Accelerated Communication

Distribution of the Phosphatidylinositol 3-Kinase Inhibitors Pictilisib (GDC-0941) and GNE-317 in U87 and GS2 Intracranial Glioblastoma Models—Assessment by Matrix-Assisted Laser Desorption Ionization Imaging

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults, and the limited available treatment options have not meaningfully impacted patient survival in the past decades. Such poor outcomes can be at least partly attributed to the inability of most drugs tested to cross the blood-brain barrier and reach all areas of the glioma. The objectives of these studies were to visualize and compare by matrix-assisted laser desorption ionization (MALDI) imaging mass spectrometry the brain and tumor distribution of the phosphatidylinositol 3-kinase (PI3K) inhibitors pictilisib (GDC-0941, 2-{1H-indazol-4-yl}-6-{4-methanesulfonyl-piperazin-1-ylmethyl}-4-morpholin-4-yl-thieno[3,2-d] pyrimidine) and GNE-317 [5-{6-[3-methoxyoxetan-3-yl]-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-2-yl}pyrimidin-2-amine] in U87 and GS2 orthotopic models of GBM, models that exhibit differing blood-brain barrier characteristics. Following administration to tumor-bearing mice, pictilisib was readily detected within tumors of the contrast-enhancing U87 model whereas it was not located in tumors of the nonenhancing GS2 model. In both GBM models, pictilisib was not detected in the healthy brain. In contrast, GNE-317 was uniformly distributed throughout the brain in the U87 and GS2 models. MALDI imaging revealed also that the pictilisib signal varied regionally by up to 6-fold within the U87 tumors whereas GNE-317 intratumor levels were more homogeneous. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analyses of the nontumored half of the brain showed pictilisib had brain-to-plasma ratios lower than 0.03 whereas they were greater than 1 for GNE-317, in agreement with their brain penetration properties. These results in orthotopic models representing either the contrast-enhancing or invasive areas of GBM clearly demonstrate the need for whole-brain distribution to potentially achieve long-term efficacy in GBM.

Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults. This tumor type, diagnosed in more than 10,000 patients each year (CBTRUS, 2012), is characterized by rapid growth and diffuse invasiveness, and presents very few treatment options. Disease progression is controlled for only a limited time, with median survival less than 2 years after initial diagnosis (Adamson et al., 2009). Despite the emergence and clinical testing of various targeted agents expected to act on pathways deregulated in GBM, few successes have not meaningfully impacted patient survival in the past decades. Such poor outcomes can be at least partly attributed to the inability of most drugs tested to cross the blood-brain barrier and reach all areas of the glioma. The objectives of these studies were to visualize and compare by matrix-assisted laser desorption ionization (MALDI) imaging mass spectrometry the brain and tumor distribution of the phosphatidylinositol 3-kinase (PI3K) inhibitors pictilisib (GDC-0941, 2-{1H-indazol-4-yl}-6-{4-methanesulfonyl-piperazin-1-ylmethyl}-4-morpholin-4-yl-thieno[3,2-d] pyrimidine) and GNE-317 [5-{6-[3-methoxyoxetan-3-yl]-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-2-yl}pyrimidin-2-amine] in U87 and GS2 orthotopic models of GBM, models that exhibit differing blood-brain barrier characteristics. Following administration to tumor-bearing mice, pictilisib was readily detected within tumors of the contrast-enhancing U87 model whereas it was not located in tumors of the nonenhancing GS2 model. In both GBM models, pictilisib was not detected in the healthy brain. In contrast, GNE-317 was uniformly distributed throughout the brain in the U87 and GS2 models. MALDI imaging revealed also that the pictilisib signal varied regionally by up to 6-fold within the U87 tumors whereas GNE-317 intratumor levels were more homogeneous. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analyses of the nontumored half of the brain showed pictilisib had brain-to-plasma ratios lower than 0.03 whereas they were greater than 1 for GNE-317, in agreement with their brain penetration properties. These results in orthotopic models representing either the contrast-enhancing or invasive areas of GBM clearly demonstrate the need for whole-brain distribution to potentially achieve long-term efficacy in GBM.

ABBRVIATIONS: BBB, blood-brain barrier; BCRP, breast cancer resistance protein; DCE-MRI, dynamic contrast enhanced magnetic resonance imaging; EGFR, epidermal growth factor receptor; GBM, glioblastoma multiforme; GDC-0941, pictilisib, 2-{1H-indazol-4-yl}-6-{4-methanesulfonyl-piperazin-1-ylmethyl}-4-morpholin-4-yl-thieno[3,2-d]pyrimidine; GNE-317, 5-{6-[3-methoxyoxetan-3-yl]-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-2-yl}pyrimidin-2-amine; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; MALDI, matrix-assisted laser desorption ionization; MRI, magnetic resonance imaging; MS, mass spectrometry; NEX, number of excitations; PI3K, phosphatidylinositol 3-kinase; Pgp, P-glycoprotein; ROIs, regions of interest.
GBM (Network TCGAR, 2008), leading to persistent activation of the pathway. Epidermal growth factor receptor (EGFR) amplification and/or mutation, mutation of the PI3K catalytic and regulatory units, and loss of PTEN (phosphatase and tensin homolog) protein are observed in 45%, 10%, and 50% of GBM cases, respectively (Akhavan et al., 2010). Thus, inhibition of PI3K signaling represents an attractive therapeutic approach against GBM. However, most agents tested that target this pathway, such as erlotinib (EGFR), gefitinib (EGFR), lapatinib (EGFR), pictilisib (GDC-0941 [2-(1H-indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin-4-yl-thieno[3,2-d]pyrimidine]; PI3K), or everolimus (mTor), have been shown to be substrates of Pgp and/or BCRP (Chu et al., 2009; Polli et al., 2009; Agarwal et al., 2010; Salpatic et al., 2010; de Vries et al., 2012), limiting their brain penetration and ability to reach areas beyond the tumor core. Previous studies showed efficacy of the PI3K/mTor inhibitor GNE-317 [5-(6-(3-methoxyoxetan-3-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-2-yl)pyrimidin-2-amine] in an intracranial GBM tumor model presenting an intact BBB, whereas pictilisib displayed activity only in the U87 model, which presents a compromised BBB (Salpatic et al., 2012). However, although differences in brain and tumor penetration were likely responsible for the contrasts in efficacy, compound tumor distribution or concentrations were not evidenced.

