Development of Anti-Glutaminolysis Drugs

Subjects: Oncology

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Glutaminolysis has emerged in recent years as an effective therapeutic focus in the treatment of cancer. In order to restrict the proliferation of glutamine-addicted cancer cells, researchers have created various drugs that target different stages in glutamine metabolism.

Keywords: cancer stem cells ; glutaminolysis ; glutaminase ; metabolic compensation

1. Glutamine Depletion

Because cancer cells' reliance on glutamine metabolism is generally greater than in normal tissues, and glutamine deprivation in cancer cell cultures frequently results in cell death, scientists have explored depleting glutamine as a cancer therapy strategy. Multiple compounds, such as bacterial L-glutaminase and phenylbutyrate derivatives, that induce systemic depletion of glutamine were evaluated as potential therapeutic agents [1][2]. However, relevant research has ebbed due to the limitation of these agents, which is the easy acquisition of tumor resistance by de novo glutamine synthesis in cancer or stromal cells. Another strategy to achieve glutamine deprivation in cancer is by depleting sources of glutamine in the tumor microenvironment. Inhibition of glutamine synthesis in cancer-associated fibroblasts was found to limit ovarian tumor growth [3]. Moreover, exosomes derived from the tumor microenvironment were shown to support tumor growth under nutritional stress [4]. However, particular inhibitors of these pathways are not yet available since therapeutically relevant targets have not been unequivocally identified.

2. Inhibitors of Glutamine Uptake

In one case, the presence of several kinds of glutamine transporters in the plasma membrane made it difficult to accomplish selective blocking of glutamine absorption [5]. Nevertheless, the link between the transporter SLC1A5 and oncogenic MYC expression and adverse prognosis in a variety of cancers prompted the development of the SLC1A5 inhibitor as a target of pharmacological blockage [6][7][8]. L-Glutamyl-p-nitroanilide (GPNA), an analog of glutamine, was developed into a first-generation SLC1A5 inhibitor [9][10]. Despite early studies showing favorable results in limiting tumor growth and producing synergistic effects with other cancer therapies, GPNA has been identified by growing evidence to have low selectivity as a transporter inhibitor [10][11]. Recent research has shown that GPNA can inhibit multiple other amino acid transporters and that GPNA's inhibition of cell viability is likely due to off-target effects from the activity of the γ-Glutamyltransferase enzyme rather than the disruption of glutamine metabolism [12].

Consequently, V-9302, a small-molecule antagonist derived from GPNA, was subsequently developed. In comparison with GPNA, V-9032 has approximately 100-fold increased potency in blocking cellular glutamine uptake, and inhibition of SLC1A5 with V-9032 was demonstrated to cause cell death, disrupt redox equilibrium, and result in disrupted development and progression of cancer [13]. However, V-9032 faced similar challenges as GPNA in selectivity and off-target effect. The mechanism underlying V-9032's effect was questioned since the knockout of SLC1A5 did not result in decreased sensitivity to V-9302 [14]. The tumor suppression effect of V-9032 was likely due to the combined blockage of other glutamine transporters, such as SNAT2 and LAT1 [14]. In this regard, a specific SLC1A5 inhibitor remains unidentified. Further investigation is warranted to unveil the mechanism of action and optimize the structure of these lead compounds.

3. Antagonists of Glutamine

For decades, researchers have been searching for ways to develop effective glutamine antagonists that block glutamine metabolism in cancer cells [15][16][17][18][19]. Compounds such as DON (6-Diazo-5-oxo-L-norleucine), acivicin, and azaserine have been shown to inhibit the growth of a variety of cancers in various clinical studies [20]. However, most of

the above glutamine analogs exhibit severe toxicity and are not recognized as favorable cancer medications despite their strong effectiveness in halting the proliferation of cancer cells [21].

In this context, scientists sought to develop DON prodrugs that maximized the transport of DON to tumors while limiting its exposure to gastrointestinal (GI) tissues. Some also proposed administering these prodrugs at a low dose so as to reduce the toxicities associated with the GI system [16][22]. Noticeably, JHU083, a prodrug that releases DON, has received much attention as a novel glutamine antagonist. JHU083 is cleaved by cathepsins and other enzymes in tumors, which helps to reduce the adverse effects that it has on other organs [23]. In addition, treatment of MC38 colon cancer cells with JHU083 not only resulted in a reduction in glycolysis and oxidative phosphorylation but also enhanced the oxidative metabolism in effector T cells and thereby boosted anti-tumor immunity [24]. Taken together, prodrugs of DON, such as JHU083, have been refined in terms of their delivery strategies and doses and may show encouraging results in patients with glutamine-dependent cancers in future studies.

