouse stromal cells (TME) and GLS in tumor cells, using GLS siRNA and GLUL siRNA, respectively, as well as a chitosan nanoparticle delivery system [69].

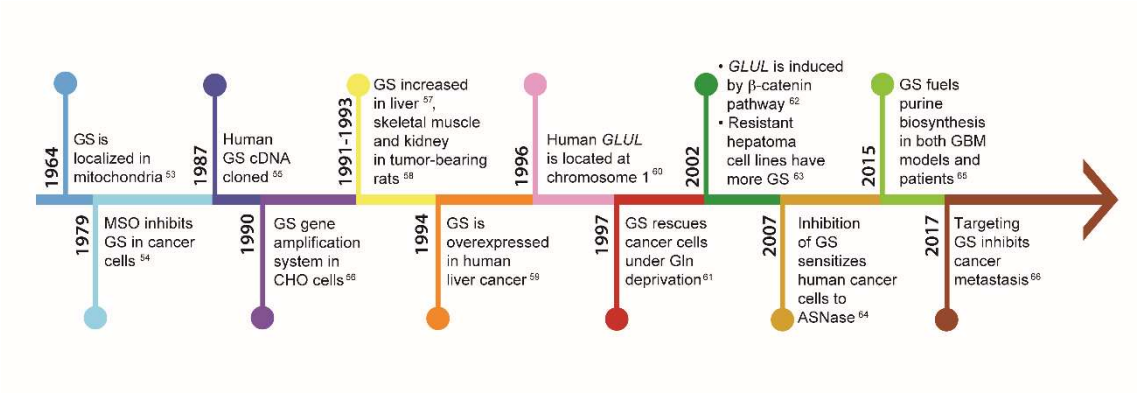

Fig. 3. Timeline of milestones for GS enzyme in cancer. Most relevant findings in scientific history that point out GLUL as a key enzyme in cancer growth are briefly depicted. ASNase, L-asparaginase; CHO, chinese hamster ovary; GBM, glioblastoma; Gln, glutamine; GLS, glutaminase isoenzyme; GLUL, γ-glutamyl:ammonia ligase gene; GS, glutamine synthetase; MSO, L-methionine sulfoximine.

Glutamine and glutamine synthetase

Some cancer cells can synthesize Gln *de novo* because of GS activity, but others import extracellular Gln, or use both metabolic capacities (GS and GLS) to produce and process Gln as a fuel for mitochondria [2]. Anyway, the absence of GS, associated to high GLS activity, makes tumor cells addicted to Gln, and this is usually linked with a more invasive, aggressive and resistant phenotype [51]. However, there are examples in which cancer cells express high levels of GS, such as papillary thyroid carcinoma [70], prostate neuroendocrine [71], prostate [72], breast [62,73] or liver cancers [74]. Cox et al. investigated zebrafish with hepatocyte-specific transgenic expression of mutated YAP (Yes-associated protein) that developed liver cancer as adults. In this model, GS increased Gln levels for nucleotide biosynthesis and the growth of liver cancer cells in a YAP-dependent manner [74]. GBM is another example where GS was upregulated in cancer [75]. These aggressive tumors have scarce GLS relative to surrounding brain tissue and show Gln independence [68]. GBM use Glc oxidation through pyruvate carboxylase (PC) during accelerated tumor growth [76]. These findings were confirmed by Tardito et al., who found that Gln-based anaplerosis was not essential for the proliferation of GBM. Experiments with both orthotopic GBM models and in patients demonstrated that GS produces Gln from TCA cycle-derived carbons. In fact, Gln required for the growth of GBM tumours is either synthesized by GS-positive glioma cells, or provided by surrounding astrocytes in a parasitic behavior [66]. Oligodendroglioma cells lack GS but are Gln-dependent [77]. As GBM, oligodendroglioma cells do not use Gln for anaplerosis. However, Gln starving induced a nutritional stress, blocking mTOR and Wnt/ β-catenin pathways, that was rescued by artificial GLUL overexpression [77]. Notably, GLUL is a characterized downstream target in the Wnt/β-

catenin signaling pathway *in vitro* and *in vivo* [63]. In addition, inhibitors of Gln transporters (ASCT2, SNAT2 and LAT2) elicited anti-proliferative effects on oligodendroglioma cells [77]. Different patterns (cancer heterogeneity) have been described in other tumor types [51]. Resistant leukemia cells depend more on glycolysis and TCA but less on GS [78]. This authors stated that reductions in GLUL expression and Gln dependence reflect an adaptation based on metabolic rewiring, that accompanies drug resistance in many cancer cells. Conversely, myeloma cells showed low expression of GLUL but high expression of Gln transporters, i.e.: ASCT2, that hint that these cells depend on extracellular Gln and use it for anaplerosis. In fact, stable ASCT2 silencing inhibited multiple myeloma growth in a murine xenograft model [79].

Glutamine synthetase expression as a therapeutic target against cancer

Remarkably, synergic antitumor capacity was achieved *in vitro* and *in vivo* by combination treatment with ASCT2 inhibition with benzylserine and the selective irreversible inhibitor of GS, L-methionine sulfoximine (MSO, Fig. 2). Ye et al. (2018) found a reinforced therapeutic efficacy of Gln-targeted treatment ASCT2-dependent in a gastric model [80]. Collectively, these results support the use of this combination therapy for treatment of patients whose gastric tumors express both ASCT2 and GLUL. Structural analog of Glu phosphinothricin and alendronate (Fig. 2), acting as GS inhibitors, have shown potential anti-cancer activity against lung cancer A-549 cell and breast cancer MCF-7 cell line [81]. Eisenberg and colleagues reviewed the GS inhibitors [82]. In SK-BR-3 breast cancer cells, it was demonstrated that knockdown of GLUL inhibited cell proliferation through inhibition of p38 mitogen-activated protein kinase (MAPK) and ERK1/2 signaling pathways [83]. In this research, the analysis of GLUL expression in clinical samples showed that GLUL expression was positively related with poor prognosis in breast cancer patients. In sarcoma cells, GS mediated proliferation of Gln-deprived cancer cells evoked nucleotide synthesis and mitochondrial bioenergetics [84]. Significantly, pharmacological (MSO) and shRNA-mediated inhibition of GLUL diminished xenograft tumor growth. Supporting results were found in ovarian cancer: knockdown of GLUL decreased the proliferation through inhibition of the p38-MAPK signaling pathway [85]. Besides, the analysis of GLUL expression in patient samples showed that high expression of GLUL was associated with poor prognosis in ovarian cancer patients. In ovarian cancer stem-like cells, GS triggered cell proliferation under ammonia stress, which enhanced tumor initiation *in vitro* and *in vivo* [86]. Mechanism was favored through hypoxia-inducible factor-1α (HIF-1α) stabilization by facilitating glycolysis and elevating Glc dependency.

Tumor-associated macrophages (TAMs) can act as metastasis promoters by creating an immune-suppressive environment, for which GLUL activity is critical [87]. GS deficiency in TAMs, induced either by GLUL silencing or MSO, led to lower T-cell suppression, reduction in angiogenesis, and tumor vessel normalization [87]. Conversely, when other types of ovarian cancer GS-overexpressing cells were subcutaneously injected into nude mice, tumor growth was fully inhibited [88]. Furthermore, in this model, the siRNA-mediated inhibition of the Gln transporter ASCT2 was more effective against tumor proliferation in the GS-deficient-ovarian cancer cells than in GLUL-expressing-ovarian cancer cells. Thus, downregulation of GLUL expression led to increased sensitivity to Gln depletion in these ovarian cancer cells.

