Pharmacological characterization of TQ05310, a potent inhibitor of isocitrate dehydrogenase 2 R140Q and R172K mutants

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Abstract
Isocitrate dehydrogenase 2 (IDH2), an important mitochondrial metabolic enzyme involved in the tricarboxylic acid cycle, is mutated in a variety of cancers. AG-221, an inhibitor primarily targeting the IDH2-R140Q mutant, has shown remarkable clinical benefits in the treatment of relapsed or refractory acute myeloid leukemia patients. However, AG-221 has weak inhibitory activity toward IDH2-R172K, a mutant form of IDH2 with more severe clinical manifestations. Herein, we report TQ05310 as the first mutant IDH2 inhibitor that potently targets both IDH2-R140Q and IDH2-R172K mutants. TQ05310 inhibited mutant IDH2 enzymatic activity, suppressed (R)-2-hydroxyglutarate (2-HG) production and induced differentiation in cells expressing IDH2-R140Q and IDH2-R172K, but not in cells expressing wild-type IDH1/2 or mutant IDH1. TQ05310 bound to both IDH2-R140Q and IDH2-R172K, with Q316 being the critical residue mediating the binding of TQ05310 with IDH2-R140Q, but not with IDH2-R172K. TQ05310 also had favorable pharmacokinetic characteristics and profoundly inhibited 2-HG production in a tumor xenografts model. The results of the current study establish a solid foundation for further clinical investigation of TQ05310, and provide new insight into the development of novel mutant IDH2 inhibitors.

KEYWORDS
AG-221, IDH2, leukemia, R140Q, R172K

1 | INTRODUCTION

Isocitrate dehydrogenase is a critical metabolic enzyme that catalyzes the conversion of isocitrate to α-KG in the tricarboxylic acid cycle. IDH occurs in three isoforms: IDH1, IDH2 and IDH3.¹ Mutations in IDH1 and IDH2 have been found in a variety of human cancers, including AML, malignant gliomas, intrahepatic cholangiocarcinoma, chondrosarcoma, and thyroid carcinomas.²⁻⁴ IDH mutations gain neomorphic activity, which catalyzes the conversion of α-KG to 2-HG, resulting in an intracellular concentration of 2-HG that is over 100-fold higher in mIDH cells than in wild-type cells.²⁻⁷ 2-HG is a competitive inhibitor of multiple α-KG-dependent dioxygenases, including JmjC domain-containing enzymes.
histone demethylases,\textsuperscript{8,9} tet methylcytosine dioxygenase 2 (TET2) and 5-methylcytosine hydroxylases,\textsuperscript{9,10} thereby linking metabolism to epigenetics and subsequent blockade of cell differentiation.\textsuperscript{9,11}

Several studies have suggested that IDH mutations play a key role in driving leukemogenesis and potentially solid cancers.\textsuperscript{12-14} These findings have stimulated interest in the development of inhibitors of mIDH as anticancer agents. The mIDH2 inhibitor AG-221 (enasidenib) and mIDH1 inhibitor AG-120 (ivosidenib) have been approved for the treatment of patients with relapsed or refractory AML with a susceptible IDH mutation. Several other compounds, including AG-B81, IDH305, BAY1436032, FT-2102 and AGI-6780, are in development.\textsuperscript{15} To date, AG-221 and AGI-6780 are the only two inhibitors developed against mIDH2. In particular, both compounds selectively bind to and inhibit the IDH2-R140Q enzyme, but show weak inhibitory effects on IDH2-R172K enzymatic activity.\textsuperscript{15,16} The IDH2-R140Q mutation occurs more frequently than IDH2-R172K in AML, which may be the reason IDH2-R140Q was selected as the primary initial target. However, recent large-scale genomic classification and prognosis studies of AML have suggested that the IDH2-R172K mutation is associated with a unique gene expression and DNA methylation profile that results in more severe aberrations in central metabolism.\textsuperscript{14,17,18}

In a separate retrospective study carried out by the UK Medical Research Council, the CR rate was 88% among R140Q patients and only 48% in those with an R172K mutation, indicating a relatively poor response in IDH2-R172K patients.\textsuperscript{19} Thus, developing an inhibitor that targets IDH2-R172K, or ideally one that targets both IDH2-R140Q and IDH2-R172K, would have significant clinical value.

