A Potent Blood–Brain Barrier-Permeable Mutant IDH1 Inhibitor Suppresses the Growth of Glioblastoma with IDH1 Mutation in a Patient-Derived Orthotopic Xenograft Model

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ABSTRACT

Gliomas are the second most common primary brain tumors in adults. They are treated with combination therapies, including surgery, radiotherapy, and chemotherapy. There are currently limited treatment options for recurrent gliomas, and new targeted therapies need to be identified, especially in glioblastomas, which have poor prognosis. Isocitrate dehydrogenase (IDH) mutations are detected in various tumors, including gliomas. Most patients with IDH mutant glioma harbor the IDH1R132H subtype. Mutant IDH catalyzes the conversion of α-ketoglutarate to the oncometabolite 2-hydroxyglutarate (2-HG), which induces aberrant epigenetic status and contributes to malignant progression, and is therefore a potential therapeutic target for IDH mutant tumors. The present study describes a novel, orally bioavailable selective mutant IDH1 inhibitor, DS-1001b. The drug has high blood–brain barrier (BBB) permeability and inhibits IDH1R132H. Continuous administration of DS-1001b impaired tumor growth and decreased 2-HG levels in subcutaneous and intracranial xenograft models derived from a patient with glioblastoma with IDH1 mutation. Moreover, the expression of glial fibrillary acidic protein was strongly induced by DS-1001b, suggesting that inhibition of mutant IDH1 promotes glial differentiation. These results reveal the efficacy of BBB-permeable DS-1001b in orthotopic patient-derived xenograft models and provide a preclinical rationale for the clinical testing of DS-1001b in recurrent gliomas.

Introduction

Gliomas are the second most common primary brain tumors in adults and comprise three major histologic subtypes: astrocytomas, oligodendrogliomas, and glioblastomas (GBM). This classification is based on morphologic similarities to normal cellular counterparts.

Gliomas are classified as WHO grades I–IV on the basis of histopathologic criteria. Although grade I tumors are benign, grade II and III tumors are malignant and can progress to the highest grade. The median survival of grade IV tumors (GBMs) is only about 16 months, even after treatment with a multimodality regimen including surgery, radiotherapy, and adjuvant chemotherapy (1–3). Therefore, the development of novel targeted therapies is urgently needed.

Heterozygous mutations in isocitrate dehydrogenase (IDH) 1 and 2 were identified in various tumors including acute myeloid leukemia (AML), angioimmunoblastic T-cell lymphoma, chondrosarcoma, and cholangiocarcinoma (4–7). These mutations also occur in the great majority of grade II–III astrocytomas and oligodendrogliomas (53–83%) and secondary GBMs (54%; refs. 8, 9). Missense mutations in the IDH1 R132 (R132) codon cause a single amino acid substitution, most commonly to histidine (H), but also to cysteine (C), serine (S), glycine (G), leucine (K), and isoleucine (I). In gliomas, the most common mutation is IDH1R132H, which represents approximately 90% of all IDH mutations, whereas IDH2 mutations are rarely detected, unlike in AML (4.4%; refs. 1, 9). Wild-type IDH is the enzyme that converts isocitrate to α-ketoglutarate (α-KG) using NADP+”, and IDH1 and IDH2 are localized in the cytosol and mitochondria, respectively. Mutant IDH catalyzes the conversion of α-KG to the oncometabolite 2-hydroxyglutarate (2-HG; ref. 10), which competitively inhibits α-KG-dependent dioxygenases including epigenetic regulators (ten-eleven translocations and histone demethylases) and others (egl nine homologs and collagen prolyl 4-hydroxylases; refs. 11–16). The inhibitory effects of 2-HG lead to aberrant DNA methylation in CpG islands and histone methylation (11–13, 17) and the stabilization of hypoxia-inducible factor 1α, thus promoting tumorigenesis (18). The roles of mutant IDH in tumorigenesis have been analyzed extensively in preclinical...
models of AML and glioma (16, 19, 20). Previous work from our group showed that mutation of IDH2 is critical for the development and maintenance of AML stem-like cells (21). Similar results, in which mutant IDH1 (IDH1R132H) in the central nervous system (CNS) impairs the differentiation of neural progenitor cells, were reported for gliomas (16, 20), suggesting that 2-HG produced by mutant IDH inhibits normal differentiation and promotes leukemogenesis and gliomagenesis. Furthermore, analysis of clinical samples shows that TP53 mutation or 1p/19q deletion, and cause malignant transformation (22). This evidence suggests that IDH mutation is a gain-of-function mutation and that targeting mutant IDH enzymes is a potential strategy for anticancer therapy. Several mutant IDH inhibitors have been developed, and some of them are currently being tested in clinical trials for the treatment of various diseases including gliomas (24, 25). Indeed, a first-in-human phase I/II trial of AG-221 (enasidenib) in patients with relapsed or refractory AML demonstrated that the drug induced differentiation of mutant IDH2 myeloblasts into functional neutrophils and achieved a high overall and complete remission rate (26, 27), resulting in FDA approval for the treatment of IDH2 mutant AML (28). However, preclinical data of mutant IDH1 inhibitors in intracranial models of glioma showed variable results, and the effectiveness of these drugs in glioma is controversial (29–31). Success in drug development for CNS tumors requires compounds that can permeate the blood–brain barrier (BBB). The low BBB penetration of mutant IDH1 inhibitors is considered to be one of the major reasons for their insufficient antitumor effects (24).