SLC7A8 gene encodes L-type amino acid transporter 2 (LAT2), that is responsible for transporting neutral amino acids, including Gln, and functions as an oncogenic protein through Gln-dependent activation of mTOR pathway in pancreatic cancer [89]. LAT2 was upregulated, *in vitro* and *in vivo*, in gemcitabine-resistant pancreatic cancer cells, while artificial LAT2 overexpression decreased the level of GLS and increased that level of GS, as well as reduced

gemcitabine sensitivity *in vitro* and *in vivo* [89]. Consequently, the LAT2-mTOR-GS pathway might be another interesting therapeutic target in pancreatic cancer. Notably, GLUL expression was upregulated in gefitinib-sensitive NSCLCs, but it was downregulated in gefitinib-resistant NSCLCs [90]. In this model, the increased Gln anabolism promoted by GLUL expression sensitized cancer cells to gefitinib by diminishing both ATP and GSH production, leading to cell death and lower invasive capacity [90]. Accordingly, GLUL knock out provoked gefitinib resistance [90]. In other study, Gln-addicted NSCLC cell lines were highly sensitive to treatment with tumor necrosis factor receptor associated protein 1 (TRAP1) inhibitor gamitrinib triphenylphosphonium (G-TPP, Fig. 2) [91]. G-TPP treatment released GS enzymatic activity in those Gln-addicted NSCLCs through phosphorylation of AMPK. Hence, this drug might be useful for therapeutic targeting of other Gln auxotrophic cancers.

Within the same tumor, stromal cells are a key element to provide Gln requirements [69]. Stromal cells, which show high GS to compensate for the low GS of tumor cells, include cancer associated fibroblasts, adipocytes, regulatory T cells and TAMs [51]. TAMs play key roles in angiogenesis, tumor cell invasion, and metastasis formation [87]. In fact, GS acts as mediator of the proangiogenic, immunosuppressive, and pro-metastatic function of macrophages, highlighting the possibility of GS targeting against cancer metastasis [67]. In addition, adipocytes in a leukemia microenvironment produced Gln by upregulating GS *in vitro*, *in vivo* and in human samples of ALL [92]. Adipocytes also secrete Gln by overexpressing GS to promote PDA cell proliferation. In return, PDA cancer cells down-regulate adipocyte GLS expression [93]. On the other hand, there exists an inter-cellular metabolic Gln symbiosis described in brain [7], kidney [94], liver [95], and breast [96]. Through the analysis of how different breast cancer cells respond to Gln deprivation, Kung et al. found a striking difference in the Gln requirement among different breast cancer cells, which tracks with the luminal *versus* basal type. In the luminal cells, GATA3 triggers expression of GLUL and contributes to Gln independence. High GS represses GLS (low expression) which would also help to maintain the cell-type specific phenotype. Basal-specific expression (high GLS, low GLUL) is supported by the absence of GATA3 and higher activities of c-Myc in the basal type cells. In addition, GLUL expression in tumor cells can vary depending on many different mechanisms, including upregulation of thymine DNA glycosylase through c-Myc, which leads to demethylation of the GLUL gene promoter and its higher expression in subsets of cancer cell lines [97]. Moreover, GLUL was upregulated by Wnt pathway in c-Myc/E2F1 hepatocarcinogenesis [98]. GS was subject to feedback control by Gln, which promoted its post-translational acetylation and ubiquitination before its degradation in HCC cells [99]. On the other hand, phosphorylation and enhanced expression of liver GS was found in HCCs [100]. Besides, Gln, insulin and glucocorticoids regulated GLUL in HCCs and adipocytes [101]. Of note, DNA hypermethylation influenced dependency on Gln availability as exemplified by Gln auxotrophy in different cancer cell lines [102].

Bode and colleagues found that resistant and more aggressive hepatoma cell lines (HepG2, Hep3B, and Huh-7) had compensatory mechanisms for Gln supply, including higher GLUL expression, than the less differentiated hepatomas (SK-Hep, FOCUS, and PLC/PRF/5) [63]. Many studies have highlighted the critical role of GS and Gln for mTOR activation in HCC development [103]. So, several sets of HCCs are characterized by mutations of β-catenin and overexpression of GLUL [98,104,105].

State of the art and perspectives

(R)-Etodolac (Fig. 2) lowered proliferation of HCC cells by diminishing GLUL expression [106]. Similarly, sorafenib (Fig. 2) decreased GS, through interfering Wnt/β-catenin signaling pathway

in HepG2 cells and in HepG2 xenografts in mice, diminishing tumor volume and increasing median survival of sorafenib treated mice [107]. The antitumor enzyme L-asparaginase (ASNase) has been employed for many years in the treatment of ALL [92]. In that research, adipocytes protected leukemia cells from ASNase via Gln production through GS overexpression. Very recently it has been stated the possibility of anticancer combination therapy using two or more ASNases from different bacteria to achieve better therapeutic outcomes with lower side effects [108]. ASNase also increased GLUL expression and GS activity in several HCC lines [109]. This authors showed that ASNase had a significant antiproliferative effect in the β-catenin mutated HepG2 cell line through higher expression and activity of sodium-coupled neutral amino acid transporter 2 (SNAT2). Importantly, ASNase lowered the availability of extracellular Gln, and the GS inhibitor MSO prevented the intracellular synthesis of the amino acid. When simultaneously treated with ASNase and MSO, undetectable intracellular Gln and proliferative arrest and apoptosis was achieved in human osteosarcoma cells [65] and HepG2 cells [109]. The key role of β-catenin and GS for establishment and progression of HCC has been characterized *in vitro* [110] and *in vivo* [111]. Very recently, targeting β-catenin-GS-mTORC1 axis in HCC ameliorated cancer growth [112]. In this research, since no anti-β-catenin inhibitors are currently available in the clinic, mTOR inhibition with rapamycin in combination with MET-β-catenin inhibitor CG1 (sobetirome) were used. Successfully, this combined treatment synergistically reduced HCC burden. Strikingly, GS and mTOR use to be differently located at midlobular liver zone in HCC, instead of pericentral liver zone of normal hepatocytes, following a dysregulated mutated β-catenin profile in Wnt-driven liver cancer [113]. In patients suffering from HCC, after liver transplantation, immunohistochemical marker GS showed a positive correlation with better survival ratios [114]. However, in another study, GS-positive staining of HCC tumors by itself, was not associated with any changes in intratumoral proliferation in mice neither in human patients [115].

