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## Inhibition of glutaminolysis in combination with other therapies to improve cancer treatment

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### Abstract

Targeting glutamine catabolism has been attracting more research attention on the development of successful cancer therapy. Catalytic enzymes such as glutaminase (GLS) in glutaminolysis, a series of biochemical reactions by which glutamine is converted to glutamate and then alpha-ketoglutarate, an intermediate of the tricarboxylic acid (TCA) cycle, can be targeted by small molecule inhibitors, some of which are undergoing early phase clinical trials and exhibiting promising safety profiles. However, resistance to glutaminolysis targeting treatments has been observed, necessitating the development of treatments to combat this resistance. One option is to use synergy drug combinations, which improve tumor chemotherapy's effectiveness and diminish drug resistance and side effects. This review will focus on studies involving the glutaminolysis pathway and diverse combination therapies with therapeutic implications.

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Declaration of Competing Interest

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## Keywords

Metabolic reprogramming; Glutaminolysis; Glutaminase; Therapeutic resistance; Combination therapy

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## Introduction

Glutamine, the most abundant free amino acid in plasma, is the quintessential substrate in several biosynthesis processes, such as the synthesis of nucleotides (purines and pyrimidines), fatty acids, antioxidants, and different nonessential amino acids (NEAAs), many of which are vital for rapidly proliferating cells such as cancer cells [1]. Forty years ago, scientists revealed that glutamine serves as the major energy source in cultured cervical cancer cells (HeLa cells) [2]. The dependence on glutamine has subsequently been seen in other cancer types, such as pancreatic cancer, ovarian cancer, breast cancer, and glioblastoma multi-forme (GBM) [3–7]. While glutamine is a nonessential amino acid in most cases, it is indispensable for glutamine-addicted cancer cells, and glutamine deprivation impairs cancer growth [1]. Glutamine can also activate the mammalian target of rapamycin complex 1 (mTORC1) pathway by being exchanged for leucine through the large neutral amino acid transporter (LAT1) or by contributing to the production of more  $\alpha$ -ketoglutarate after the conversion to glutamate, which eventually leads to cell growth and suppression of autophagy [8–10]. Inhibiting glutaminolysis, coupled with chemo medications or other metabolic inhibitors, triggers synergy drug combinations in glutamine-dependent tumors. Specifically, GLS inactivation causes redox imbalance, suppresses the synthesis of nucleotides, and induces replication stress, making cancer cells more dependent on poly (ADP-ribose) polymerase (PARP) DNA repair and thus sensitizing them to the PARP inhibitor [11]. On the other hand, pancreatic tumors were found to escape GLS inhibition by elevating glutamate production, such as through the glutaminase II pathway, where glutamine is converted to glutamate via the intermediates  $\alpha$ -ketoglutarate (KGM) and  $\alpha$ -ketoglutarate ( $\alpha$ -KG) [12]. Knockdown of glutamine transaminase K (GTK), a key enzyme of the glutaminase II pathway, in combination with GLS inhibition, can impair both metabolic pathways [12]. Pancreatic tumors undergo metabolic compensation to rely more on glucose metabolism after GLS inhibition [13]. Cancer cells that survive GLS inhibition tend to be more susceptible to metformin, a drug used to treat diabetes [13]. Moreover, even if glutamate production from other sources is limited, N-acetylaspartylglutamate (NAAG) can serve as an essential reservoir to provide glutamate to cancer cells via carboxypeptidase II (GCP2), thereby making GCP2 a viable target for cancer therapy, either alone or in combination with GLS inhibition [14]. As a result, a combination of GLS inhibitors and other treatments could be a potential avenue to successful cancer therapy.

### The crucial role of glutamine metabolism in cancer growth and survival

In order to decipher the fate of glutamine in cancer cells, glutamine metabolism, including glutaminolysis, has been an important and vigorously researched topic. Cancer cells transport exogenous glutamine into the cytoplasm via alanine, serine, cysteine transporter 2 (ASCT2, also called SLC1A5), and glutamine can subsequently be converted to glutamate via glutaminase 1 (GLS1 or GLS) or glutaminase 2 (GLS2) (Figure 1). Glutamate

then undergoes a deamidation through two different possible pathways to produce  $\alpha$ -ketoglutarate ( $\alpha$ -KG), which is incorporated into the TCA cycle. The first pathway is catalyzed by glutamate dehydrogenase (GDH/GLUD1), which converts glutamate into ammonium and  $\alpha$ -KG. The second pathway is catalyzed by a number of transaminases, such as glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase.

The transaminase reactions can contribute to the production of other nonessential amino acids, including alanine and aspartate. Apart from its incorporation into the TCA cycle for the subsequent production of energy,  $\alpha$ -KG can be exported from the mitochondria, where the TCA cycle occurs, to the cytosol and be converted to isocitrate by isocitrate dehydrogenase 1 (IDH1), which is then converted to citrate for *de novo* fatty acid synthesis [9,15]. Other TCA cycle metabolites produced from glutamine can be released into the cytosol and be converted into other molecules that also assist proliferating cells. For example, pyruvate can be generated from malate, concomitant with the production of NADPH via malic enzyme 1 (ME1) to replenish cell reduction capacity. Oxaloacetate (OAA) and glutamate can undergo a transamination reaction and generate  $\alpha$ -KG and aspartate, which can be transported to the cytosol and participate in nucleotide synthesis [16–18]. Furthermore, glutamine is also the precursor (via glutamate) of glutathione (GSH), which is an endogenous antioxidant that shields cancer cells from excessive oxidative stress and sustains their survival [1]. Glutamine provides nitrogen for nucleobase synthesis, carbon for the TCA cycle [19], and participates in lipid synthesis and redox hemostasis. These are necessary for the survival and proliferation of cancer cells, making glutaminolysis an attractive target for developing new small molecule anticancer drugs [20,21].

### Glutamine-addicted cancers

While an accurate proportion of human cancers that display glutamine addiction is yet to be defined, it has been noted that particular cancer types or cancer cell lines may be more prone to glutamine addiction than others, such as pancreatic cancer, acute myelogenous leukemia, GBM, myeloma, clear cell renal cell carcinoma (ccRCC), lung cancer and triple-negative breast cancer (TNBC) [22–26]. The dependency on glutamine in glioblastoma was interpreted by the high concentration of glutamine in glioblastoma of patients injected with  $^{13}\text{C}_6$ -glucose [27]. Lung cancer cells become addicted to glutamine as a result of aberrantly triggered oncogenes and the lack of tumor suppressors in contrast to normal counterparts [28]. Glutamine addiction may be specific to a particular breast cancer subtype; for instance, the TNBC MDA-MB-453 cell line is tamine dependent, while the luminal A subtype MCF-7 breast cancer cell line is not [29].