To resolve this challenging problem, we developed a novel, orally available, selective mutant IDH1 inhibitor, DS-1001b, which showed high permeability through the BBB and potent inhibitory activity against mutant IDH1. We also succeeded in the development of orthotopic glioma patient-derived xenograft (PDX) models, which enabled us to evaluate the efficacy of DS-1001b in vivo. In this study, we present the details of the chemical structure and characteristics of DS-1001b, and provide useful preclinical data in GBMs using intracranial PDX models harboring IDH1 mutation.

Materials and Methods

Compounds

The synthesis and characterization of DS-1001b (Daichi Sankyo) are described in a Patent Cooperation Treaty application (publication number: WO2016052697 A). For in vivo administration, DS-1001b was mixed with sterilized pellet food (CRF-1; Oriental Yeast) and administered ad libitum. DS-1001a is a salt-free form of DS-1001b. [14C]DS-1001a is a radiolabeled, salt-free form of DS-1001b. [14C]DS-1001a was synthesized at LSI Medicine Corporation.

Crystallization

Cocrystallization of a tertiary complex composed of compound A, a compound with a chemical structure similar to that of DS-1001b, NADPH, and IDH1(R132C), was performed using the sitting-drop vapor diffusion method. Prior to drop setup, the protein sample was mixed with a molar excess of NADPH and compound A. After incubation on ice for 1 hour, an equal volume of precipitant solution [1.5 mol/L ammonium citrate tribasic (pH was adjusted to pH 7.0 using HCl) and 2.5 mmol/L DTT] was added, and the mixture was subjected to vapor diffusion. To obtain high-quality crystals reproducibly, microseeding methods were employed.

X-Ray data collection and structure determination

Crystals were cryoprotected in 1.8 mol/L ammonium citrate tribasic, pH 7.0/25% glycerol, and flash-frozen in nitrogen gas stream at 100 K. X-ray diffraction data were collected using the X-ray generator FR-E (Rigaku Corporation) and the imaging plate detector R-Axis 4 (Rigaku Corporation), and were processed with mosflm (32) and aimless (33). Phase refinement was performed using Refmac5 (34) and coat (35). Data collection and phase-refinement statistics are summarized in Supplementary Table S1. Figures representing the crystal structure were prepared with PyMol (Schrödinger). Atomic coordinates and structure factors have been deposited in the Protein Data Bank with accession code 6I0O.