Concluding remarks and future prospects

Different therapeutic targets and specific inhibitors dealing with metabolic dysregulation have been very recently described [116]. In Table 1 are depicted GLS and GS inhibitors described in this review, and their roles in corresponding cancer types. As stated by many authors (see this review and this special issue), inhibiting a single metabolic target is not sufficient to block cancer growth in preclinical trials. In Table 1 some multi therapy treatments which synergistically inhibit some types of cancer are outlined. Future prospects include diagnostic signature of GLS inhibition predicting *in vivo* efficacy profile of BPTES/CB-839 treatments. Recently, drug response in mesenchymal breast and lung tumors was envisioned, and confirmed in lung patient-derived xenograft models [117]. In this research, a co-dependency of GLS and the γ -glutamylcysteine synthetase (GCS) inhibitor BSO was established, suggesting that the control of redox balance is a fundamental role of GLS. On the other hand, for overcoming drug resistance by specific metabolic inhibition, double or triple combination therapies have been proposed [116]. Among key metabolic targets, Gln metabolism has been extensively tackled, as reported in last years [2,3,55,118].

Table 1. Glutaminase and glutamine synthetase specific inhibitors with anticancer properties.

Drug	Inhibition	Multi therapy	Cancer type	Ref.
DON Acivicin	GLS/GLS2	ASNase	P388 and L1210 leukemia cells	17
DON	GLS/GLS2	Lonidamine, orlistat	Colon	21
BPTES	GLS	Metformin	Pancreas	2
BPTES	GLS	*Gls* silencing	HCC, lymphoma	20
BPTES	GLS	5-FU	NSCLC	26
BPTES	GLS	Cisplatin	TNBC	27
BPTES	GLS	DBZ	T-ALL	28
BPTES	GLS	ARQ761	PDA	43
BPTES	GLS	Shikonin	Gastric cancer cells	44
BPTES	GLS	BSO	Breast/lung cancer cells	117
CB-839	GLS	TXNIP repression	PC3/DU145 prostate cancer cells	22
CB-839	GLS	AZD8055	TNBC	23
CB-839	GLS	MLN128	Lung SCC, head and neck SCC, osteosarcoma	24
CB-839	GLS	THZ1	NSCLC	25
CB-839	GLS	Phenformin/metformin	ESCC	31
CB-839	GLS	Etomoxir/BSO	PDA	42
CB-839	GLS	ARQ761, albendazole, MTX, MG-132	PDA	43
CB-839	GLS	BSO	Breast/lung cancer cells	117
968	GLS	DHA	HCC	29
968	GLS	Metformin, ribavirin, oxaliplatin	CRC	30
968	GLS	CQ	NSCLC	31
968	GLS	PP242	GBM	32
CPD-3B	GLS/GLUD	----	A-549 lung, HCT116 colon, H22 liver, U251 GBM, caki-1 renal cells	33, 34
CPD-20	GLS	----	H22 liver/HCT116 colon	35
CPD-5	GLS/GLS2	Doxorubicin	MDA-MB-231 breast, AsPC-1 pancreas cells	36
3B	GLS	----	HCT116 colon cells	37
MSO	GS	Benzylserine	Gastric cancer cells	79
MSO	GS	----	Sarcoma cells	80
ALD/PPT	GS	----	A-549 lung, MCF-7 breast cancer cells	83
G-TPP	GS/AMPK	*Gls* silencing	NSCLC	90
Etodolac	GS/Wnt/β-catenin	----	HepG2/Hep3B liver cells	105
Sorafenib	GS/Wnt/β-catenin	----	HCC	106

ALD, alendronate; AMPK, adenosine monophosphate-activated protein kinase; ASNase, L-asparaginase; DBZ, dibenzazepine; BSO, L-buthionine-(S,R)-sulfoximine; CQ, chloroquine; CRC, colorectal cancer; DHA, dihydroartemisinin; DON, 6-diazo-5-oxo-L-norleucine; ESCC, esophageal squamous cell carcinoma; 5-FU, 5-fluorouracil; GBM, glioblastoma; *Gls*, glutaminase gene; GLS, glutaminase isoenzyme; GLS2, glutaminase 2 isoenzyme; GLUD, glutamate dehydrogenase; GS, glutamine synthetase; G-TPP, gamitrinib triphenylphosphonium; HCC, hepatocellular carcinoma; MTX, methotrexate; MSO, L-methionine sulfoximine; NSCLC, non-small-cell lung cancer; PDA, pancreatic ductal adenocarcinoma; PPT, phosphinothricin; SCC, squamous cell carcinoma; T-ALL, T-cell acute lymphoblastic leukemia; TNBC, triple negative breast cancer; TXNIP, thioredoxin-interacting protein.

Ralph DeBerardinis and colleagues confirmed that Gln is not only essential for feeding the biosynthesis of proteins, nucleotides and fatty acids, but also supports NADPH production and anaplerosis in tumor cells [119]. Although many cancer studies draw conclusions from a number of cell lines, large-scale metabolomic characterization of tumor samples will have direct implications for therapeutics targeting metabolism [102]. As found in most cancer models, a high ratio of GLS/GLUL expression in a tumor is indicative of extracellular Gln-dependent metabolism for cell proliferation and survival [120]. On the other hand, immunotherapy has a great potential to rewire the immune cycle through the generation of a bunch of alternative immune responses, and it has been established as another critical tool in the metabolic network of tumors to struggle against cancer [52]. Importantly, GLS controlled *in vitro* and *in vivo* for inflammatory effector T cell responses, enhancing mTORC1 and phosphatidylinositide 3-kinase (PI3K) signaling, to support glutaminolysis that integrates with glycolysis [121]. In a very recent research, GS and GLS have been characterized as basic targets in oral cancer [122]. Both metabolic enzymes are positively correlated with c-Myc overexpression, as well as with tumor growth and metastasis stage. Therefore, GLS and GS can be pointed out as essential metabolic enzymes to be targeted for cancer therapy. Anyhow, accurate patient stratification based on individual mutations and respective metabolic profiles is required to better plan the optimal combinations of targeted chemotherapies and immunotherapies needed for the success of personalized oncology [52,123].

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

Thanks are due to RTI2018-096866-B-I00 from Ministerio de Ciencia, Innovación y Universidades, Spain. JDS-J is granted by FPU17/04084, Ministerio de Ciencia, Innovación y Universidades.

References

1. Matés JM, Segura JA, Campos-Sandoval JA, Lobo C, Alonso L, Alonso FJ, Márquez J. Glutamine homeostasis and mitochondrial dynamics. Int J Biochem Cell Biol. 2009; 41: 2051-2061.

2. Matés JM, Di Paola FJ, Campos-Sandoval JA, Mazurek S, Márquez J. Therapeutic targeting of glutaminolysis as an essential strategy to combat cancer. Semin Cell Dev Biol. 2019: pii: S1084-9521(19)30073-4. doi: 10.1016/j.semcdb.2019.05.012.

3. Matés JM, Campos-Sandoval JA, Márquez J. Glutaminase isoenzymes in the metabolic therapy of cancer. Biochim Biophys Acta Rev Cancer. 2018; 1870: 158-164.

4. Matés JM, Segura JA, Martín-Rufián M, Campos-Sandoval JA, Alonso FJ, Márquez J. Glutaminase isoenzymes as key regulators in metabolic and oxidative stress against cancer, Curr Mol Med. 2013; 13: 514-534.

5. Jacque N, Ronchetti AM, Larrue C, Meunier G, Birsen R, Willems L, Saland E, Decroocq J, Maciel TT, Lambert M, Poulain L, Hospital MA, Sujobert P, Joseph L, Chapuis N, Lacombe

C, Moura IC, Demo S, Sarry JE, Recher C, Mayeux P, Tamburini J, Bouscary D. Targeting glutaminolysis has antileukemic activity in acute myeloid leukemia and synergizes with BCL-2 inhibition. Blood. 2015; 126: 1346-1356.