We analyzed the fractions of cancer cell lines that are more dependent on the glutaminolysis pathway using data collected from DepMap (<https://depmap.org/portal> [30] Table 1). The data collected from DepMap are recorded using CERES dependency scores [32] that are based on data from cell depletion arrays. Glutamine-addicted cancer cells may express a higher level of the following genes: GLS1, GLS2, MYC, ASCT2, and GLUD1. Consequently, we calculated the dependent rate of cell lines toward certain genes by dividing the number of cell lines dependent on the genes (as the CERES score of 0 indicates that the gene is not essential for the cell line, and a lower score indicates that the gene

is more likely to be crucial for the survival of the cell line, a cell line with a CERES score below 0 are taken as gene dependent [31,32]) by the total number of cell lines. Since glutamine synthetase (GLUL) catalyzes the synthesis of glutamine from glutamate and ammonia, GLS1/GLUL, GLUD1/GLUL, and (GLS1 + GLUD1)/GLUL were used to show the activity of glutamine catabolism as compared to glutamine anabolism [5]. Acute myeloid leukemia (AML), cervical squamous, esophagus adenocarcinoma, nonsmall cell lung cancer (NSCLC), non-Hodgkin's lymphoma, ovary adenocarcinoma, thyroid carcinoma, and several types of soft tissue sarcoma manifest high ratios of GLS1/GLUL, GLUD1/GLUL, and (GLS1 + GLUD1)/GLUL suggesting a greater reliance on extracellular glutamine catabolism (Table 1).

### **Biomarkers determining glutamine addiction in cancer**

In order to select patients for future clinical trials evaluating the efficacy of glutaminolysis inhibitors, certain biomarkers need to be identified to determine the cancers that are glutamine addicted. The study by Yuneva et al. showed that MYC expression results in a strong dependence on glutamine in cancer cells, so much so that upon removal of glutamine, these cells undergo apoptosis [33]. This is because, largely glutaminolysis is stimulated by an MYC-regulated transcriptional program. Thus, MYC causes the mitochondrial TCA cycle to become reliant on glutamine [34]. MYC is also responsible for activating SLC38A5 (SN2), a major transporter of glutamine, and SLC1A5 (ASCT2), an amino acid transporter that is involved in glutamine-dependent mTORC1 activation. mTORC1, a protein translation regulator, has been shown to be responsive to the levels of glutamine in the cell [22]. Furthermore, in cells that are dependent on glutamine, higher than average levels of SLC1A5 are often present [35]. For example, SLC1A5 is highly expressed in TNBC patients, and knocking it down improved survival in tumor-bearing mice [26]. Additionally, it has been discovered that in cells displaying an overexpression of MYC, glutaminase levels are higher than those in cells with low MYC expression levels [11,36]. Thus, all of these—MYC, SLC1A5, and mTORC1 levels in addition to levels of glutaminase—can be used in future clinical trials as biomarkers to select patients when evaluating the potential efficacy of glutaminolysis inhibitors as they all are present at greater levels in glutamine-dependent cells.

### **Targeting glutaminolysis in combination with other therapies for cancer treatment**

Decades ago, several glutamine antagonists, such as DON (6-diazo-5-oxo-L-norleucine), acivicin, and azaserine, were developed by researchers to inhibit glutamine metabolism in cancer cells (Table 2) [37–41]. Although such glutamine analogs demonstrate high efficiency in obstructing cancer cell growth, they are not ideal cancer drugs because of their high toxicity [1]. To alleviate gastrointestinal (GI) toxicities, scientists developed DON prodrugs that optimized the delivery of DON to tumors with limited exposure to GI tissues and suggested administering these prodrugs in a low dosage [38,42]. Moreover, treating MC38 colon cancer cells with JHU083, a prodrug of DON developed by Leone et al., not only decreased glycolysis and oxidative phosphorylation but also promoted oxidative metabolism of effector T cells [43]. Taken together, prodrugs of the glutamine antagonist DON together with optimized delivery strategies and dosages may

show promising outcomes in patients with glutamine-addicted tumors in clinical trials in the future.

On the other hand, glutaminolysis has been an attractive cancer therapy target for the past decade (Figure 1). Researchers have developed multiple medications blocking various steps of glutamine catabolism pathways to impede glutamine-addicted cancer cells' growth. Targeting amino acid transporter SLC1A5 plays a vital role in importing extracellular glutamine. High SLC1A5 expression level has been documented to be correlated to poor overall survival in various types of cancers, including nonsmall cell lung cancer, liver cancer, and breast cancer [44–46]. The SLC1A5 inhibitor L- $\gamma$ -glutamyl-p-nitroanilide (GPNA) is an analog of glutamine that blocks SLC1A5 [47,48]. V-9302, a small molecule antagonist, is another SLC1A5 inhibitor. In a previous study, blocking SLC1A5 with V-9032 diminished cancer growth, disturbed redox homeostasis, and inflicted cell death in preclinical models [49]. Small-molecule allosteric inhibitors of GLS have been identified, including compound 968, BPTES (bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide), and CB-839 (Telaglenastat). Compound 968 can interact with kidney glutaminase (KGA or GLS1) and its slicing variant, glutaminase C (GAC) [50]. It inactivates GAC by inhibiting nonactive monomeric GAC from forming enzymatically active GAC tetramer [51]. Compound 968 also inhibits the liver-type glutaminase (GLS2 or LGA) and its slicing variant, glutaminase B (GAB), in luminal-subtype breast cancer [52]. While the inhibitory potency of BPTES is substantial, its low solubility makes it difficult to deliver *in vivo* [1,53]. Hence, scientists are now vigorously engaged in concerted efforts to refine BPTES and create BPTES analogs in the hope of boosting its efficacy for cancer therapy [53,54]. Although these small molecule drugs showed profound efficiency in inhibiting glutaminolysis and are capable of hampering cancer cell growth in preclinical models, the emergence of therapeutic resistance has been a refractory issue. It is common in real-world scenarios to find heterogeneity within a single tumor relying on different metabolic pathways [55–57]. Solely inhibiting glutaminolysis is not enough to cure patients whose tumors exhibit high intratumoral heterogeneity. Therefore, scientists now aim to target additional pathways besides glutaminolysis to optimize cancer treatments.

### Targeting GLS1 by CB-839 synergizes with other clinical drugs

CB-839, another important GLS inhibitor, selectively inhibits both KGA and GAC [58]. CB-839 has undergone many clinical trials for many cancer types, including clear cell renal cell carcinoma (ccRCC), colorectal cancer, ovarian cancer ([NCT03875313](#), [NCT02861300](#), [NCT03944902](#)). CB-839 is also currently undergoing a phase I clinical trial to treat patients with relapsed and/or treatment-refractory leukemia ([NCT02071927](#)).