BBB penetration in mice

[14C]DS-1001a or DS-1001a was administered orally to male mice (BALB/CAnNCrCrlj, 7 weeks of age, Charles River Japan, Inc.) at doses of 1, 3, 10, 30, 100, and 300 mg/kg. At 2, 6, and 24 hours, plasma and whole brain were collected (n = 3). Radioactivity was measured by a liquid scintillation counter (2900TR, PerkinElmer Inc.). The concentrations of DS-1001a in plasma and brain were measured by LC-MS/MS (Prominence UFLC, Shimadzu Corporation and API4000, Applied Biosystems/MDI SCIX) in a system equipped with Shim-pack XR-ODS, 2.1 mm I.D. × 30 mm (Shimadzu Corp.). Elution was performed using a mixture of solvent A (consisting of acetonitrile containing 5% 0.1 mol/L ammonium acetate) and solvent B (consisting of 5% acetonitrile containing 5% 0.1 mol/L ammonium acetate) as a mobile phase. The proportion of solvent B was 50% from 0 to 0.5 minutes, and from 50% to 100% from 0.5 to 1 minute (linear gradient).

Mice

NOD.Cg-Prkdcsidcl Il2rgtm1Sug/jc (NOG) and CB17.Cg- Prkdcsidcl<Scid>Lyst<cbd>–>/CelCrj (SCID-Beige) mice were purchased from the Central Institute for Experimental Animals and Charles River Laboratories Japan, Inc., respectively. All animal procedures were performed in accordance with the guidelines for the care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee of the National Cancer Center. Each experiment was performed in a pathogen-free environment at the animal facility of the National Cancer Center according to institutional guidelines.

GBM PDX model

Two primary GBM PDX lines were kindly provided by Dr. K. Ichimura (the cells' characteristics are provided in Supplementary Table S2). A1074 (GB181 passage 7) harbors a heterozygous IDH1 mutation (IDH1R132H), and A1056 (GB138 passage 12) has wild-type IDH (36). This mutation was confirmed by pyrosequencing and IHC for anti-IDH1R132H. Tumor pieces were implanted subcutaneously into the left flank of 6-week-old female NOG or SCID-Beige mice, and the tumors were passaged to other mice when the diameter reached 1 cm. Tumor-bearing mice were randomized into two groups, and treatment was started 2 and 4 weeks after implantation of A1056 and A1074 cells, respectively. DS-1001b mixed with sterilized pellet food was administered ad libitum for 4 weeks. The tumor volume was measured at weekly intervals with calipers and calculated as 1/2 × (tumor length) × (tumor width)2. For event-free survival (EFS) analysis, an event was defined as a tumor size that exceeded 250 mm3 or any animal discomfort resulting from the tumor or the treatment.
Peripheral blood was collected every 2 weeks from treated and untreated mice for analysis of serum 2-HG levels. Mice were sacrificed when treatment was completed, and subcutaneous tumors were collected for the analysis of intratumoral 2-HG by LC-MS/MS and immunostaining of GFAP and IDH1R132H.

**Orthotopic PDX model**

A1074 tumors from PDX mice were minced to 1 mm³ cubes with a surgical knife. Cells were maintained in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 2 mg/mL collagenase (SIGMA), and 2 mmol/L CaCl₂. They were incubated at 37°C in a humidified incubator with 95% air and 5% CO₂ for 1 hour. Cubes were pipetted occasionally and filtered using a 100-μm mesh. To hemolyze the red blood cells in the tissues, 170 mmol/L Tris-HCl (pH 7.65) supplemented with 0.92% NH₄Cl was added and incubated for 10 minutes at room temperature. Then, cells were washed with 1 unit/mL of DNase (M030S, BioLabs) containing PBS and incubated with Accutase (GIBCO) for 1 minute. A small hole was bored into the skull (2.0 mm lateral to the bregma) using a dental drill in 4- to 6-week-old female SCID-beige mice. Three microliters of tumor cells (from 1 to 2 x 10⁶ cells) was injected into the right hemisphere 3 mm below the cortex using a Hamilton syringe with an unbeveled 30-gauge needle. The injection was performed over 3 minutes, with an additional 2-minute pause before removing the syringe. The hole was sealed with bone wax. Tumor-bearing mice were randomized into two groups, and treatment was administered via orthotopic brain injection. The 2-HG injection was performed over 3 minutes, with an additional 2-minute pause before removing the syringe. The hole was sealed with bone wax. Treatment was started immediately or 4 weeks after inoculation of A1074 cells. DS-1001b mixed with sterilized pellet food (CRF-1; Oriental Yeast) was administered ad libitum for 10 weeks. The tumor volume was monitored at 8 and 10 weeks after transplantation by MRL. Peripheral blood was collected every 4 weeks from treated and untreated mice for analysis of serum 2-HG levels. Mice were sacrificed when treatment was completed, and brain tumors were collected for the analysis of intratumoral 2-HG by LC-MS/MS and immunostaining of GFAP and IDH1R132H. All images were captured with BZ-9000 (KEYENCE), and the tumor area was calculated using BX-X analysis software (KEYENCE).