6. Ganapathy-Kanniappan, S. Molecular intricacies of aerobic glycolysis in cancer: current insights into the classic metabolic phenotype. Crit Rev Biochem Mol Biol. 2019; 22: 1-16.

7. Márquez J, Alonso FJ, Matés JM, Segura JA, Martín-Rufián M, Campos-Sandoval JA. Glutamine Addiction In Gliomas. Neurochem Res. 2017; 42: 1735-1746.

8. Alam MM, Lal S, FitzGerald KE, Zhang L. A holistic view of cancer bioenergetics: mitochondrial function and respiration play fundamental roles in the development and progression of diverse tumors. Clin Transl Med. 2016; 5: 3.

9. Kalyanaraman B, Cheng G, Hardy M, Ouari O, Lopez M, Joseph J, Zielonka J, Dwinell MB. A review of the basics of mitochondrial bioenergetics, metabolism, and related signaling pathways in cancer cells: Therapeutic targeting of tumor mitochondria with lipophilic cationic compounds. Redox Biol. 2018; 14: 316-327.

10. Rodrigues MF, Obre E, de Melo FH, Santos GC Jr, Galina A, Jasiulionis MG, Rossignol R, Rumjanek FD, Amoêdo ND. Enhanced OXPHOS, glutaminolysis and β-oxidation constitute the metastatic phenotype of melanoma cells. Biochem J. 2016; 473: 703-715.

11. DeBerardinis, RJ, Chandel, NS. Fundamentals of cancer metabolism. Sci Adv. 2016; 2: e1600200.

12. Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature. 2012; 491: 364-373.

13. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, Wellen KE, O'Rourke DM, Berger SL, Chan TA, Levine RL, Mellinghoff IK, Thompson CB. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012; 483: 474-478.

14. Yuneva MO, Fan TW, Allen TD, Higashi RM, Ferraris DV, Tsukamoto T, Matés JM, Alonso FJ, Wang C, Seo Y, Chen X, Bishop JM. The metabolic profile of tumors depends on both the responsible genetic lesion and tissue type. Cell Metab. 2012; 15: 157-170.

15. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016; 16: 619-634.

16. Le Page GA, Loo TL. Purine antagonists. In: Holland JF and Frei III, E (eds.), Cancer Medicine. Philadelphia: Lea and Febiger. 1973, pp. 754-756.

17. Rosenfeld H, Roberts J. Enhancement of antitumor activity of glutamine antagonists 6-diazo-5-oxo-L-norleucine and acivicin in cell culture by glutaminase-asparaginase. Cancer Res. 1981; 41: 1324-1328.

18. Matés JM, Campos-Sandoval JA, de Los Santos-Jiménez J, Segura JA, Alonso FJ, Márquez J. Metabolic reprogramming of cancer by chemicals that target glutaminase isoenzymes. Curr Med Chem. 2019; 26: doi: 10.2174/0929867326666190416165004.

19. Márquez J, Matés JM, Campos-Sandoval JA. Glutaminases. Adv Neurobiol. 2016; 13: 133-171.

20. Xiang Y, Stine ZE, Xia J, Lu Y, O'Connor RS, Altman BJ, Hsieh AL, Gouw AM, Thomas AG, Gao P, Sun L, Song L, Yan B, Slusher BS, Zhuo J, Ooi LL, Lee CG, Mancuso A, McCallion AS, Le A, Milone MC, Rayport S, Felsher DW, Dang CV. Targeted inhibition of tumor-specific

glutaminase diminishes cell-autonomous tumorigenesis. J Clin Invest. 2015; 125: 2293–2306.

21. Cervantes-Madrid D, Dominguez-Gomez G, Gonzalez-Fierro A, Perez-Cardenas E, Taja-Chayeb L, Trejo-Becerril C, Duenas-Gonzalez, A. Feasibility and antitumor efficacy in vivo, of simultaneously targeting glycolysis, glutaminolysis and fatty acid synthesis using lonidamine, 6-diazo-5-oxo-L-norleucine and orlistat in colon cancer. Oncol Lett. 2017; 13: 1905-1910.

22. Qu X, Sun J, Zhang Y, Li J, Hu J, Li K, Gao L, Shen L. c-Myc-driven glycolysis via TXNIP suppression is dependent on glutaminase-MondoA axis in prostate cancer. Biochem Biophys Res Commun. 2018; 504: 415-421.

23. Lampa M, Arlt H, He T, Ospina B, Reeves J, Zhang B, Murtie J, Deng G, Barberis C, Hoffmann D, Cheng H, Pollard J, Winter C, Richon V, Garcia-Escheverria C, Adrian F, Wiederschain D, Srinivasan L. Glutaminase is essential for the growth of triple-negative breast cancer cells with a deregulated glutamine metabolism pathway and its suppression synergizes with mTOR inhibition. PLoS One. 2017; 12: e0185092.

24. Momcilovic M, Bailey ST, Lee JT, Fishbein MC, Braas D, Go J, Graeber TG, Parlati F, Demo S, Li R, Walser TC, Gricowski M, Shuman R, Ibarra J, Fridman D, Phelps ME, Badran K, St. John M, Bernthal NM, Federman N, Yanagawa J, Dubinett SM, Sadeghi S, Christofk HR, Shackelford, DB. The GSK3 Signaling Axis Regulates Adaptive Glutamine Metabolism in Lung Squamous Cell Carcinoma. Cancer Cell. 2018; 33: 905-921.

25. Cheng ZJ, Miao DL, Su QY, Tang XL, Wang XL, Deng LB, Shi HD, Xin HB. THZ1 suppresses human non-small-cell lung cancer cells in vitro through interference with cancer metabolism. Acta Pharmacol Sin. 2019; 40: 814-822.

26. Lee JS, Kang JH, Lee SH, Lee CH, Son J, Kim SY. Glutaminase 1 inhibition reduces thymidine synthesis in NSCLC. Biochem Biophys Res Commun. 2016; 477: 374-382.

27. Chen L, Cui H, Fang J, Deng H, Kuang P, Guo H, Wang X, Zhao L. Glutamine deprivation plus BPTES alters etoposide- and cisplatin-induced apoptosis in triple negative breast cancer cells. Oncotarget. 2016; 7: 54691-54701.

28. Herranz D, Ambesi-Impiombato A, Sudderth J, Sánchez-Martín M, Belver L, Tosello V, Xu L, Wendorff AA, Castillo M, Haydu JE, Márquez J, Matés JM, Kung AL, Rayport S, Cordon-Cardo C, DeBerardinis RJ, Ferrando AA. Metabolic reprogramming induces resistance to anti-NOTCH1 therapies in acute lymphoblastic leukemia. Nat Med. 2015; 21: 1182-1189.

29. Wang D, Meng G, Zheng M, Zhang Y, Chen A, Wu J, Wei J. The Glutaminase-1 Inhibitor 968 Enhances Dihydroartemisinin-Mediated Antitumor Efficacy in Hepatocellular Carcinoma Cells. PLoS One. 2016; 11: e0166423.