Herein, we rationally designed and synthesized a series of derivatives based on the chemical structure of AG-221. One derivative, TQ05310 (Figure 1), stood out in initial screens and was selected for further evaluation. TQ05310 was superior to AG-221 in inhibition of IDH2-R140Q enzymatic activity; more importantly, TQ05310 also potently inhibited IDH2-R172K and had no apparent inhibitory effects on wild-type IDH1/2 enzymes. In this report of major preclinical pharmacological results of TQ05310, we show that TQ05310 is the first mIDH2 inhibitor that potently targets both IDH2-R140Q and IDH2-R172K mutations.

2 | MATERIALS AND METHODS

2.1 | Reagents and antibodies

TQ05310 was provided by Jiangsu Chia-Tai Tianqing Pharmaceutical Co., Ltd (Nanjing, China). AG-221 and doxycycline hyclate were purchased from Selleckchem (Boston, MA, USA). Monoclonal antibodies against IDH1 and Myc-tag were purchased from Cell Signaling Technology (Danvers, MA, USA). Monoclonal antibodies against IDH2, IDH2-R172K,\textsuperscript{20} and GAPDH were purchased from Abcam (Cambridge, UK). MBL International Corporation (Woburn, MA, USA) and AbSci (College Park, MD, USA), respectively. rhEPO was obtained from 3SBio Inc. (Shenyang, China). dl-Isocitric acid trisodium salt hydrate was purchased from Sigma-Aldrich (St Louis, MO, USA).

2.2 | Expression plasmids and viruses

Human IDH1 and IDH2 mutants were constructed using standard techniques and subcloned into pLenti-C-Myc-DDK-IRES-Puro or tetracycline-inducible lentiviral pLVX-TRE3G expression vectors. Primers for site-directed mutagenesis are listed in Data S1. Sequences were verified by Sanger sequencing (Data S1). Lentiviral particles were produced from transiently transfected HEK 293FT cells.

2.3 | Cells and lentiviral transfection

HEK 293FT, TF-1, and U-87 MG cells were obtained from ATCC, and cell identities were confirmed by short tandem repeat (STR) DNA profiling analysis in Genesky. HEK 293FT and U-87 MG cells were maintained in DMEM supplemented with 10% FBS. TF-1 cells were maintained in RPMI-1640 medium supplemented with 10% FBS and 2 ng/mL hGM-CSF.

TF-1 cells were infected with tetracycline-inducible pLVX-TRE3G lentiviral particles (IDH2-WT, IDH2-R140Q, IDH2-R172K) to generate TF-1/IDH2-WT, TF-1/IDH2-R140Q, and TF-1/IDH2-R172K cells. U-87 MG cells were infected with pLenti-C-Myc-DDK-IRES-Puro lentiviral particles (IDH1-WT, IDH1-R132C, IDH1-R132H, IDH2-WT, IDH2-R140Q, IDH2-R172K) to generate U-87 MG/IDH1-WT, U-87 MG/IDH1-R132C, U-87 MG/IDH1-R132H, U-87 MG/IDH2-WT, U-87 MG/IDH2-R140Q, and U-87 MG/IDH2-R172K cells.

2.4 | Western blotting

Western blot analysis was conducted as previously described.\textsuperscript{21} After drug treatment, cells were lysed in SDS sample buffer. Proteins in lysates were separated by SDS-PAGE electrophoresis and then transferred to PVDF membranes. After incubating with primary antibodies and secondary antibodies, proteins were visualized using the Western Blot Imaging System (Clinx Science Instruments, Shanghai, China).
2.5 | Cell-based assays for measuring inhibition of 2-HG production

Cells were treated with TQ05310 or AG-221 for 72 hours. Media were removed and 2-HG was extracted using 80% aqueous methanol and determined by LC-MS/MS.

2.6 | Cell-based enzyme assays

Cells were lysed with RIPA buffer (20 mmol/L Tris-HCl, 100 mmol/L NaCl, 1% NP-40, 1 mmol/L EDTA), and cell lysates were centrifuged at 13 000 x g for 40 minutes at 4°C. Supernatants were collected and used to assay IDH oxidation activity, measured with 25 μmol/L NADPH, 0.8 mmol/L α-KG, 150 mmol/L NaCl, 10 mmol/L MgCl₂, 0.5% βSA, 2 mmol/L β-mercaptoethanol, and 20 mmol/L Tris-HCl (pH 7.5). Activity of mIDH enzymes was measured with 100 μmol/L NADP, 100 μmol/L isocitrate, 150 mmol/L NaCl, 10 mmol/L MgCl₂, 0.5% βSA, 2 mmol/L β-mercaptoethanol, and 20 mmol/L Tris-HCl (pH 7.5). NADPH was detected at 340 nm using a Synergy H4 Hybrid Microplate Reader (BioTek Instruments, Winooski, VT, USA). All reactions were carried out at room temperature for 4 hours.