**Results**

**DS-1001b is a potent and specific mutant IDH1 inhibitor that permeates the BBB**

A novel, orally bioavailable mutant IDH1 inhibitor, DS-1001b, was developed (Fig. 1A). The inhibitory effect of DS-1001b on mutant IDH1 was examined. Cell-free enzymatic assays conducted without any preincubation of the enzyme and compound in the absence of substrate demonstrated that DS-1001b strongly inhibited mutant IDH1, but not wild-type IDH1 or mutant IDH2 (Table 1). Although DS-1001b showed activity toward wild-type IDH1 in assays conducted with a 2-hour preincubation step, it remained more potent against IDH1R132H and IDH1R132C than wild-type IDH1 in these conditions. To analyze the mechanism of action of the compound, we tested DS-1001b against IDH1R132H with various substrate concentrations and without preincubation (Fig. 1B–D). Higher concentrations of α-KG or MgCl₂ decreased the activity of DS-1001b against the IDH1R132H enzyme, whereas NADPH concentration did not affect DS-1001b activity. To investigate the inhibition mechanism of DS-1001b, we performed cocystal analysis of the tertiary complex of IDH1R132C, NADPH, and compound A, a compound with a similar chemical structure to DS-1001b (Fig. 1E). Compound A showed inhibitory activity against IDH1(R132C) (IC₅₀ = 130 nmol/L), suggesting that DS-1001b and compound A share a similar inhibition mechanism. IDH1 formed a dimer. Two molecules of compound A were bound in the allosteric pocket located at the dimer surface and seemed to stabilize the conformation of IDH1R132C to the ‘open’ inactive form (Fig. 1F–G; refs. 37, 38). This conformational change disrupted the spatial arrangement of Asp residues (Asp 275, Asp 279, and Asp 252 in another protomer), which form the binding site of a catalytically important divalent cation. This conformational change also reduced the affinity for the substrate α-KG, because coordinate bond formation with the divalent cation is necessary for α-KG binding. As a result, compound A and DS-1001b inhibit the overall catalytic activity of IDH1 mutants by lowering the binding affinity of both divalent cation and substrate. Binding of compound A is stabilized by a number of hydrophobic interactions together with salt-bridges formed between the carbonyl moiety and Arg 119 (Fig. 1H). DS-1001b showed good cellular potency in several models. DS-1001b inhibited 2-HG production in TF-1 cells stably transfected with IDH1R132H or IDH1R132C at around 30 nmol/L (Table 2). DS-1001b showed similar inhibition of 2-HG production in 293A cells transiently transfected with IDH1R132H or IDH1R132C. The IC₅₀ values were the lowest in IDH1R132H mutant cells in both stable cells and transiently transfected cells. The activity of DS-1001b against IDH1R132G, IDH1R132L, or IDH1R132S was around 200 nmol/L, and no inhibition was observed against IDH2R140Q or IDH2R172K. We next examined whether DS-1001b could permeate the BBB by measuring the radioactivity of [¹⁴C]DS-1001a in mice. The cerebrum time–concentration curve of [¹⁴C]DS-1001a was similar to that observed in plasma (Fig. 2A). In addition, the brain concentration of DS-1001a measured by LC-MS/MS showed a strong positive correlation with the plasma concentration of DS-1001a (Fig. 2B; Supplementary Tables S3 and S4). Specifically, the AUC of DS-1001a in brain (8,140 ng·eq./h/g) was approximately 65% of that of DS-1001a in plasma (12,500 ng·eq./h/mL), demonstrating that DS-1001a penetrated the BBB. These results suggest that this drug is suitable for the treatment of IDH1 mutant gliomas, most of which harbor the IDH1R132H mutation.