30. Richard SM, Martinez Marignac VL. Sensitization to oxaliplatin in HCT116 and HT29 cell lines by metformin and ribavirin and differences in response to mitochondrial glutaminase inhibition. J Cancer Res Ther. 2015; 11: 336-340.

31. Qie S, Yoshida A, Parnham S, Oleinik N, Beeson GC, Beeson CC, Ogretmen B, Bass AJ, Wong KK, Rustgi AK, Diehl JA. Targeting glutamine-addiction and overcoming CDK4/6 inhibitor resistance in human esophageal squamous cell carcinoma. Nat Commun. 2019; 10: 1296.

32. Han T, Guo M, Zhang T, Gan M, Xie C, Wang JB. A novel Glutaminase inhibitor-968 inhibits the migration and proliferation of non-small cell lung cancer cells by targeting EGFR/ERK signaling pathway. Oncotarget. 2017; 8 : 28063-28073.

33. Tanaka K, Sasayama T, Irino Y, Takata K, Nagashima H, Satoh N, Kyotani K, Mizowaki T, Imahori T, Ejima Y, Masui K, Gini B, Yang H, Hosoda K, Sasaki R, Mischel PS, Kohmura E. Compensatory glutamine metabolism promotes glioblastoma resistance to mTOR inhibitor treatment. J Clin Invest. 2015; 125: 1591-1602.

34. Zhu M, Fang J, Zhang J, Zhang Z, Xie J, Yu Y, Ruan JJ, Chen Z, Hou W, Yang G, Su W, Ruan BH. Biomolecular Interaction Assays Identified Dual Inhibitors of Glutaminase and Glutamate Dehydrogenase That Disrupt Mitochondrial Function and Prevent Growth of Cancer Cells. Anal Chem. 2017; 89: 1689-1696.

35. Ruan JJ, Yu Y, Hou W, Chen Z, Fang J, Zhang J, Ni M, Li D, Lu S, Rui J, Wu R, Zhang W, Ruan BH. Kidney Type Glutaminase Inhibitor Hexylselen Selectively Kills Cancer Cells via a Three-Pronged Mechanism. ACS Pharmacol Transl Sci. 2019; 2: 18-30.

36. Chen Z, Li D, Xu N, Fang J, Yu Y, Hou W, Ruan H, Zhu P, Ma R, Lu S, Cao D, Wu R, Ni M, Zhang W, Su W, Ruan BH. Novel 1,3,4-Selenadiazole-Containing Kidney-Type Glutaminase Inhibitors Showed Improved Cellular Uptake and Antitumor Activity. J Med Chem. 2019; 62: 589-603.

37. Yeh TK, Kuo CC, Lee YZ, Ke YY, Chu KF, Hsu HY, Chang HY, Liu YW, Song JS, Yang CW, Lin LM, Sun M, Wu SH, Kuo PC, Shih C, Chen CT, Tsou LK, Lee SJ. Design, Synthesis, and Evaluation of Thiazolidine-2,4-dione Derivatives as a Novel Class of Glutaminase Inhibitors. J Med Chem. 2017; 60: 5599-5612.

38. Hou W, Zhou Y, Rui J, Bai R, Bhasin AKK, Ruan BH. Design and synthesis of novel tellurodibenzoic acid compounds as kidney-type glutaminase (KGA) inhibitors. Bioorg Med Chem Lett. 2019; 29: 1673-1676.

39. Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. J Clin Invest. 2013: 123: 3678-3684.

40. Yang L, Venneti S, Nagrath D. Glutaminolysis: A Hallmark of Cancer Metabolism. Annu Rev Biomed Eng. 2017; 19: 163-194.

41. Zhou R, Pantel AR, Li S, Lieberman BP, Ploessl K, Choi H, Blankemeyer E, Lee H, Kung HF, Mach RH, Mankoff DA. [^{18}F](2S,4R)4-Fluoroglutamine PET Detects Glutamine Pool Size Changes in Triple-Negative Breast Cancer in Response to Glutaminase Inhibition. Cancer Res. 2017; 77: 1476-1484.

42. Hensley CT, DeBerardinis RJ. In vivo analysis of lung cancer metabolism: nothing like the real thing. J Clin Invest. 2015; 25: 495-497.

43. Biancur DE, Paulo JA, Małachowska B, Quiles Del Rey M, Sousa CM, Wang X, Sohn ASW, Chu GC, Gygi SP, Harper JW, Fendler W, Mancias JD, Kimmelman AC. Compensatory metabolic networks in pancreatic cancers upon perturbation of glutamine metabolism. Nat Commun. 2017; 8: 15965.

44. Chakrabarti G, Moore ZR, Luo X, Ilcheva M, Ali A, Padanad M, Zhou Y, Xie Y, Burma S, Scaglioni PP, Cantley LC, DeBerardinis RJ, Kimmelman AC, Lyssiotis CA, Boothman DA. Targeting glutamine metabolism sensitizes pancreatic cancer to PARP-driven metabolic catastrophe induced by ß-lapachone. Cancer Metab. 2015; 3: 12.

45. Kitayama K, Yashiro M, Morisaki T, Miki Y, Okuno T, Kinoshita H, Fukuoka T, Kasashima H, Masuda G, Hasegawa T, Sakurai K, Kubo N, Hirakawa K, Ohira M. Pyruvate kinase isozyme M2 and glutaminase might be promising molecular targets for the treatment of gastric cancer. Cancer Sci. 2017; 108: 2462-2469.

46. Lu WQ, Hu YY, Lin XP, Fan W. Knockdown of PKM2 and GLS1 expression can significantly reverse oxaliplatin-resistance in colorectal cancer cells. Oncotarget. 2017; 8: 44171-4418.

47. Krajewski WW, Collins R, Holmberg-Schiavone L, Jones TA, Karlberg T, Mowbray SL. Crystal structures of mammalian glutamine synthetases illustrate substrate-induced conformational changes and provide opportunities for drug and herbicide design. J Mol Biol. 2008; 375: 217-228.

48. Moreira C, Ramos MJ, Fernandes PA. Clarifying the Catalytic Mechanism of Human Glutamine Synthetase: A QM/MM Study. J Phys Chem B. 2017; 121: 6313-6320.

49. Eelen G, Dubois C, Cantelmo AR, Goveia J, Brüning U, DeRan M, Jarugumilli G, van Rijssel J, Saladino G, Comitani F, Zecchin A, Rocha S, Chen R, Huang H, Vandekeere S, Kalucka J, Lange C, Morales-Rodriguez F, Cruys B, Treps L, Ramer L, Vinckier S, Brepoels K, Wyns S, Souffreau J, Schoonjans L, Lamers WH, Wu Y, Haustraete J, Hofkens J, Liekens S, Cubbon R, Ghesquière B, Dewerchin M, Gervasio FL, Li X, van Buul JD, Wu X, Carmeliet P. Role of glutamine synthetase in angiogenesis beyond glutamine synthesis. Nature. 2018; 561: 63-69.

50. Zhuang X, Zhang H, Hu G. Cancer and Microenvironment Plasticity: Double-Edged Swords in Metastasis. Trends Pharmacol Sci. 2019; 40: 419-429.

51. Castegna A, Menga A. Glutamine Synthetase: Localization Dictates Outcome. Genes (Basel). 2018;9: pii: E108.

52. Ramapriyan R, Caetano MS, Barsoumian HB, Mafra ACP, Zambalde EP, Menon H, Tsouko E, Welsh JW, Cortez MA. Altered cancer metabolism in mechanisms of immunotherapy resistance. Pharmacol Ther. 2019; 195: 162-171.