Over the past decade, CB-839 has been used in various preclinical studies and clinical trials in combination with various clinical drugs, and some of them have shown a promising synergistic effect (Table 3). In a patient-derived TNBC xenograft model and a basal HER2<sup>+</sup> cell line xenograft model, CB-839 resulted in significant reductions in tumor growth, either alone or in combination with paclitaxel, which showed strong synergy to suppress the regrowth of the tumors [59]. A phase II combination trial of CB-839 and paclitaxel in patients with advanced TNBC was also performed ([NCT03057600](#)). In addition, the

combined use of CB-839 and olaparib, a poly (ADP-ribose) polymerase (PARP) inhibitor, resulted in potently synergistic cell death presumably due to extreme oxidative stress and DNA replication stress in tumors, reinforcing the rationale for a clinical trial setting to incorporate GLS inhibitors with PARP inhibitors (Figure 2) [11,60]. Furthermore, CB-839 could act synergistically with the epidermal growth factor receptor (EGFR) inhibitor erlotinib or the EGFR-targeted monoclonal antibody cetuximab for cancer therapy [61,62]. Specifically, CB-839, in combination with erlotinib, synergistically triggered *in vitro* apoptosis and reduced *in vivo* tumor growth in EGFR mutant nonsmall cell lung cancer [61]. A combination of CB-839 and cetuximab also showed a synergistic effect and decreased cell viability in colorectal cancer cells [62]. CB-839 is currently undergoing clinical trials, both alone or in conjunction with everolimus (NCT03163667), cabozantinib (NCT03428217), or nivolumab (NCT02771626), for various cancers [63]. The phase I or phase II clinical trials showed positive results and demonstrated that all the drug combinations could be tolerated [63]. CB-839 has a synergistic effect of promoting ER stress and apoptosis caused by carfilzomib in treating myeloma cell lines with the proteasome inhibitor-resistant phenotype [64]. By activating mitochondrial-mediated apoptosis, CB-839 displayed good synergism with ABT-199, a BCL-2 inhibitor, killing acute myeloid leukemia blasts [65]. In the tumor microenvironment [66], tumor cells outcompete T-cells for scarce nutrients such as glutamine. By diverting glutamine from cancer cells to active T-cells, CB-839 is proposed to have synergistic effects with nivolumab, an immunoglobulin G4 PD-1 immune checkpoint inhibitor (Figure 2) [63]. Inhibition of glutamine metabolism can hamper oxidative and glycolytic metabolism in cancer cells while stimulating oxidative metabolism in effector T cells and rendering these T cells highly activated and long-lived [67]. These findings signify that targeting glutamine addiction through GLS1 inhibition in combination with other drugs provides a promising therapeutic strategy for several types of cancers.

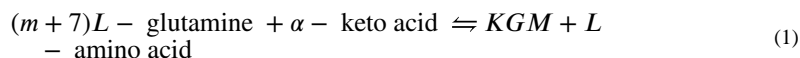
### Dual inhibition of glutaminase I and glutaminase II pathways

A study recently discovered that the inhibition of GLS1 could only partially reduce tumor growth and not entirely hinder cancer cell proliferation [12]. By following glutamine metabolism in mice bearing patient-derived pancreatic cancer using (m+7)  $^{13}\text{C}_5^{15}\text{N}_2$ -labeled glutamine [68], the authors observed an increase in (m+5) glutamate production upon GLS1 inhibition using BPTES-NP (nanoparticles encapsulated BPTES). As  $^{13}\text{C}_4^{15}\text{N}_1$  glutamate and  $^{13}\text{C}_5$  glutamate are the only two possible (m+5) glutamate produced from  $^{13}\text{C}_5^{15}\text{N}_2$ -labeled glutamine, and  $^{13}\text{C}_4^{15}\text{N}_1$  glutamate is highly improbable, GLS1 inhibition should lead to a reduction in (m+5) glutamate production if it is the only pathway for the generation of glutamate. This is because the formation of  $^{13}\text{C}_5$  glutamate requires a transamination reaction from  $^{13}\text{C}_5$   $\alpha$ -KG, which requires the production of  $^{13}\text{C}_5^{15}\text{N}_1$  glutamate via GLS1. The result obtained thus indicates the presence of a different glutamate generation pathway other than glutaminolysis [12]. The study indicated that the  $^{13}\text{C}_5$  glutamate production occurs by the transamination of  $^{13}\text{C}_5$   $\alpha$ -KG derived from an alternative pathway: the glutaminase II pathway [12]. The glutaminase II pathway begins with a glutamine transaminase (most notably glutamine transaminase K; GTK) that catalyzes the transamination of glutamine to  $\alpha$ -ketoglutarate (KGM) in the presence

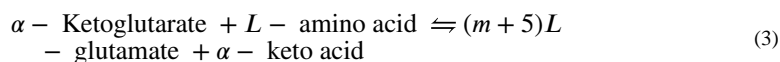


of a suitable  $\alpha$ -keto acceptor. In the second step, the enzyme  $\omega$ -amidodicarboxylate amidohydrolase then catalyzes the conversion of KGM to  $\alpha$ -KG (Figure 2). Glutamate can be further derived from  $\alpha$ -KG by a transamination reaction or by GDH [12]. The carbon backbone for glutamate is derived from  $\alpha$ -KG in the transamination reaction. This sequence of reactions thereby helps to demonstrate how both  $^{15}\text{N}$ -labels are lost.

#### Glutaminase II Pathway



#### Transamination Reaction



KGM, the glutaminase II pathway intermediate, was studied to further validate that the glutaminase II pathway is responsible for the increased (m+5) glutamate production. Since  $\omega$ -amidase only catalyzes reactions involving the open form of KGM (note that at physiological pH, 99.7% of KGM is cyclized to a lactam and only 0.3% is in the open-chain (substrate) form) [69], mass spectrometry and  $^1\text{H}$  NMR were employed to quantify the levels of KGM in the tumors, where the KGM structure could only be adequately identified in  $^1\text{H}$  NMR. Elevated KGM levels were found in tumors treated with BPTES-NP as compared to tumors treated with blank-NP. For further verification, GTK, the primary enzyme for the glutaminase II pathway that is responsible for generating KGM, was then analyzed. In eight pancreatic cancer cell lines derived from patients, the highest expression of GTK was found in P198 cells. shRNA knockdown of GTK in P198 resulted in reduced cell numbers, indicating that GTK is essential for the proliferation of cancer cells. Moreover, knockdown of GTK led to the complete suppression of *in vivo* pancreatic tumorigenesis [70]. With the role of the glutaminase II pathway in cancer now uncovered, a novel class of specific small molecule inhibitors with dual targeting of both the GLS1 and glutaminase II pathways could be a potential therapeutic strategy for cancer therapy.