4. Glutaminase Inhibitors

Glutaminase is an amidohydrolase that catalyzes the conversion of glutamine (GLN) into glutamate (GLU) and ammonium ions in the first step of glutaminolysis. GLS1 is a critical enzyme in the growth and proliferation of many types of cancer and is thus a potential therapeutic target. Two lead compounds, bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl) ethyl sulfide (BPTES) and compound 968, were first developed as allosteric glutaminase inhibitors around a decade ago [25][26]. BPTES is a selective allosteric inhibitor of GLS1. Despite showing high anti-proliferative efficacy in vitro, the application of BPTES in vivo was restricted by its low solubility [27][28]. To address this problem, various delivery strategies and structural modifications were sought to improve its bioavailability [29][30][31]. One of the derivatives, Telaglenastat (CB-839), was found to be a potent hit and has advanced to clinical trials as monotherapy or combination therapy with other treatments [25]. On the other hand, compound 968 is a pan-glutaminase inhibitor with a four-fold higher potency in inhibiting GLS2 than GLS1 [32]. Recently, many other glutaminase inhibitors, such as C9.22 [33], compound 27 (IPN60090) [34], alkyl benzoquinones [35], and thiazolidine-2,4-dione compounds [36] were developed and are undergoing evaluation.

4.1. BPTES

The inhibitory effect of BPTES on GLS1 was achieved by affecting the dimer–tetramer equilibrium of GLS1 [37][38]. However, the fact that BPTES has poor solubility (0.144 μg/mL) makes it challenging to administer in vivo [21][28]. Accordingly, novel techniques have allowed the encapsulation of BPTES in sub-100 nm nanoparticles, which demonstrated better pharmacokinetic properties and efficacy compared to unencapsulated BPTES [31]. Further metabolomic analyses revealed that tumor cells that survived after glutaminase inhibition depend on glycolysis and glycogen synthesis for energy production. Consistent with this finding, combination therapy of BPTES nanoparticles and metformin, a mitochondrial complex I inhibitor that blocks glycogen synthesis, resulted in greatly increased inhibition of tumor growth compared to monotherapy with either agent alone [31]. Safety evaluation using a patient-derived pancreatic cancer mouse model found no weight loss or signs of liver or kidney toxicity with the combination of BPTES-NPs and metformin [31]. The combined targeting of multiple metabolic pathways with small-molecule drugs showed remarkable efficacy in disturbing the proliferation of cancer cells in preclinical models and holds promise for future cancer therapy.

4.2. CB-839

Although derived from BPTES, the potency and kinetic behavior of CB-839 differed from those of BPTES. The effect of CB-839 is time-dependent and slowly reversible. Compared to BPTES, CB-839 has increased potency and distinct kinetic behavior, showing a slow-on/slow-off mechanism [39]. The early preclinical results of CB-839 were promising. For example, one recent study found that CB-839 had a selective inhibitory effect on PIK3CA-mutant, but not WT, colorectal malignancies [40]. In addition, an additive impact on apoptosis was demonstrated when CB-839 was coupled with 5-FU, camptothecin, oxaliplatin, and regorafenib in HCT116 colorectal cells. These findings showed that the addition of CB-839 to current cancer therapy might greatly improve the treatment of patients with PIK3CA-mutant colorectal tumors [40].

As the most studied glutaminase inhibitor with high expectations, dozens of clinical trials are now underway to evaluate CB-839′s effect in vivo. However, early trials showed inconsistent results. While one phase I (NCT02071862) and one phase II (NCT03163667) trial reported enhanced anti-tumor effects for CB-839 when combined with cabozantinib [41] or everolimus [41][42] in metastatic renal cell carcinoma, a recently published phase II randomized controlled trial (NCT03428217) failed to demonstrate improved progression-free survival with CB-839 + cabozantinib compared to placebo + cabozantinib [43]. Despite the controversial results in efficacy, most trials reported tolerable adverse events in response to CB-839 treatment [41][42][43].