53. Weiskirchen R. Intratumor heterogeneity, variability and plasticity: questioning the current concepts in classification and treatment of hepatocellular carcinoma. Hepatobiliary Surg Nutr. 2016; 5: 183-187.

54. Hsu L, Tappel AL. The intracelular distibution of glutamine synthetase in rat liver and the effect of metals on its activity. J Cell Comp Physiol. 1964; 64: 265-270.

55. Meister A, Griffith OW. Effects of methionine sulfoximine analogs on the synthesis of glutamine and glutathione: possible chemotherapeutic implications. Cancer Treat Rep. 1979; 63: 1115-1121.

56. Gibbs CS, Campbell KE, Wilson RH. Sequence of a human glutamine synthetase cDNA. Nucleic Acids Res. 1987; 15: 6293.

57. Cockett MI, Bebbington CR, Yarranton GT. High level expression of tissue inhibitor of metalloproteinases in Chinese hamster ovary cells using glutamine synthetase gene amplification. Biotechnology (N Y). 1990; 8: 662-667.

58. Chen MK, Salloum RM, Austgen TR, Bland JB, Bland KI, Copeland EM 3rd, Souba WW. Tumor regulation of hepatic glutamine metabolism. JPEN J Parenter Enteral Nutr. 1991; 15: 159-164.

59. Chen MK, Espat NJ, Bland KI, Copeland EM 3rd, Souba WW. Influence of progressive tumor growth on glutamine metabolism in skeletal muscle and kidney. Ann Surg. 1993; 217: 655-666.

60. Christa L, Simon MT, Flinois JP, Gebhardt R, Brechot C, Lasserre C. Overexpression of glutamine synthetase in human primary liver cancer. Gastroenterology. 1994; 106: 1312-1320.

61. Clancy KP, Berger R, Cox M, Bleskan J, Walton KA, Hart I, Patterson D. Localization of the L-glutamine synthetase gene to chromosome 1q23. Genomics. 1996; 38: 418-420.

62. Collins CL, Wasa M, Souba WW, Abcouwer SF. Regulation of glutamine synthetase in human breast carcinoma cells and experimental tumors. Surgery. 1997; 122:451–463.

63. Cadoret A, Ovejero C, Terris B, Souil E, Lévy L, Lamers WH, Kitajewski J, Kahn A, Perret C. New targets of beta-catenin signaling in the liver are involved in the glutamine metabolism. Oncogene. 2002; 21: 8293-8301.

64. Bode BP, Fuchs BC, Hurley BP, Conroy JL, Suetterlin JE, Tanabe KK, Rhoads DB, Abcouwer SF, Souba WW. Molecular and functional analysis of glutamine uptake in human hepatoma and liver-derived cells. Am J Physiol Gastrointest Liver Physiol. 2002; 283: G1062-G1073.

65. Tardito S, Uggeri J, Bozzetti C, Bianchi MG, Rotoli BM, Franchi-Gazzola R, Gazzola GC, Gatti R, Bussolati O. The inhibition of glutamine synthetase sensitizes human sarcoma cells to L-asparaginase. Cancer Chemother Pharmacol. 2007; 60: 751-758.

66. Tardito, S.; Oudin, A.; Ahmed, S.U.; Fack, F.; Keunen, O.; Zheng, L.; Miletic, H.; Sakariassen, P.Ø.; Weinstock, A.; Wagner, A.; Lindsay, S.L.; Hock, A.K.; Barnett, S.C.; Ruppin, E.; Mørkve, S.H.; Lund-Johansen, M.; Chalmers, A.J.; Bjerkvig, R.; Niclou, S.P.; Gottlieb, E. Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma. Nat Cell Biol. 2015; 17: 1556-1568.

67. Palmieri EM, Menga A, Martín-Pérez R, Quinto A, Riera-Domingo C, De Tullio G, Hooper DC, Lamers WH, Ghesquière B, McVicar DW, Guarini A, Mazzone M, Castegna A. Pharmacologic or Genetic Targeting of Glutamine Synthetase Skews Macrophages toward an M1-like Phenotype and Inhibits Tumor Metastasis. Cell Rep. 2017; 20: 1654-1666.

68. Cluntun AA, Lukey MJ, Cerione RA, Locasale JW. Glutamine Metabolism in Cancer: Understanding the Heterogeneity. Trends Cancer. 2017; 3: 169-180.

69. Yang L, Achreja A, Yeung TL, Mangala LS, Jiang D, Han C, Baddour J, Marini JC, Ni J, Nakahara R, Wahlig S, Chiba L, Kim SH, Morse J, Pradeep S, Nagaraja AS, Haemmerle M, Kyunghee N, Derichsweiler M, Plackemeier T, Mercado-Uribe I, Lopez-Berestein G, Moss T, Ram PT, Liu J, Lu X, Mok SC, Sood AK, Nagrath D. Targeting Stromal Glutamine Synthetase in Tumors Disrupts Tumor Microenvironment-Regulated Cancer Cell Growth. Cell Metab. 2016; 24: 685-700.

70. Huang K, Cui M, Ye F, Li Y, Zhang D. Global profiling of the signaling network of papillary thyroid carcinoma. Life Sci. 2016; 147: 9-14.

71. Ippolito JE, Piwnica-Worms D. A fluorescence-coupled assay for gamma aminobutyric acid (GABA) reveals metabolic stress-induced modulation of GABA content in neuroendocrine cancer. PLoS One. 2014; 9: e88667.

72. Shi X, Zhang X, Yi C, Liu Y, He Q. [^{13}N]Ammonia positron emission tomographic/computed tomographic imaging targeting glutamine synthetase expression in prostate cancer. Mol Imaging. 2014; 13.

73. Selicharová I, Smutná K, Sanda M, Ubik K, Matousková E, Bursíková E, Brozová M, Vydra J, Jirácek J. 2-DE analysis of a new human cell line EM-G3 derived from breast cancer progenitor cells and comparison with normal mammary epithelial cells. Proteomics. 2007; 7: 1549-1559.

74. Cox AG, Hwang KL, Brown KK, Evason K, Beltz S, Tsomides A, O'Connor K, Galli GG, Yimlamai D, Chhangawala S, Yuan M, Lien EC, Wucherpfennig J, Nissim S, Minami A, Cohen DE, Camargo FD, Asara JM, Houvras Y, Stainier DYR, Goessling W. Yap reprograms glutamine metabolism to increase nucleotide biosynthesis and enable liver growth. Nat Cell Biol. 2016; 18: 886-896.

75. Krall AS, Christofk HR. Rethinking glutamine addiction. Nat Cell Biol. 2015; 17: 1515-1517.

76. Marin-Valencia I, Yang C, Mashimo T, Cho S, Baek H, Yang XL, Rajagopalan KN, Maddie M, Vemireddy V, Zhao Z, Cai L, Good L, Tu BP, Hatanpaa KJ, Mickey BE, Matés JM, Pascual JM, Maher EA, Malloy CR, Deberardinis RJ, Bachoo RM. Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain *in vivo*. Cell Metab. 2012; 15: 827-837.