**DS-1001b impairs the proliferation of IDH1 mutant GBM cells and induces differentiation in a subcutaneous PDX model**

We utilized GBM PDX models expressing wild-type or mutant IDH1 (38) to test the efficacy of DS-1001b in vivo. Pyrosequencing was performed to assess the presence of IDH mutations in the models. Heterozygous IDH1R132H was detected in the A1074 cells, whereas the A1056 cells had no mutation in either allele (Supplementary Fig. S1). To test the effect of DS-1001b on mutant IDH1 GBM in vivo, the A1074 tumor xenograft model was used. Mice harboring A1074 subcutaneous tumors were treated with control CRF-1 or DS-1001b 4 weeks after transplantation. Continuous administration of DS-1001b for 4 weeks significantly impaired tumor growth and prolonged EFS in A1074 xenografted mice (Fig. 3A and B; Supplementary Fig. S2). Tumor weight was also significantly decreased in treated mice at the final evaluation (Fig. 3C). Measurement of intratumoral 2-HG after completion of the 4-week treatment demonstrated that 2-HG levels were markedly lower in DS-1001b–treated A1074-bearing mice than in untreated A1074-bearing mice, whereas 2-HG levels were significantly higher in A1074-untreated tumors than in A1056-untreated tumors, in which 2-HG was barely detectable (Fig. 3D). DS-1001b also suppressed the increase in plasma 2-HG levels in the A1074 model, which increased 2 weeks after vehicle administration in untreated mice (Fig. 3E). These results are similar to those previously observed in chondrosarcoma (39). Furthermore, because
Figure 1.
DS-1001b is a potent and specific mutant IDH1 inhibitor. A, Chemical structure of DS-1001b. B–D, DS-1001b activity against IDH1R132H using various substrate concentrations. Various concentrations of α-KG (B), MgCl₂ (C), or NADPH (D) were used for the enzymatic assays, and the relative enzyme activity is shown. E, Chemical structure of compound A. F, Overall structure of IDH1R132C/NADPH/compound A complex. Compound A and NADPH are shown in sphere- and stick-representations, respectively. Two molecules of compound A are bound in the allosteric pocket located at the dimer surface of the "open" inactive form of IDH1 (37). G, Electron density (Fo-Fc, contoured at 3.0σ) of compound A bound to chains A (left) and B (right). H, Intermolecular interactions observed between compound A and amino acids in the allosteric pocket. Salt-bridges are shown in dashed lines. Labels represent amino acids interacting with compound A.
previous reports demonstrated that inhibition of mutant IDH induces the differentiation of AML and glioma cells, we hypothesized that neural differentiation may be induced by DS-1001b in this PDX model. We therefore performed IHC analysis of GFAP. GFAP was more strongly expressed in the cytoplasm of DS-1001b–treated A1074 cells than in the cytoplasm of untreated A1074 cells (Fig. 3F). These results suggest that inhibition of mutant IDH1 by DS-1001b promotes neural differentiation and impairs cell proliferation in IDH1 mutant subcutaneous tumors.

**Growth of IDH wild-type GBM is not affected by DS-1001b treatment in subcutaneous PDX model**

To assess the effect of DS-1001b on wild-type IDH GBM, the A1056 model was used. Mice harboring A1056 subcutaneous tumors were treated with control CRF-1 or DS-1001b 2 weeks after transplantation. Continuous administration of DS-1001b for 4 weeks showed no significant difference in subcutaneous tumor growth or tumor weight (Supplementary Fig. S3A–S3C). The levels of 2-HG did not differ significantly between untreated and DS-1001b–treated A1056 tumors (Fig. 3D). Furthermore, the expression of GFAP was not induced at all by DS-1001b treatment in A1056 tumors (Supplementary Fig. S3D). These results suggest that DS-1001b selectively impairs cell proliferation in IDH1 mutant GBM cells and has little off-target effect on IDH wild-type cells.