2.7 | Cell differentiation

TF-1/IDH2-R140Q and TF-1/IDH2-R172K cells were treated with compounds for 7 days in RPMI-1640 supplemented with 10% FBS and 2 ng/mL hGM-CSF. Erythroid differentiation of cells was induced by replacing GM-CSF with EPO (2 IU/mL) for another 7 days in culture medium containing compounds. After induction, cell pellets were collected for analysis of the expression of HBG.

2.8 | RNA isolation and quantitative reverse transcription-polymerase chain reaction chain reaction

Total RNA was extracted and reverse transcribed as previously described. qRT-PCR was carried out on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) according to the instructions for the SYBR Premix Ex Taq II kit (TaKaRa, Kusatsu, Japan). Sequences of primers used for qRT-PCR were as follows: HBG, 5’-TGT GCC TTG ACT TTG GGG TT-3’ (forward) and 5’-CGT TCT CAG CCT T-3’ (reverse); GAPDH, 5’-GGG GAA GGT GAA GGT CGG AGT C-3’ (forward) and 5’-CAA GCT TCC CGT TCT CAG CCT T-3’ (reverse).

2.9 | Cell proliferation assay

TF-1/IDH2-R140Q and TF-1/IDH2-R172K cells were treated with compounds for 7 days in medium with 2 ng/mL hGM-CSF, and another 7 days without hGM-CSF. At the end of treatment, MTT solution was added to each well, and plates were incubated for 4 hours at 37°C. Formazan crystals formed were then solubilized in acidic isopropanol, and the optical density of the resulting solution was read at 570 nm using a Synergy H4 microplate reader (BioTek).

2.10 | Cellular thermal shift assay

Experiments were carried out using standard techniques. Briefly, after treatment with compounds for 1 hour, cells were harvested and washed with PBS. Cells were then distributed into different tubes (10⁶ cells/tube), heated at different temperatures for 3 minutes and lysed using two freeze-thaw cycles with liquid nitrogen. Soluble fractions were isolated and analyzed by western blotting.

2.11 | Molecular docking

The IDH2-R140Q structure from a crystal of the IDH2-R140Q/AG-221 complex was used as a template. The X-ray crystal structure of this IDH2-R140Q/AG-221 complex (PDB code: 5l96) was obtained from the Protein Data Bank. Molecular docking was carried out using Glide v6.9 in SP mode. LigPrep v3.6 was applied to pre-process the compound using default parameters. The obtained docked poses were analyzed with Maestro, PyMOL, and LigPlot.

2.12 | Pharmacokinetic/pharmacodynamic studies

Female nude mice (Balb/cA-nude, 5-6 weeks old) were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. Tumor models were established by s.c. inoculation of U-87 MG or U-87 MG/IDH2-R140Q cells into nude mice. When tumors reached a volume of 100-300 mm³, mice bearing U-87 MG or U-87 MG/IDH2-R140Q tumors received a single oral dose of TQ05310 (18.05 or 54.14 mg/kg), and then tumor tissue and blood were collected at multiple time points (0, 0.5, 2, 6, 12, 24, 48, and 72 hours) post-dosing. TQ05310 and 2HG levels were analyzed by LC/MS-MS. All animal experiments were carried out in accordance with guidelines of the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.13 | Statistical analysis

Data were analyzed with GraphPad Prism software. Two-tailed Student’s t tests were used to determine the statistical significance of differences between two groups.