77. Chiu M, Taurino G, Bianchi MG, Ottaviani L, Andreoli R, Ciociola T, Lagrasta CAM, Tardito S, Bussolati O. Oligodendroglioma Cells Lack Glutamine Synthetase and Are Auxotrophic for Glutamine, but Do not Depend on Glutamine Anaplerosis for Growth. Int J Mol Sci. 2018; 19: pii: E1099.

78. Stäubert C, Bhuiyan H, Lindahl A, Broom OJ, Zhu Y, Islam S, Linnarsson S, Lehtiö J, Nordström A. Rewired metabolism in drug-resistant leukemia cells: a metabolic switch hallmarked by reduced dependence on exogenous glutamine. J Biol Chem. 2015; 290: 8348-8359.

79. Bolzoni M, Chiu M, Accardi F, Vescovini R, Airoldi I, Storti P, Todoerti K, Agnelli L, Missale G, Andreoli R, Bianchi MG, Allegri M, Barilli A, Nicolini F, Cavalli A, Costa F, Marchica V, Toscani D, Mancini C, Martella E, Dall'Asta V, Donofrio G, Aversa F, Bussolati O, Giuliani N. Dependence on glutamine uptake and glutamine addiction characterize myeloma cells: a new attractive target. Blood. 2016; 128: 667-679.

80. Ye J, Huang Q, Xu J, Huang J, Wang J, Zhong W, Chen W, Lin X, Lin X. Targeting of glutamine transporter ASCT2 and glutamine synthetase suppresses gastric cancer cell growth. J Cancer Res Clin Oncol. 2018; 144: 821-833.

81. Sakr TM, Khedr MA, Rashed HM, Mohamed ME. In Silico-Based Repositioning of Phosphinothricin as a Novel Technetium-99m Imaging Probe with Potential Anti-Cancer Activity. Molecules. 2018; 23: pii: E496.

82. Eisenberg D, Gill HS, Pfluegl GM, Rotstein SH. Structure-function relationships of glutamine synthetases. Biochim Biophys Acta. 2000; 1477: 122–45.

83. Wang Y, Fan S, Lu J, Zhang Z, Wu D, Wu Z, Zheng Y. GLUL promotes cell proliferation in breast cancer. J Cell Biochem. 2017; 118: 2018-2025.

84. Issaq SH, Mendoza A, Fox SD, Helman LJ. Glutamine synthetase is necessary for sarcoma adaptation to glutamine deprivation and tumor growth. Oncogenesis. 2019; 8: 20.

85. Fan S, Wang Y, Zhang Z, Lu J, Wu Z, Shan Q, Sun C, Wu D, Li M, Sheng N, Xie Y, Zheng Y. High expression of glutamate-ammonia ligase is associated with unfavorable prognosis in patients with ovarian cancer. J Cell Biochem. 2018; 119: 6008-6015.

86. Kitajima S, Lee KL, Hikasa H, Sun W, Huang RY, Yang H, Matsunaga S, Yamaguchi T, Araki M, Kato H, Poellinger L. Hypoxia-inducible factor-1α promotes cell survival during ammonia stress response in ovarian cancer stem-like cells. Oncotarget. 2017; 8: 114481-114494.

87. Mazzone M, Menga A, Castegna A. Metabolism and TAM functions-it takes two to tango. FEBS J. 2018; 285: 700-716.

88. Furusawa A, Miyamoto M, Takano M, Tsuda H, Song YS, Aoki D, Miyasaka N, Inazawa J, Inoue J. Ovarian cancer therapeutic potential of glutamine depletion based on GS expression. Carcinogenesis. 2018; 39: 758-766.

89. Feng M, Xiong G, Cao Z, Yang G, Zheng S, Qiu J, You L, Zheng L, Zhang T, Zhao Y. LAT2 regulates glutamine-dependent mTOR activation to promote glycolysis and chemoresistance in pancreatic cancer. J Exp Clin Cancer Res. 2018; 37: 274.

90. Wang L, Peng W, Wu T, Deng P, Zhao YL. Increased glutamine anabolism sensitizes non-small cell lung cancer to gefitinib treatment. Cell Death Discov. 2018; 4: 24.

91. Vo VTA, Choi JW, Phan ANH, Hua TNM, Kim MK, Kang BH, Jung SH, Yong SJ, Jeong Y. TRAP1 inhibition increases glutamine synthetase activity in glutamine auxotrophic non-small cell lung cancer cells. Anticancer Res. 2018; 38:2187-2193.

92. Ehsanipour EA, Sheng X, Behan JW, Wang X, Butturini A, Avramis VI, Mittelman SD. Adipocytes cause leukemia cell resistance to L-asparaginase via release of glutamine. Cancer Res. 2013; 73: 2998-3006.

93. Meyer KA, Neeley CK, Baker NA, Washabaugh AR, Flesher CG, Nelson BS, Frankel TL, Lumeng CN, Lyssiotis CA, Wynn ML, Rhim AD, O'Rourke RW. Adipocytes promote pancreatic cancer cell proliferation via glutamine transfer. Biochem Biophys Rep. 2016; 7: 144-149.

94. Burch HB, Choi S, McCarthy WZ, Wong PY, Lowry OH. The location of glutamine synthetase within the rat and rabbit nephron. Biochem Biophys Res Commun. 1978; 82:498–505.

95. Brosnan ME, Brosnan JT. Hepatic glutamate metabolism: a tale of 2 hepatocytes. Am J Clin Nutr. 2009; 90: 857S–861S.

96. Kung HN, Marks JR, Chi JT. Glutamine synthetase is a genetic determinant of cell type-specific glutamine independence in breast epithelia. PLoS Genet. 2011; 7:e1002229.

97. Bott AJ, Peng IC, Fan Y, Faubert B, Zhao L, Li J, Neidler S, Sun Y, Jaber N, Krokowski D, Lu W, Pan JA, Powers S, Rabinowitz J, Hatzoglou M, Murphy DJ, Jones R, Wu S, Girnun G, Zong WX. Oncogenic Myc induces expression of glutamine synthetase through promoter demethylation. Cell Metab. 2015; 22: 1068–1077.

98. Calvisi DF, Conner EA, Ladu S, Lemmer ER, Factor VM, Thorgeirsson SS. Activation of the canonical Wnt/beta-catenin pathway confers growth advantages in c-Myc/E2F1 transgenic mouse model of liver cancer. J Hepatol. 2005; 42: 842-849.

99. Nguyen TV, Lee JE, Sweredoski MJ, Yang SJ, Jeon SJ, Harrison JS, Yim JH, Lee SG, Handa H, Kuhlman B, Jeong JS, Reitsma JM, Park CS, Hess S, Deshaies RJ. Glutamine triggers

acetylation-dependent degradation of glutamine synthetase via the thalidomide receptor cereblon. Mol Cell. 2016; 61: 809–820.

100. Kuramitsu Y, Harada T, Takashima M, Yokoyama Y, Hidaka I, Iizuka N, Toda T, Fujimoto M, Zhang X, Sakaida I, Okita K, Oka M, Nakamura K. Increased expression and phosphorylation of liver glutamine synthetase in well-differentiated hepatocellular carcinoma tissues from patients infected with hepatitis C virus. Electrophoresis. 2006; 27: 1651-1658.