4.3. Compound 968

Compound 968 is a pan-glutaminase inhibitor that interacts with both kidney-type glutaminase (KGA) and glutaminase C (GAC) isoforms of GLS1 by preventing the combination of inactive monomers of GLS1 into an active tetramer [33][44]. Moreover, compound 968 has an inhibitory effect on GLS2, which was previously identified as being essential in the tumorigenesis of luminal-type breast cancers [45]. Research on breast cancer cell lines discovered that basal- and luminal-type breast cancer, although both rely on glutamine metabolism, adopt distinct pathways for glutaminolysis [45]. The glutamine utilization in luminal-subtype breast cancers is mediated by GLS2, rendering them insensitive to many commonly used GLS1 inhibitors. In line with this finding, targeting GLS2 with compound 968 successfully suppressed the proliferation and tumorigenesis of BPTES-resistant luminal-type breast cancer [45]. Aside from breast cancers, compound 968 was found to be effective against various cancers in preclinical studies, including endometrial [46], ovarian [47][48], hepatocellular [49][50], non-small cell lung cancer [51], and multiple myeloma [52].

4.4. C9.22

Recently, a high-throughput screening study using the coupled enzyme-based fluorescent glutaminase activity assay to screen a library of about 30,000 compounds was conducted [33]. As a result, 11 glutaminase inhibitors were found to be hits, and they were further characterized by in silico, biochemical, and glutaminase-based cellular assays [33]. The structure-activity relationship research on the most promising hit (C9) led to the identification of C9.22, a derivative with improved in vitro and cellular glutaminase-inhibiting activity [33]. C9.22 inhibited GAC selectively, presumably through a mechanism similar to BPTES and CB-839. The new glutaminase inhibitor C9.22, which has a unique structure and prevents cells from using glutamine, leads to suppression of propagation in three dimensions [33].

4.5. Combination Therapies

Due to the pivotal role of GLS in glutaminolysis and its influence on multiple inter-related pathways, GLS inhibitors have the potential to exhibit synergistic effects with many cancer therapies that target different pathways. Various combination regimens were proposed. Metformin, an antidiabetic drug that was found to have an anticancer effect in multiple malignancies [53], has a tendency to be more effective against cancer cells that have survived GLS inhibition [54]. Moreover, as inactivation of GLS produces a redox imbalance, reduces the production of nucleotides, and generates replication stress, cancer cells become more dependent on poly (ADP-ribose) polymerase (PARP) DNA repair. This therefore sensitizes them to PARP inhibitors, suggesting the consideration of the combined therapy of GLS inhibitors and PARP inhibitors as a novel therapeutic regimen [55].

On the other hand, it was shown that pancreatic cancers might circumvent GLS inhibition by increasing their glutamate synthesis through the glutaminase 2 route [56]. Inhibition of glutamine transaminase K, a major enzyme of the glutaminase 2 pathway, in conjunction with GLS inhibition, can dampen both metabolic pathways [56]. Another study reported that N-acetylaspartylglutamate can act as a crucial reservoir, supplying glutamate to cancer cells via carboxypeptidase II (GCPII), even when glutamate production from exogenous glutamine is restricted. This makes GCPII a feasible target for cancer therapy, either alone or in combination with GLS inhibition [57]. Taken together, the use of GLS inhibitors in conjunction with other therapies may provide a viable strategy for the effective treatment of cancer.

In recent years, there has been a growing focus placed on the relationship between the inhibition of glutaminase and the activation of the immune system brought about by immunotherapies. As a glutamine-rich tumor microenvironment was suggested to be essential for CD8 T cell activation and effector function, the combination of glutamine inhibition with immunotherapy was called into question [58][59]. This was corroborated by a recent study, which demonstrated that glutaminase inhibition with CB-839 impaired the clonal expansion and activation of CD8 T cells in *Lkb1*-deficient lung cancer [58]. Although glutaminase inhibition or anti-PD1 immunotherapy may be beneficial to this subset of cancer as monotherapy, their combination could lead to a contradictory effect [58]. Since several clinical trials have been launched to examine the combination of CB-839 with anti-PD1 immunotherapies (NCT03894540, NCT04265534, and NCT02771626), the importance of potential interference should be carefully considered.

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