3 | RESULTS

3.1 | Establishment of cell models exogenously expressing mIDH genes

Isocitrate dehydrogenase mutations are heterozygous, and the most common mutational types are IDH1-R132C, IDH1-R132H, IDH2-R140Q, and IDH2-R172K. Accordingly, we transfected exogenous mIDH genes (Table 1) into cells endogenously expressing wild-type IDH. Two sets of models were constructed: TF-1 AML cells transfected with inducibly expressed IDH (IDH2-WT, IDH2-R140Q, IDH2-R172K; Figure 2A), and U-87 MG glioma cells transfected with constitutively expressed IDH (IDH2-WT, IDH2-R140Q,
TABLE 1  Genetic mutations in isocitrate dehydrogenase (IDH)1/2

| IDH1/IDH2 | Amino acid change | Mutation\a |
|-----------|-------------------|------------|
| IDH1      | R132C             | c.394C > T, CGT to TGT |
| IDH1      | R132H             | c.395G > A, CGT to CAT |
| IDH2      | R140Q             | c.419G > A, CGG to CAG |
| IDH2      | R172K             | c.515G > A, AAG to AAG |
| IDH2-R140Q| Q316A             | c.946C > G, c.947A > C, CAG to GCG |
| IDH2-R172K| Q316I             | c.946C > A, c.947A > T, c.948G > C, CAG to ATC |

\aNucleotide numbering is based on NM_005896.2 for IDH1 and NM_002168.2 for IDH2.

3.2 | TQ05310 inhibits mIDH enzymatic activity and 2-HG production in both IDH2-R140Q- and IDH2-R172K-expressing cells

We first evaluated the inhibitory activity of TQ05310 against wild-type and mIDH enzymes using cell-based enzyme assays. As shown in Figure 3A, TQ05310 was more potent than AG-221 in the inhibition of IDH2-R140Q (IC\_50: 136.9 ± 15.8 nmol/L vs 229.3 ± 23.2 nmol/L). More importantly, TQ05310 was significantly more potent than AG-221 in the inhibition of IDH2-R172K (IC\_50: 37.9 ± 7.3 nmol/L vs 624.5 ± 146.3). Both TQ05310 and AG-221 had no apparent inhibitory effects on IDH2-WT, IDH1-WT, IDH1-R132C or IDH1-R132H enzymatic activity.

We then examined 2-HG levels in cells expressing mIDH2. Consistent with the inhibitory effects on enzymatic activity, both

![Figure 2](image-url)
TQ05310 (0.2 μmol/L) and AG-221 (0.2 μmol/L) inhibited 2-HG production in U-87 MG/IDH2-R140Q cells (78.2% vs 67.3%), and only TQ05310 (0.2 μmol/L) inhibited 2-HG production in U-87 MG/IDH2-R172K cells (74.7%) (Figure 3B). These effects were also confirmed in TF-1 models. Compared with AG-221, TQ05310 showed more potent anti-2-HG activity in TF-1/IDH2-R140Q cells (IC₅₀: 9.9 ± 2.8 vs 24.7 ± 2.3) and significantly more potent anti-2-HG activity in TF-1/IDH2-R172K cells (IC₅₀: 40.9 ± 1.6 vs 732.0 ± 331.4) (Figure 3C).

Taken together, these data indicate that, unlike AG-221, a selective inhibitor of IDH2-R140Q, TQ05310 is a mIDH2 inhibitor with potent inhibitory effects on both IDH2-R140Q and IDH2-R172K.

### 3.3 TQ05310 relieves differentiation block in both IDH2-R140Q- and IDH2-R172K-expressing cells

(R)-2-hydroxylutarate blocks differentiation of hematopoietic progenitor cells, and IDH inhibition has been reported to induce differentiation of mIDH-expressing hematopoietic cells. To examine the effects of TQ05310 on cell differentiation, we measured levels of HBG, a marker of differentiation to erythroid-lineage, in TF-1 AML cells harboring an IDH2 mutation under EPO induction. As shown in Figure 4A, DOX-induced expression of IDH2-R140Q or IDH2-R172K in TF-1 cells decreased HBG levels, indicating blockage...
of cell differentiation by mIDH2. Treatment with TQ05310 caused a concentration-dependent increase in HBG levels in both TF-1/IDH2-R140Q and TF-1/IDH2-R172K cells, indicating induction of cell differentiation by TQ05310. Unlike TQ05310, AG-221 increased HBG only in TF-1/IDH2-R140Q cells, confirming its selective inhibition of IDH2-R140Q. We then examined the effects of TQ05310 on cell proliferation. As shown in Figure 4B, TQ05310 and AG-221 did not significantly inhibit proliferation in both TF-1/IDH2-R140Q and TF-1/IDH2-R172K cells. These results suggest that TQ05310 mainly induces cell differentiation but does not inhibit cell proliferation in TF-1/IDH2-R140Q and TF-1/IDH2-R172K cells in this experimental condition.