101. Wang Y, Watford M. Glutamine, insulin and glucocorticoids regulate glutamine synthetase expression in C2C12 myotubes, Hep G2 hepatoma cells and 3T3 L1 adipocytes. Biochim Biophys Acta. 2007; 1770: 594-600.

102. Li H, Ning S, Ghandi M, Kryukov GV, Gopal S, Deik A, Souza A, Pierce K, Keskula P, Hernandez D, Ann J, Shkoza D, Apfel V, Zou Y, Vazquez F, Barretina J, Pagliarini RA, Galli GG, Root DE, Hahn WC, Tsherniak A, Giannakis M, Schreiber SL, Clish CB, Garraway LA, Sellers WR. The landscape of cancer cell line metabolism. Nat Med. 2019; 25: 850-860.

103. Chiu M, Tardito S, Pillozzi S, Arcangeli A, Armento A, Uggeri J, Missale G, Bianchi MG, Barilli A, Dall'Asta V, Campanini N, Silini EM, Fuchs J, Armeanu-Ebinger S, Bussolati O. Glutamine depletion by crisantaspase hinders the growth of human hepatocellular carcinoma xenografts. Br J Cancer. 2014; 111: 1159-1167.

104. Austinat M, Dunsch R, Wittekind C, Tannapfel A, Gebhardt R, Gaunitz F. Correlation between beta-catenin mutations and expression of Wnt-signaling target genes in hepatocellular carcinoma. Mol Cancer. 2008; 7: 21.

105. Nakamoto Y. Promising new strategies for hepatocellular carcinoma. Hepatol Res. 2017; 47: 251-265.

106. Behari J, Zeng G, Otruba W, Thompson MD, Muller P, Micsenyi A, Sekhon SS, Leoni L, Monga SP. R-Etodolac decreases beta-catenin levels along with survival and proliferation of hepatoma cells. J Hepatol. 2007; 46: 849-857.

107. Lachenmayer A, Alsinet C, Savic R, Cabellos L, Toffanin S, Hoshida Y, Villanueva A, Minguez B, Newell P, Tsai HW, Barretina J, Thung S, Ward SC, Bruix J, Mazzaferro V, Schwartz M, Friedman SL, Llovet JM. Wnt-pathway activation in two molecular classes of hepatocellular carcinoma and experimental modulation by sorafenib. Clin Cancer Res. 2012; 18: 4997-5007.

108. Ghasemian A, Al-Marzoqi AH, Al-Abodi HR, Alghanimi YK, Kadhum SA, Shokouhi Mostafavi SK, Fattahi A. Bacterial l-asparaginases for cancer therapy: Current knowledge and future perspectives. J Cell Physiol. 2019; 234: 19271-19279.

109. Tardito S, Chiu M, Uggeri J, Zerbini A, Da Ros F, Dall'Asta V, Missale G, Bussolati O. L-Asparaginase and inhibitors of glutamine synthetase disclose glutamine addiction of β-catenin-mutated human hepatocellular carcinoma cells. Curr Cancer Drug Targets. 2011; 11: 929-943.

110. Zeng G, Apte U, Cieply B, Singh S, Monga SP. siRNA-mediated beta-catenin knockdown in human hepatoma cells results in decreased growth and survival. Neoplasia. 2007; 9: 951-959.

111. Tao J, Zhang R, Singh S, Poddar M, Xu E, Oertel M, Chen X, Ganesh S, Abrams M, Monga SP. Targeting β-catenin in hepatocellular cancers induced by coexpression of mutant β-catenin and K-Ras in mice. Hepatology. 2017; 65: 1581-1599.

112. Adebayo Michael AO, Ko S, Tao J, Moghe A, Yang H, Xu M, Russell JO, Pradhan-Sundd T, Liu S, Singh S, Poddar M, Monga JS, Liu P, Oertel M, Ranganathan S, Singhi A, Rebouissou S, Zucman-Rossi J, Ribback S, Calvisi D, Qvartskhava N, Görg B, Häussinger D, Chen X, Monga SP. Inhibiting Glutamine-Dependent mTORC1 Activation Ameliorates Liver Cancers Driven by β-Catenin Mutations. Cell Metab. 2019; 29: 1135-1150.

113. Goessling W. Position Is Destiny: Metabolism and Cell Identity. Cell Metab. 2019; 29: 1017-1019.

114. Ataide EC, Perales SR, Silva MG, Filho FC, Sparapani AC, Latuf Filho PF, Stucchi RSB, Vassallo J, Escanhoela CAF, Boin IFSF. Immunoexpression of Heat Shock Protein 70, Glypican 3, Glutamine Synthetase, and Beta-Catenin in Hepatocellular Carcinoma After Liver Transplantation: Association Between Positive Glypican 3 and Beta-Catenin with the Presence of Larger Nodules. Transplant Proc. 2017; 49: 858-862.

115. Lee JM, Yang J, Newell P, Singh S, Parwani A, Friedman SL, Nejak-Bowen KN, Monga SP. β-Catenin signaling in hepatocellular cancer: Implications in inflammation, fibrosis, and proliferation. Cancer Lett. 2014; 343: 90-97.

116. Kim SY. Targeting cancer energy metabolism: a potential systemic cure for cancer. Arch Pharm Res. 2019; 42: 140-149.

117. Daemen A, Liu B, Song K, Kwong M, Gao M, Hong R, Nannini M, Peterson D, Liederer BM, de la Cruz C, Sangaraju D, Jaochico A, Zhao X, Sandoval W, Hunsaker T, Firestein R, Latham S, Sampath D, Evangelista M, Hatzivassiliou G. Pan-Cancer Metabolic Signature Predicts Co-Dependency on Glutaminase and De Novo Glutathione Synthesis Linked to a High-Mesenchymal Cell State. Cell Metab. 2018; 28: 383-399.

118. Bott AJ, Maimouni S, Zong WX. The Pleiotropic Effects of Glutamine Metabolism in Cancer. Cancers (Basel). 2019; 11: pii: E770.

119. DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci U S A. 2007; 104: 19345-19350.

120. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci USA. 2008; 105: 18782–18787.

121. Johnson MO, Wolf MM, Madden MZ, Andrejeva G, Sugiura A, Contreras DC, Maseda D, Liberti MV, Paz K, Kishton RJ, Johnson ME, de Cubas AA, Wu P, Li G, Zhang Y, Newcomb DC, Wells AD, Restifo NP, Rathmell WK, Locasale JW, Davila ML, Blazar BR, Rathmell JC. Distinct Regulation of Th17 and Th1 Cell Differentiation by Glutaminase-Dependent Metabolism. Cell. 2018; 175: 1780-1795.

122. Wang T, Cai B, Ding M, Su Z, Liu Y, Shen L. c-Myc Overexpression Promotes Oral Cancer Cell Proliferation and Migration by Enhancing Glutaminase and Glutamine Synthetase Activity. Am J Med Sci. 2019; 358: 235-242.

123. Sicklick JK, Kato S, Okamura R, Schwaederle M, Hahn ME, Williams CB, De P, Krie A, Piccioni DE, Miller VA, Ross JS, Benson A, Webster J, Stephens PJ, Lee JJ, Fanta PT, Lippman SM, Leyland-Jones B, Kurzrock R. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. Nat Med. 2019; 25: 744-750.