### Glutaminase 1 (GLS1) and glutaminase 2 (GLS2)

There are two isoenzymes of glutaminase, glutaminase 1 (GLS1 or GLS) and glutaminase 2 (GLS2 or LGA), which were initially found in the kidney and the liver, respectively [71]. Both are essential enzymes required in the regulation of glutamine metabolism that catalyzes the hydrolysis of glutamine to glutamate [72,73]. However, in tumorigenesis, GLS1 and GLS2 lead to opposite effects [74,75]. Studies have shown the significant role of GLS2 as a tumor suppressor in cancers such as gastric cancer [76,77]. The idea that GLS2 functions as a tumor suppressor while GLS1 promotes cancer growth has led to the assumption that inhibiting the GLS1 pathway is a sufficient anticancer strategy [74]. However, this is not

completely true, as emerging evidence illustrates the importance of GLS2 in cancer cell proliferation, and a study on luminal-subtype breast cancer suggests a dual-GLS1/GLS2 inhibition to be more effective than GLS1 inhibition alone [52]. GLS2 is also expressed, though downregulated, in pancreatic cancer cells [78]; hence, it is possible that the resistance to BPTES-NP in pancreatic cancer could come from the compensation of GLS2. Although compound 968 inhibits both GLS1 and GLS2, this inhibition will not prevent the generation of glutamate via the glutaminase II pathway, as discussed in section 2.2. Therefore, dual glutaminase I and II pathway inhibition would be a better option.

### **Combined treatment of GLS1 inhibitor with blockage of glutamate release from its reservoir**

N-Acetyl-aspartyl-glutamate (NAAG), the third most abundant neurotransmitter in the human body, has been studied thoroughly with respect to its function in several neurological disorders [79]. However, its role in cancer metabolism, its feasibility as a cancer therapy target, and its capacity as a noninvasive cancer progression indicator have only recently been discovered. A study by Nguyen et al. revealed that NAAG is a quintessential glutamate reservoir and that GCPII is responsible for the hydrolysis of NAAG to N-acetyl-aspartate (NAA) and glutamate (Figure 2) [14]. Knocking down the GCPII gene in pancreatic cancer P198 cells, using both shRNA lenti-virus and CRISPR, and treating them with BTPES, showed accentuation of decreased intracellular glutamate level and an inhibitory effect on cell proliferation compared to the control cells [14]. The possibility of targeting GCPII as a means of cancer therapy was then further tested using 2-(phosphonomethyl)-pentanedioic acid (2-PMPA), which was found to be a selective competitive inhibitor of GCPII, to treat patient-derived ovarian and orthotopic pancreatic tumors, and this treatment resulted in a significant reduction in tumor sizes [14]. A combination therapy using CB-839 and 2-PMPA was then tested, and, importantly, the decrease in tumor growth was found to be greater than when either treatment was used alone [14]. This is because these two compounds target two different glutamate metabolic pathways, which reduces drug resistance. Nguyen et al. also found that the concentrations of NAAG in *MYC*-transformed lymphoma B cells [80–82], high-grade ovarian tumors, and brain tumors are higher than those in low-grade cancers of the same type [14]. This is corroborated with other studies showing that GCPII exists at higher levels and exhibits greater activity in metastatic prostate cancer cells than in either benign or normal cells by at least a factor of ten [83]. NAAG also has the potential to be used as a noninvasive marker of predicting tumor growth. The study done by Nguyen et al. found that increases and decreases in NAAG concentrations in plasma took place before corresponding increases and decreases in tumor growth, respectively [14,84]. This correlation may allow physicians to monitor and stay one step ahead of tumor development and should have translational implications by introducing a new strategy for future clinical testing.

In short, NAAG's role in glutamine metabolism and its correlation with cancer progression make this compound and the proteins involved in its metabolism likely candidates for future research and potential therapeutic targets. However, due to the propensity of many cancers for drug resistance, any future therapies targeting NAAG metabolism will likely be more effective when combined with other metabolic inhibitors, especially glutaminase inhibitors.



### Combination therapy with GLS inhibitor and other metabolic inhibitors

BPTES-nanoparticles, where nanoparticles made up of poly (ethylene glycol) (PEG) and poly (lactic-co-glycolic acid) (PLGA) encapsulate the drug to increase blood circulation time and tumor uptake, was an attempt to overcome the solubility limitation of BPTES. Indeed, it was shown to have better pharmacokinetics than unencapsulated BPTES [13]. The combined treatment of BPTES-NPs and gemcitabine, the current standard post-PDAC surgery adjuvant chemotherapy, did not result in improved tumor reduction [13]. Upon BPTES-NPs treatment, the residual tumor relies on glycolysis and glycogen synthesis, as evidenced by increases in the levels of lactate, glycogen, glucose-6-phosphate, glucose-1-phosphate, and UDP-glucose. Elgogary et al. then tested the combination of BPTES-NP and metformin, a diabetic drug currently in phase III clinical trials for cancer therapy (NCT01101438). Metformin has the ability to reduce glucose metabolism [13,85]. It has been previously shown that by inhibiting hexokinase-II, which catalyzes glucose phosphorylation, metformin reduces the supply of cellular energy and glucose metabolism [86]. With the combined therapy of BPTES-NPs and metformin, a greater decrease tumor growth as compared to either drug acting alone, as well as a decreased level of lactate, glucose-6-phosphate, glucose-1-phosphate, and UDP-glucose was detected.

Combination therapy has the ability to combat intratumoral metabolic heterogeneity and has important implications for the design of more effective treatment strategies. The rationale for combination therapy is to use drugs that work via different mechanisms, thereby decreasing the likelihood that resistant cancer cells will develop. With many types of combination therapies investigated, the combination of BPTES and metformin was selected by tracking the metabolic pathways utilized in the tumor cells remaining after GLS inhibition and identifying the best-suited drugs for combined synergistic therapy.

### Conclusion

Resistance to therapy is a major impediment in the treatment of cancer, especially in heterogeneous cancers. In comparison, malignant cells rely more on glucose, glutamine, and other substrates than their noncancer counterparts. Given that glutamine metabolism underpins human malignancies, and GLS expression in cancer cells can determine their response toward GLS inhibiting drugs, biomarkers such as GLS can be used to select patients who are most likely to respond to and benefit from the anti-GLS therapy [87]. The utilization of L-[<sup>11</sup>C]glutamine positron emission tomography (PET) imaging demonstrates the value of noninvasive biomarkers that may be paired with therapies targeting tumor metabolism [88]. Future analysis of the molecular-based metabolic vulnerabilities of cancer cells will allow for the stratification of additional resistant patient populations who may benefit from GLS inhibitor treatment in combination with other anti-cancer drugs. Since combination therapy could decrease the likelihood of the development of resistant cancer cells through the inhibition of several different pathways, it is by far the most effective cancer treatment [89]. Future investigations that will provide more mechanistic insights into the metabolic adaptation of glutamine-dependent tumors will further highlight and potentiate novel strategies for cancer therapy.