**DS-1001b permeates the BBB and suppresses tumor growth in an orthotopic PDX model**

Targeted therapy for brain tumors requires drugs that can permeate the BBB. Because DS-1001b showed high BBB permeability (Fig. 2A and B), we tested the effect of DS-1001b on mutant IDH1 A1074 GBM model cells implanted orthotopically. In this experiment, A1074 tumors were implanted orthotopically into the right hemisphere, and treatment with control CRF-1 or DS-1001b was performed for 10 weeks beginning right after transplantation (n = 7 control group, n = 6 DS-1001b group). After treatment ended, all mice were sacrificed and the tumor size was evaluated by hematoxylin and eosin (H&E) staining. Large masses were detected in the right brain of vehicle-treated mice, whereas only small masses were detected in DS-1001b–treated mice (Fig. 4A). In addition, the tumor area was dramatically reduced by DS-1001b treatment (Fig. 4B). We also succeeded in evaluating TV in vivo by 9.4 tesla MRI in 2 mice (control and DS-1001b-treated). This analysis revealed that DS-1001b markedly impaired tumor progression compared with the control (Fig. 4C). Furthermore, the effect of DS-1001b in this orthotopic model was confirmed by another protocol, in which DS-1001b treatment was started 4 weeks after transplantation (n = 3 per group). Intracranial tumors derived from the A1074 model cells showed complete positivity for mutant IDH using anti-IDH1R132H antibody, suggesting that these tumors were all derived from IDH1 mutant cells (Supplementary Fig. S4A). H&E staining showed that DS-1001b also

**Table 1. Biochemical activity of DS-1001b against several IDH1 or IDH2 enzymes.**

<table>
<thead>
<tr>
<th>Enzymatic activity</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nmol/L; 2-hour preincubation)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nmol/L; without preincubation)</th>
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</thead>
<tbody>
<tr>
<td>IDH1R132H</td>
<td>8.4</td>
<td>15</td>
</tr>
<tr>
<td>IDH1R132C</td>
<td>11</td>
<td>130</td>
</tr>
<tr>
<td>IDH2R140Q</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>IDH2R172Q</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>IDH1WT</td>
<td>180</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>IDH2WT</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
</tbody>
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**Table 2. Inhibition of 2-HG production by DS-1001b in several cell models.**

<table>
<thead>
<tr>
<th>Cells (IDH mutation)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nmol/L)</th>
</tr>
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<tbody>
<tr>
<td>Stable lines</td>
<td></td>
</tr>
<tr>
<td>TF1 (IDH1R132H)</td>
<td>29</td>
</tr>
<tr>
<td>TF1 (IDH1R132C)</td>
<td>35</td>
</tr>
<tr>
<td>Transient transfection</td>
<td></td>
</tr>
<tr>
<td>293A (IDH1R132H)</td>
<td>29</td>
</tr>
<tr>
<td>293A (IDH1R132C)</td>
<td>42</td>
</tr>
<tr>
<td>293A (IDH1R132G)</td>
<td>208</td>
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<tr>
<td>293A (IDH1R132L)</td>
<td>165</td>
</tr>
<tr>
<td>293A (IDH1R132S)</td>
<td>175</td>
</tr>
<tr>
<td>293A (IDH2R140G)</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>293A (IDH2R172K)</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>
Figure 3.
DS-1001b impairs the proliferation of glioblastoma with IDH1R132H and induces differentiation in a subcutaneous PDX model. A1074 (IDH1R132H) cells were implanted subcutaneously into mice, and continuous administration of DS-1001b was started 4 weeks after implantation. A, Tumor volume in mice implanted subcutaneously with A1074 cells (n = 5 per group). **P = 0.030 and ***P < 0.001. B, Images of DS-1001b–treated and untreated subcutaneous A1074 tumors. Scale bar, 1 cm. C, Tumor weight in mice implanted subcutaneously with A1074 cells. Mice were treated with DS-1001b or vehicle control. D, Bar graphs show the levels of intratumoral 2-HG in A1056 (IDH wild-type) and A1074 (IDH1R132H) subcutaneous tumors at the end of treatment in control or DS-1001b–treated mice. E, Line graph representing the levels of plasma 2-HG measured every 2 or 3 weeks in control or DS-1001b–treated mice. **P = 0.005 and ***P = 0.042. F, IHC of GFAP and IDH1R132H in DS-1001b–treated or untreated A1074 subcutaneous tumors. Scale bar, 100 μm.
significantly suppressed tumor progression using this treatment regimen (Supplementary Fig. S4A and S4B). GFAP was more strongly expressed in the cytoplasm of DS-1001b-treated A1074 cells than in the cytoplasm of untreated A1074 cells (Fig. 4D), suggesting that DS-1001b also promotes neural differentiation in IDH1 mutant brain tumors. In this experiment, intratumoral 2-HG levels were measured at the end of treatment to examine the effect of DS-1001b on 2-HG production. DS-1001b significantly reduced 2-HG levels, achieving the same level as that observed in subcutaneous tumors (Fig. 4E). Plasma 2-HG in orthotopic tumor-bearing mice was hardly detected even in the absence of DS-1001b, as previously reported (refs. 40, 41; Fig. 4F). These results demonstrate that DS-1001b permeates the BBB and suppresses tumor growth in an orthotopic GBM model.