3.4 Structural basis for the inhibition of IDH2-R140Q and IDH2-R172K by TQ05310

To determine whether TQ05310 inhibited mIDH2 by directly binding to mIDH2 protein, we then carried out CETSA, a method for evaluating drug-target interactions. As shown in Figure 5A, TQ05310 exerted strong thermal-stabilizing effects on both IDH2-R140Q and IDH2-R172K, indicating binding of TQ05310 to both proteins; AG-221 had an apparent thermal-stabilization effect on IDH2-R140Q (weaker than TQ05310) and a weak thermal-stabilization effect on IDH2-R172K, indicating preferential binding of AG-221 to IDH2-R140Q. Neither TQ05310 nor AG-221 stabilized wild-type IDH2.

Next, we further explored the binding sites of TQ05310 by structural modeling using the IDH2-R140Q structure from a crystal of the IDH2-R140Q/AG-221 complex as a template. TQ05310 was found to occupy the same active pocket of IDH2-R140Q as AG-221 (Figure 5B). Modeling showed that nitrogen on the diaminotriazine core accepts hydrogen bonds from the amino side chain of the Q316 residue, and linker nitrogens donate hydrogen bonds to the Q316 carbonyl side chain (Figure 5B). Thus, Q316 is the critical residue that mediates binding of TQ05310 with IDH2-R140Q. Because there is no reported crystal structure of IDH2-R172K in complex with inhibitor, we did not conduct structural modeling of TQ05310 using this mutant.
To confirm the critical role of the Q316 residue, we carried out site-directed mutagenesis (predicted with SIFT: https://sift.bii.a-star.edu.sg/) to construct plasmids for IDH2-R140Q-Q316A, IDH2-R140Q-Q316I, IDH2-R172K-Q316A and IDH2-R172K-Q316I, and transfected these plasmids into U-87 MG glioma cells. Exogenous expression of IDH2-R140Q-Q316A or IDH2-R172K-Q316A led to significant increases in cellular levels of 2-HG. In contrast, IDH2-R140Q-Q316I and IDH2-R172K-Q316I had no such effect, indicating that the Q316A mutant, but not the Q316I mutant, retained its activity (Figure 5C). We then carried out CETSA in cells transfected with IDH2-R140Q-Q316A or IDH2-R172K-Q316A led to significant increases in cellular levels of 2-HG. In contrast, IDH2-R140Q-Q316I and IDH2-R172K-Q316I had no such effect, indicating that the Q316A mutant, but not the Q316I mutant, retained its activity (Figure 5C). We then carried out CETSA in cells transfected with IDH2-R140Q-Q316A or IDH2-R172K-Q316A. As shown in Figure 5D, the thermal-stabilizing effects of both TQ05310 and AG-221 were lost in U-87 MG/IDH2-R140Q-Q316A cells, confirming the critical role of the Q316 residue in binding of these two inhibitors with IDH2-R140Q. However, TQ05310 retained its thermal-stabilizing effects in U-87 MG/IDH2-R172K-Q316A cells, suggesting that the Q316 residue is not the only critical residue in the binding of TQ05310 with IDH2-R172K.

3.5 Pharmacodynamic and pharmacokinetic characterization of TQ05310

Given the superior activity of TQ05310 against mIDH2 in vitro, we next analyzed its pharmacodynamic and pharmacokinetic profiles in the U-87 MG/IDH2-140Q xenograft model. As shown in Figure 6A, after giving a single oral dose of TQ05310 (18.05 or 54.14 mg/kg), the \( C_{\text{max}} \) of TQ05310 in tumors was 10138 and 16700 ng/g, respectively, concentrations 4.4- and 3.5-fold higher than those in plasma (2279 and 4725 ng/mL). These tumor \( C_{\text{max}} \) values were 130.0- and 214.1-fold higher than in vitro \( IC_{50} \) values (136.9 nmol/L or 78.0 ng/mL) for inhibition of the IDH2-R140Q
enzyme in U-87 MG/IDH2-R140Q cells. Moreover, the total exposure (area under the curve [AUC] 0-24 h) of tumors to TQ05310 at a dose rate of 191.082 and 281.938 h ng/mL was 7.6- and 4.8-fold higher, respectively, than that in plasma (251.125 and 589.946 h ng/mL), and the corresponding half-lives in tumors (19.84 and 19.27 hours) were 2.7- and 2.1-fold longer than those in plasma (7.43 and 9.40 hours).