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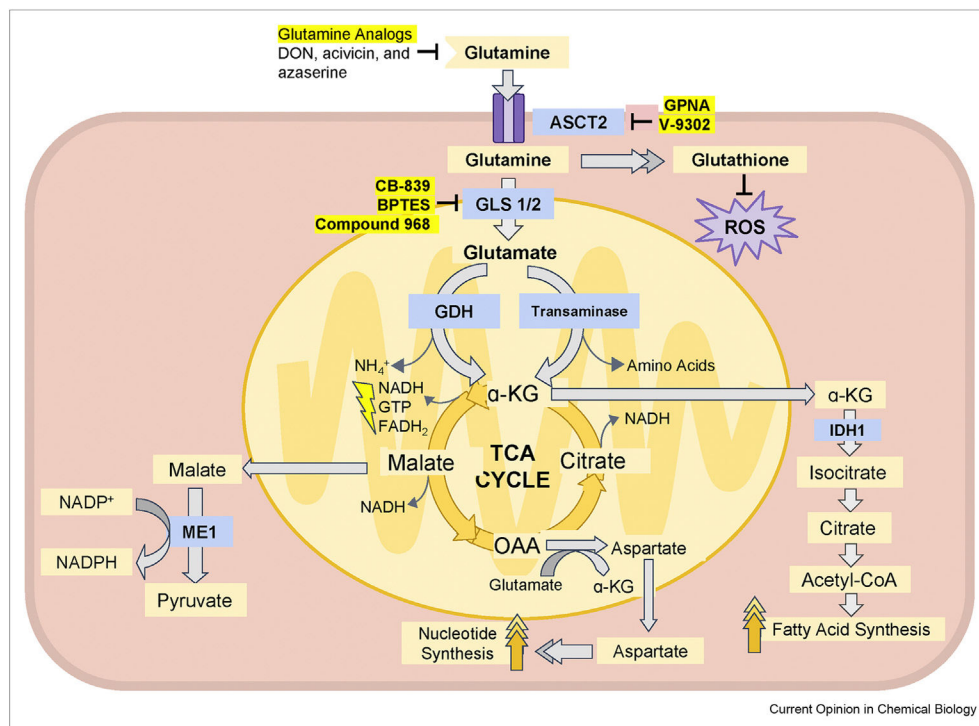
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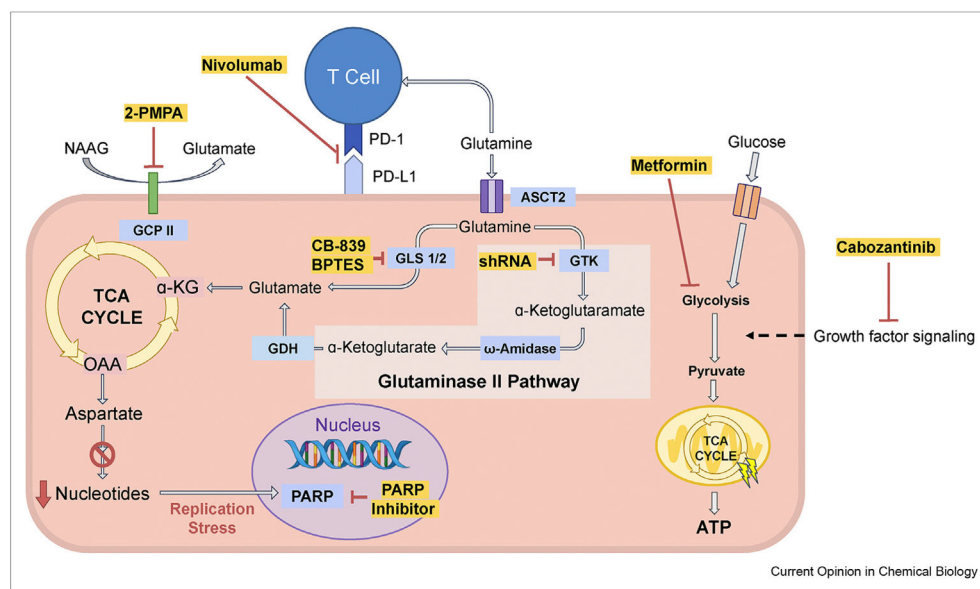
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**Figure 1.**

The glutaminolysis pathway and its inhibitors.

Glutamine is an important nitrogen and carbon source for actively proliferating cells, especially glutamine-addicted cancer cells, and is an essential building block for numerous biosynthesis processes, such as the synthesis of glutathione, NADPH, nucleotides, and fatty acids. Glutamine can be imported from the extracellular space via alanine, serine, cysteine transporter 2 (ASCT2). Glutamine is then converted to glutamate by either glutaminase 1 (GLS1) or glutaminase 2 (GLS2). Glutamate is then converted to  $\alpha$ -ketoglutarate ( $\alpha$ -KG). This reaction can be catalyzed by either glutamate dehydrogenase (GDH) generating one equivalent of ammonia or by transaminases in which the nitrogen is incorporated into other amino acids.  $\alpha$ -KG can be incorporated into the TCA cycle and its carbon converted to malate, oxaloacetate (OAA), and citrate subsequently. Malate can be transported from the mitochondrion to the cytoplasm, where it is a substrate of the malic enzyme 1 (ME1), thereby assisting in the production of NADPH from  $\text{NADP}^+$ . OAA can be converted to aspartate and transported to the cytoplasm to participate in nucleotide synthesis.  $\alpha$ -KG can also be transported to the cytoplasm as a source of acetyl-CoA, which is an essential substrate of fatty acid synthesis. There are several drugs developed to inhibit different parts of the glutaminolysis pathway. The first type is exemplified by glutamine analogs, such as DON, acivicin, and azaserine. The second group is represented by the SLC1A5 inhibitors, including GPNA and V-9302. Last, GLS1/2 is also a promising target for glutaminolysis inhibition. Several noncompetitive allosteric inhibitors have been identified, such as CB-839, BPTES, and compound 968.



**Figure 2.**

Emerging combination therapy: Combined blockage of glutaminolysis with inhibition of other pathways.