**Discussion**

In this study, we developed a novel BBB-permeable mutant IDH1 inhibitor, DS-1001b, and investigated its effect in mutant IDH1 GBM using subcutaneous and intracranial PDX models. DS-1001b showed high permeability through the BBB and significantly impaired tumor progression and reduced intratumoral 2-HG levels in both models.

Although surgery combined with radiotherapy and chemotherapy such as temozolomide plays a key role in the treatment of gliomas, there are few treatment options for recurrent cases. In addition, once lower-grade gliomas progress to secondary GBMs, patients have a dismal prognosis regardless of IDH mutation status, although IDH mutations are associated with better prognosis in gliomas (1–3).
Furthermore, the survival time from diagnosis of secondary GBMs is not different between IDH mutant and IDH wild-type gliomas (2). Therefore, the development of novel targeted therapies for the treatment of primary and secondary GBMs is eagerly awaited. Among gliomas, IDH mutations are present in astrocytoma, oligodendroglia, and secondary GBM, at a rate of no less than 50% (8, 9). Because IDH1 mutations are gain-of-function mutations and 2-HG, which is regarded as an oncometabolite, is not produced in IDH wild-type normal tissues, mutant IDH is an attractive therapeutic target for IDH mutant GBMs.

In vitro assays showed that the IDH1R132H mutation subtype has the highest sensitivity for DS-1001b compared with other IDH mutations tested. In addition, DS-1001b can penetrate the BBB and concentrations of DS-1001b in the cerebrospinal positively correlate with those in plasma, suggesting that DS-1001b is suitable as a targeted therapy for glioma. As expected, inhibition of mutant IDH1 using DS-1001b significantly impaired mutant IDH1 tumor growth in subcutaneous models. Moreover, A1074 intracranial tumors were also sensitive to DS-1001b, and intratumoral 2-HG levels were dramatically reduced by DS-1001b treatment. Low penetration rate through the BBB is considered to be the reason why other mutant IDH1 inhibitors could not sufficiently inhibit 2-HG production and suppress tumour progression in glioma models (24). Although it is difficult to compare the effect of DS-1001b directly with that of other mutant IDH1 inhibitors due to differences in the assays and samples used, DS-1001b is expected to have better BBB penetration and efficacy against CNS tumors. Tumor volumes gradually increased in size even with DS-1001b treatment, suggesting that other oncocgenic signaling pathways were recruited after inhibition of the mutant IDH1. Despite this however, DS-1001b significantly suppressed tumor progression and increased progression-free survival. In addition, DS-1001b might be more effective against low-grade glioma models than against the GBM model A1074. Furthermore, the proliferation and 2-HG levels of IDH wild-type A1056 tumors were not affected by DS-1001b at all, indicating that this drug potently and selectively inhibits GBMs with IDH1 mutations.

Our results also demonstrated that the expression of GFAP, a differentiation marker of astrocytes in GBMs, was strongly induced after inhibition of the mutant IDH1. Despite this however, DS-1001b significantly suppressed tumor progression and increased progression-free survival. In addition, DS-1001b might be more effective against low-grade glioma models than against the GBM model A1074. Furthermore, the proliferation and 2-HG levels of IDH wild-type A1056 tumors were not affected by DS-1001b at all, indicating that this drug potently and selectively inhibits GBMs with IDH1 mutations.

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A Potent Blood–Brain Barrier-Permeable Mutant IDH1 Inhibitor Suppresses the Growth of Glioblastoma with IDH1 Mutation in a Patient-Derived Orthotopic Xenograft Model

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