Finally, we evaluated inhibition of 2-HG production by TQ05310 in the U-87 MG/IDH2-140Q xenograft model. TQ05310 at doses of 18.05 and 54.14 mg/kg reduced 2-HG to low levels (47.2 and 34.0 ng/mg, respectively) in tumors within 0.5 hours, and sustained this status for at least 24 hours (Figure 6B).

Collectively, these results suggest that TQ05310 has favorable pharmacokinetic characteristics and can inhibit 2-HG production in vivo.

4 | DISCUSSION

Mutant IDH2 enzymes, which lead to various metabolic alterations, are extensively expressed in AML and glioma patients. However, despite their shared 2-HG production, IDH2 mutations are not alike and differ in tumorigenic properties. AG-221, an mIDH2 inhibitor, has been approved for the treatment of patients with relapsed or refractory AML harboring an IDH2 mutation. However, despite its strong inhibition of IDH2-R140Q, AG-221 has very weak inhibitory activity against IDH2-R172K. Here, we identified TQ05310 as a novel mIDH2 inhibitor that targets both IDH2-R140Q and IDH2-R172K mutants.

TQ05310 is a novel, potent, and orally available inhibitor of IDH2 that is superior to AG-221, and the most successful IDH2 inhibitor developed to date, in several respects.

First, TQ05310 was significantly more potent than AG-221 at inhibiting IDH2-R172K-mediated processes. The structure of IDH2-R172K is very different to that of IDH2-R140Q. All mIDH2 inhibitors have been designed to target IDH2-R140Q, and show very weak inhibitory effects on IDH2-R172K. Unlike these inhibitors, TQ05310 very strongly inhibited IDH2-R172K, significantly inhibiting enzymatic activity and 2-HG production, and inducing differentiation in cells transfected with IDH2-R172K. CETSA confirmed that TQ05310 directly binds to IDH2-R172K. TQ05310 was superior to AG-221 in all of these aspects, suggesting that TQ05310 would have an enormous advantage in the treatment of patients with IDH2-R172K.

Second, TQ05310 showed potent inhibitory effects against IDH2-R140Q-mediated processes. TQ05310 was even more effective than AG-221 in inhibiting IDH2-R140Q enzymatic activity and 2-HG production, as well as in inducing differentiation of cells transfected with IDH2-R140Q. Taken together, these results provide a basis for the application of TQ05310 in the treatment of patients with IDH2-R140Q.

In addition to its remarkable in vitro characteristics, TQ05310 also had favorable pharmacokinetic and drug-like properties. In the U-87 MG/IDH2-R140Q xenograft model, TQ05310 was rapidly absorbed and showed much higher accumulation in tumors than in plasma. Moreover, TQ05310 rapidly reduced 2-HG to a low level in tumors and sustained it for at least 24 hours post-dosage.

To explore the binding mode of TQ05310, we constructed different models with IDH2-Q316 mutations. We found that the thermal-stabilizing effects of TQ05310 and AG-221 were lost in U-87 MG/IDH2-R140Q-Q316A cells, suggesting the critical role of the Q316 residue in binding these two inhibitors with IDH2-R140Q, and that the Q316 mutation may cause resistance to these inhibitors in patients with IDH2-R140Q. Notably, a study based on clinical observation reported that an AML patient with IDH2-R140Q showed an initial clinical response to AG-221, but developed drug resistance and disease progression after the emergence of the Q316E mutation in IDH2, which confirmed the critical role of the Q316 residue in drug sensitivity. Collectively, these results indicate that monitoring Q316 mutations is important to predict the effectiveness of AG-221 and TQ05310 in patients harboring the IDH2-R140Q mutation.

Taken together, the findings of the present study show that TQ05310, the first mIDH2 inhibitor having strong inhibitory effects on both IDH2-R140Q and IDH2-R172K, has superior activity compared with AG-221 in preclinical models, supporting its further clinical investigation for patients harboring IDH2-R140Q or IDH2-R172K mutations.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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