CB-839 or BPTES, small molecule inhibitors of GLS, combined with other drugs, is an emerging cancer treatment strategy. (1) CB-839 combinations: CB-839 combined with cabozantinib, a small molecule inhibitor of growth factor signaling, or with everolimus, an mTOR inhibitor, are currently undergoing clinical trials. In addition, CB-839 synergizes with nivolumab, a human IgG4 monoclonal antibody blocking PD-1, and this combination is also undergoing a Phase II clinical trial. Poly (ADP-ribose) polymerase (PARP) is important for cancer cells to reduce ROS stress and sustain genome integrity. GLS inhibition by, e.g. CB-839 treatment, suppresses the generation of nucleotides and causes replication stress, which may activate PARP-dependent DNA repairs. Treating cancer cells with a combination of CB-839 and PARP inhibitor, such as olaparib, has been shown to achieve synergistic antitumor activity. NAAG has been identified as a glutamine reservoir in cancer cells and is hydrolyzed to generate glutamate and NAA by carboxypeptidase II (GCP II), which can be inhibited by 2-PMPA. Combining 2-PMPA with CB-839 showed a more significant decrease in tumor size compared to treating the tumor with either of these drugs alone. (2) BPTES combination: BPTES combined with metformin, a glucose metabolism inhibitor, results in greater inhibition of tumor growth. The glutaminase II pathway provides cancer cells with a mechanism for bypassing GLS inhibition. Inhibition of both GLS1/2 and the glutaminase II pathway is a novel strategy for treating glutamine-addicted cancers. The effect of each drug or shRNA (highlighted in yellow) is shown in red, metabolites are shown in black, and enzymes are shown in blue boxes.

Table 1

Fraction of Cell Lines Dependent on Glutaminolysis: Data analysis is based on <https://depmap.org/portal/>.

Primary Disease	Lineage	Gene Dependency Rate									
		GLSI	GLS2	MYC	SLCIA5	GLUD1	GLUL	GLSI/GLUL	GLUD1/GLUL	(GLSI+GLUD1)/GLUL	
Bile	Cholangiocarcinoma (27)	88.89	96.30	100.00	100.00	85.19	44.44	2.00	1.92	3.92	
Duct (30)	Gallbladder Adenocarcinoma (3)	100.00	100.00	100.00	66.67	66.67	66.67	1.50	1.00	2.50	
Blood (44)	ALL (12)	75.00	100.00	100.00	100.00	66.67	41.67	1.80	1.60	3.40	
	AML (21)	95.24	95.24	100.00	100.00	85.71	38.10	2.50	2.25	4.75	
	CLL (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
	CML (7)	100.00	85.71	100.00	100.00	85.71	57.14	1.75	1.50	3.25	
	Unspecified Leukemia (3)	66.67	100.00	100.00	100.00	66.66	66.67	1.00	1.00	2.00	
Bone (28)	Chondrosarcoma (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
	Choroma (3)	100.00	100.00	100.00	100.00 (4 cell lines)	100.00	50.00 (4 cell lines)	2.00	2.00	4.00	
	Ewing Sarcoma (16)	75.00	100.00	100.00	100.00	75.00	43.75	1.71	1.71	3.43	
	Osteosarcoma (8)	62.50	100.00	100.00	100.00	62.50	75.00	0.83	0.83	1.67	
Breast (34)	Breast Adenocarcinoma (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
	Breast Carcinoma (18)	83.33	94.44	100.00	100.00	77.78	11.11	7.50	7.00	14.50	
	Breast Ductal Carcinoma (15)	100.00	93.33	100.00	100.00	86.67	46.67	2.14	1.86	4.00	
Central Nervous	Glioma (50)	90.00	94.00	100.00	100.00	94.00	56.00	1.61	1.68	3.29	
System (61)	Medulloblastoma (9)	66.67	100.00	100.00	100.00	77.78	44.44	1.50	1.75	3.25	
	Meningioma (2)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
Cervix (11)	Cervical Adenocarcinoma (3)	100.00	100.00	100.00	100.00	100.00	66.67	1.50	1.50	3.00	
	Cervical Carcinoma (4)	75.00	100.00	100.00	100.00	75.00	100.00	0.75	0.75	1.50	
	Cervical Squamous (4)	100.00	100.00	100.00	100.00	100.00	50.00	2.00	2.00	4.00	
Colorectal (37)	Colorectal Adenocarcinoma (37)	75.68	81.08	100.00	100.00	91.89	67.57	1.12	1.36	2.48	
Embryo (1)	Teratoma (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
Epidermoid Carcinoma (1)	Skin Squamous (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	

Primary Disease	Lineage	Gene Dependency Rate									
		Formula = ((cell line counts that scores of 0 and -1 represent the median effects of nonessential genes and common core essential genes)/total cell line counts) * 100%									
		GLS1	GLS2	MYC	SLCIA5	GLUD1	GLUL	GLS1/GLUL	GLUD1/GLUL	(GLS1+GLUD1)/GLUL	
Esophagus (23)	Esophagus Adenocarcinoma (3)	100.00	100.00	100.00	100.00 (5 cell lines)	66.67	40.00 (5 cell lines)	2.50	1.67	4.17	
	Esophagus Squamous (20)	90.00	100.00	100.00	100.00	95.00	55.00	1.64	1.73	3.36	
Eye (6)	Retinoblastoma (1)	0.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
	Uveal Melanoma (5)	100.00	80.00	100.00	100.00	100.00	60.00	1.64	1.73	3.36	
Fibroblast (1)	Fibroblast Soft Tissue (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
Gastric (26)	Gastric Adenocarcinoma (26)	80.77	88.46	100.00	100.00	80.77	38.46	2.10	2.10	4.20	
Kidney (23)	Malignant Rhabdoid Tumor (7)	100.00	100.00	100.00	100.00 (8 cell lines)	100.00	87.50 (8 cell lines)	1.14	1.14	2.29	
	Renal Cell Carcinoma (21)	76.19	90.48	100.00	100.00	85.71	57.14	1.33	1.50	2.83	
Liver (22)	Hepatoblastoma (1)	0.00	100.00	100.00	100.00	100.00	100.00	0.00	1.00	1.00	
	Hepatocellular Carcinoma (20)	80.00	95.00	100.00	100.00	80.00	55.00	1.45	1.45	2.91	
Lung (107)	Lung Carcinoid (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
	Mesothelioma (10)	100.00	100.00	100.00	100.00	90.00	80.00	1.25	1.13	2.38	
	NSCLC (78)	85.90	98.72	100.00	100.00	93.59	50.00	1.72	1.87	3.59	
	SCLC (18)	77.78	100.00	94.44	100.00	83.33	55.56	1.40	1.50	2.90	
Lymphocyte (19)	ATL (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
	Hodgkin Lymphoma (2)	100.00	50.00	100.00	100.00 (3 cell lines)	100.00	66.67 (3 cell lines)	1.50	1.50	3.00	
	Lymphoma Unspecified (3)	100.00	100.00	100.00	100.00	100.00	66.67	1.50	1.50	3.00	
	Non Hodgkin Lymphoma (13)	100.00	100.00	100.00	100.00 (14 cell lines)	69.23	28.57 (14 cell lines)	3.50	2.42	5.92	
Ovary (43)	Brenner Tumor (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
	Mixed Germ Cell (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
	Ovary Adenocarcinoma (38)	92.11	100.00	100.00	100.00	89.47	36.84	2.50	2.43	4.93	
	Ovary Carcinoma (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
	SCCOHT (2)	100.00	100.00	100.00	100.00	50.00	50.00	2.00	1.00	3.00	
Pancreas (34)	Exocrine (34)	88.24	100.00	100.00	100.00	88.24	52.94	1.67	1.67	3.33	
PNS (20)	Neuroblastoma (20)	85.00	90.00	100.00	100.00	95.00	60.00	1.42	1.58	3.00	

Primary Disease	Lineage	Gene Dependency Rate Formula = ((cell line counts that scores of 0 and -1 represent the median effects of nonessential genes and common core essential genes)/total cell line counts) * 100%									
		GLS1	GLS2	MYC	SLC1A5	GLUD1	GLUL	GLS1/ GLUL	GLUD1/ GLUL	(GLS1+GLUD1)/ GLUL	
Plasma (21)	Multiple Myeloma (21)	100.00	95.24	100.00	100.00	76.19	71.43	1.40	1.07	2.47	
Prostate (1)	Prostate Adenocarcinoma (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
Skin (52)	Melanoma (46)	87.00	93.48	100.00	100.00 (47 cell lines)	80.43	53.19 (47 cell lines)	1.63	1.51	3.15	
	Merkel Cell Carcinoma (2)	100.00	100.00	100.00	100.00 (3 cell lines)	100.00	33.33 (3 cell lines)	3.00	3.00	6.00	
	Skin Squamous (4)	100.00	100.00	100.00	100.00	50.00	25.00	4.00	2.00	6.00	
Soft Tissue (39)	ATRT (3)	66.67	100.00	100.00	100.00 (5 cell lines)	100.00	20.00 (5 cell lines)	3.33	5.00	8.33	
	Epithelioid Sarcoma (2)	100.00	100.00	100.00	100.00	100.00	50.00	2.00	2.00	4.00	
	Fibrosarcoma (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
	Leiomyosarcoma (1)	100.00	100.00	100.00	100.00 (2 cell lines)	100.00	50.00 (2 cell lines)	2.00	2.00	4.00	
	Liposarcoma (8)	100.00	87.50	100.00	100.00	100.00	37.50	2.67	2.67	5.33	
	Malignant Rhabdoid Tumor (7)	85.71	85.71	100.00	100.00 (8 cell lines)	100.00	37.50 (8 cell lines)	2.29	2.67	4.95	
	Pleomorphic Sarcoma (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
	Rhabdomyosarcoma (11)	81.82	90.91	100.00	100.00	54.55	36.36	2.25	1.50	3.75	
	Synovial Sarcoma (5)	80.00	100.00	100.00	100.00	80.00	60.00	1.33	1.33	2.67	
	Thyroid Sarcoma (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
	Undifferentiated Sarcoma (1)	100.00	100.00	100.00	100.00	0.00	0.00	N/A	N/A	N/A	
Thyroid (6)	Thyroid Carcinoma (5)	80.00	80.00	100.00	100.00	80.00	40.00	2.00	2.00	4.00	
	Thyroid Squamous (1)	100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
Upper Aerodigestive (32)	Upper Aerodigestive Squamous (32)	87.50	90.63	100.00	100.00 (33 cell lines)	90.63	45.45 (33 cell lines)	1.93	1.99	3.92	
Urinary Tract (29)	Bladder Carcinoma (29)	75.86	93.10	96.55	100.00	96.55	55.17	1.38	1.75	3.13	
Uterus (26)	Choriocarcinoma (2)	0.00	100.00	100.00	100.00	100.00	100.00	0.00	1.00	1.00	
	Clear Cell Carcinoma (1)	100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
	Endometrial Adenocarcinoma (15)	60.00	100.00	100.00	100.00	73.33	73.33	0.82	1.00	1.82	



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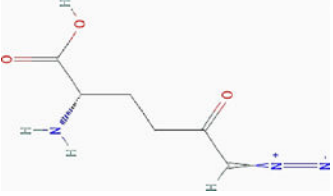
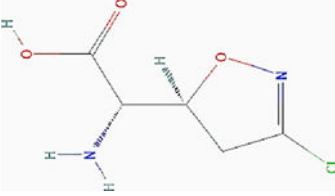
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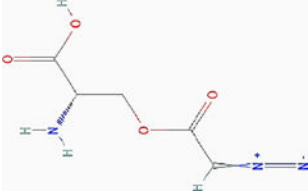
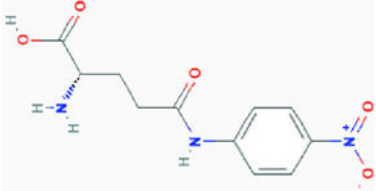
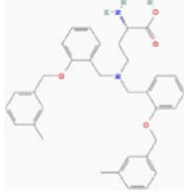
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Primary Disease	Lineage	Gene Dependency Rate Formula = ((cell line counts that scores of 0 and -1 represent the median effects of nonessential genes and common core essential genes)/ total cell line counts) * 100%									
		GLS1	GLS2	MYC	SLC1A5	GLUD1	GLUL	GLS1/ GLUL	GLUD1/ GLUL	(GLS1+GLUD1)/ GLUL	
Endometrial Adenosquamous (1)		100.00	100.00	100.00	100.00	100.00	100.00	1.00	1.00	2.00	
Endometrial Squamous (3)		66.67	100.00	100.00	100.00	100.00	66.67	1.00	1.50	2.50	
Endometrial Stromal Sarcoma (1)		100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
MMMT (1)		100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
Mullerian Carcinoma (1)		0.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	
Uterine Carcinosarcoma (1)		100.00	100.00	100.00	100.00	100.00	0.00	N/A	N/A	N/A	

Table 2

Small molecule inhibitors targeting glutaminolysis pathway.

Small Molecule Inhibitors	Chemical Structure	Inhibition Type	Enzyme Target	Inhibitory Activity	Reference
6-diazo-5-oxo-L-norleucine (DON)		Irreversible inhibition	Nonselective. Glutamine utilizing enzymes, including glutamine amidotransferases	Inhibits the enzymes by covalently modifying the cysteine residues in the glutamine active sites irreversibly.	PubChem CID: 9087 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/9087#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/9087#section=2D-Structure</a> [38,40,90]
Acivicin		Irreversible inhibition	Nonselective. Glutamine utilizing enzymes, including glutamine amidotransferases, $\gamma$ -glutamyltranspeptidase (GGT)	Inhibits the enzymes by forming an imine-thioether adduct at the cysteine residue of the active site as a result of nucleophilic substitution of the chlorine atom. Inhibits GGT by covalently modifying the Thr391 residue at the active site.	PubChem CID: 294641 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/294641#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/294641#section=2D-Structure</a> [40,91,92]

Small Molecule Inhibitors Targeting Glutaminolysis Pathway						
Small Molecule Inhibitors	Chemical Structure	Inhibition Type	Enzyme Target	Inhibitory Activity	Reference	
Azaserine		Irreversible inhibition	Nonselective. Glutamine utilizing enzymes, including glutamine amidotransferases, $\gamma$ -glutamyltranspeptidase (GGT)	Inhibits GGT by covalently modifying the Thr391 residue at the active site.	PubChem CID: 460129 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/460129#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/460129#section=2D-Structure</a> [40,92]	
L- $\gamma$ -Glutamyl-p-nitroanilide (GPNA)		Competitive inhibition	Glutamine transporter SLC1A5, sodium-dependent and independent amino acid transporters	Proposed model: Inhibits SLC1A5 by binding to the binding site with high affinity (the ligand part binds to the lipophilic pocket of SLC1A5 and forms a hydrogen bond).	PubChem CID: 81732 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/81732#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/81732#section=2D-Structure</a> [47,48]	
V-9502		Competitive inhibition	Glutamine transporter SLC1A5	<i>In silico</i> model: V-9502 docks into the orthosteric binding site situated at the transmembrane region of SLC1A5.	PubChem CID: 127035871 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/127035871#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/127035871#section=2D-Structure</a> [49]	

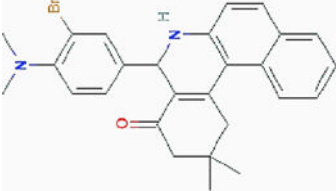
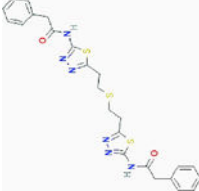

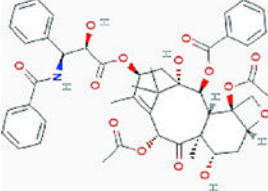
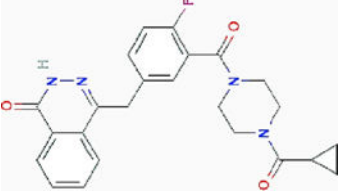
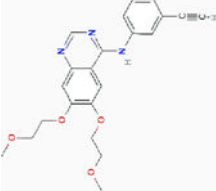
Small Molecule Inhibitors Targeting Glutaminolysis Pathway						
Small Molecule Inhibitors	Chemical Structure	Inhibition Type	Enzyme Target	Inhibitory Activity	Reference	
Compound 968		Allosteric inhibition	Glutaminase (GLS); kidney-type glutaminase; kidney glutaminase (KGA) and glutaminase C (GAC), and liver-type glutaminase (GLS2)	Inhibits glutaminases by binding to GAC monomers and preventing the formation of activated GAC tetramers.	PubChem CID: 3099980 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/3099980#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/3099980#section=2D-Structure</a> [50–52]	
bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES)		Allosteric inhibition	Selectively inhibits kidney-type glutaminase (GLS); kidney glutaminase (KGA) and glutaminase C (GAC)	Inhibits KGA and GAC by trapping their tetramers in a conformation that is nonfunctioning.	PubChem CID: 3372016 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/3372016#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/3372016#section=2D-Structure</a> [93–95]	
CB-839 (Telaglenastat)		Allosteric inhibition	Selectively inhibits kidney-type glutaminase (GLS); kidney glutaminase (KGA) and glutaminase C (GAC)	Inhibits KGA and GAC by forming hydrogen bonds with cKGA (catalytic domain Ile221-Leu533 of KGA) tetramer.	PubChem CID: 71577426 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/71577426#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/71577426#section=2D-Structure</a> [58,59]	

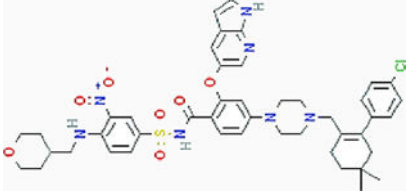
Table 3

Compounds that synergize with glutamine catabolism inhibitors.

Small Molecule Inhibitors Targeting Glutaminolysis	Compounds that Synergize with Glutamine Catabolism Inhibitors	Cell Line	Results and Cellular Target/Mechanism	Reference
BPTES [bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide]	shRNA silencing glutamine transaminase K (GTK)	Pancreatic cancer	Result: Decreased intracellular glutamate level and inhibited cell growth. Target: GTK.	[14]
	2-(Phosphonomethyl)-pentanedioic acid	Patient-derived ovarian and pancreatic orthotopic tumors	Result: Reduced tumor growth. Target: GCP11.	PubChem CID: 10130754 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/10130754#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/10130754#section=2D-Structure</a> [14]
	[2-(PMMPA)]	Pancreatic ductal adenocarcinoma (PDAC)	Result: Combination therapy did not improve single treatment efficacy. Target: DNA replication.	PubChem CID: 60750 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/60750#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/60750#section=2D-Structure</a> [13,96]
	Gemcitabine	Pancreatic and prostate cancer	Results: Greater decrease in cancer cell number and decreased levels of lactate, glucose-6-phosphate, glucose-1-phosphate, and UDP-glucose (pancreatic). Target: glucose metabolism	PubChem CID: 4091 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/4091#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/4091#section=2D-Structure</a> [13,85]
	Metformin			

Small Molecule Inhibitors Targeting Glutaminolysis	Compounds that Synergize with Glutamine Catabolism Inhibitors	Cell Line	Results and Cellular Target/Mechanism	Reference
CB-839 (Telaglenastat)		TNBC and a basal HER2 <sup>+</sup> cell line	Result: Reductions in tumor growth and suppressed regrowth of tumor. Mechanism: Inducing ER stress which promotes degradation of SLC1A5 by ubiquitin ligase RNF5	PubChem CID: 36314 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/36314#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/36314#section=2D-Structure</a> [97,98]
	Paclitaxel 	Ovarian cancer and renal cell carcinoma	Result: Potently synergistic cell death. Target: poly (ADP-ribose) polymerase (PARP)	PubChem CID: 23725625 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/23725625#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/23725625#section=2D-Structure</a> [11,60]
	Olaparib 	EGFR mutant nonsmall cell lung cancer	Result: <i>In vitro</i> apoptosis and reduced <i>in vivo</i> tumor growth. Target: EGFR tyrosine kinase	PubChem CID: 176870 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/176870#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/176870#section=2D-Structure</a> [61]
	Erlotinib			



Small Molecule Inhibitors Targeting Glutaminolysis	Compounds that Synergize with Glutamine Catabolism Inhibitors	Cell Line	Results and Cellular Target/Mechanism	Reference
	Myeloid leukemia	Result: Killing acute myeloid leukemia blasts. Target: BCL-2	PubChem CID: 49846579 URL: <a href="https://pubchem.ncbi.nlm.nih.gov/compound/49846579#section=2D-Structure">https://pubchem.ncbi.nlm.nih.gov/compound/49846579#section=2D-Structure</a> [65]	
ABT-199 Nivolumab	Clear cell renal cell carcinoma	Result: Activation of T cells and proposed synergistic effects. Target: G4 PD-1 immune checkpoint	[63]	