The goals of our studies were to visualize the spatial distribution of the PI3K inhibitors pictilisib and GNE-317 in the U87 and GS2 orthotopic models and surrounding brain by matrix-assisted laser desorption ionization (MALDI) imaging and to show the impact of different BBB statuses, assessed by magnetic resonance imaging (MRI), on the tumor penetration of these two compounds.

Materials and Methods

Chemicals

Pictilisib (Fig. 1A) and GNE-317 (Fig. 1B) were synthesized by Genentech, Inc. (South San Francisco, CA). All solvents used in analytical assays were from Thermo Fisher Scientific (Waltham, MA) and of analytical or high-performance liquid chromatography grade. All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

Tumor Models and MRI

All studies conducted were approved by the Institutional Animal Care and Use Committee at Genentech, Inc.

Two human glioma models were used in the distribution studies: U-87.MG (U87) glioblastoma cancer cells, derived from the U-87 MG cells (American Type Culture Collection, Manassas, VA), and the GS2 glioblastoma cells (Gunther et al., 2008). Six female nude mice (Charles River Laboratories, Hollister, CA) were implanted with each of the glioma cell types. The U87 (250 K) and GS2 (100 K) tumor cells were injected via stereotactic surgery into the right striatum at a volume of 3–5 μl. Nineteen to 21 days after implantation, one animal in each tumor group was selected for MRI to confirm the growth of the tumor and assess the BBB integrity. MRI was performed on a Varian 9.4T MRI system (Varian, Palo Alto, CA) with a 30-mm quadrature volume coil. During the imaging, animals were kept under anesthesia with 2% isoflurane in air. Body temperature was continuously monitored using a rectal probe and was maintained at 37°C by a heated-airflow system regulated by in-house LabVIEW controller software (National Instruments, Austin, TX).

A T2-weighted fast spin echo, multislice (FSEMS) sequence was used to detect lesions by MRI. We acquired 12 axial 0.5 mm-thick slices with a 20 × 20 mm field of view (FOV), and 128 × 128 matrix, zero-filled to 256 × 256 images; TR (repetition time) = 3500 milliseconds, TE (echo time) = 10 milliseconds, ETL (echo train length) = 8, k-zero = 4, NEX (number of excitations) = 8. The BBB integrity was evaluated by dynamic contrast enhanced MRI (DCE-MRI). Precontrast three-dimensional gradient echo (3DGE) datasets were acquired at 2° and 10° flip angles, TR (repetition time) = 8.3 milliseconds, TE (echo time) = 1.1 milliseconds, NEX = 4, FOV (field of view) = 20 × 20 × 8 mm, matrix = 64 × 64 × 16. A 50-μl bolus injection of Gd-based Gadodiamide (Omniscan; GE Healthcare, Piscataway, NJ) contrast agent was injected via a tail vein catheter after collection of the precontrast images. Postcontrast three-dimensional gradient echo (3DGE) images were then acquired approximately every 10 seconds for 30 minutes (10° flip angle, NEX = 1).

MALDI Imaging

Tissue Preparation. After the MRI experiments, a single oral dose of either pictilisib or GNE-317 was administered at 150 mg/kg and 50 mg/kg, respectively. Treatments were administered at a time that ensured that the BBB had recovered from the surgery and tumors were expanding. The formulation for both compounds was 0.5% methylcellulose/0.2% Tween 80 (MCT). Mice were euthanized at 1 hour after the dose via exsanguination by perfusion under anesthesia. Brains were excised, flash frozen in liquid N2, and stored in a −80°C freezer until analyzed. Fresh-frozen tissue sections were obtained on a cryomicrotome (CM3050S; Leica, Buffalo Grove, IL) at 12-μm thickness and thaw-mounted onto indium tin oxide–coated glass slides (Bruker Daltonics, Billerica, MA). Tissue sections were analyzed by imaging MALDI MS, providing signal intensities (and not absolute quantitation), followed by cresyl violet staining for histologic interrogation.

Imaging MALDI Magnetic Spectrometry. A 40 mg/ml solution of 2,5-dihydroxybenzoic acid (Sigma-Aldrich) was prepared in methanol-water (70:30

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time</th>
<th>Plasma Brain</th>
<th>Brain-to-Plasma Ratio</th>
<th>Free Brain-to-Free Plasma Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>h (24 h)</td>
<td>μM</td>
<td></td>
</tr>
<tr>
<td>GNE-317</td>
<td>1</td>
<td>2.01 ± 0.48</td>
<td>2.50 ± 0.24</td>
<td>1.32 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.86 ± 0.93</td>
<td>2.02 ± 1.13</td>
<td>1.10 ± 0.21</td>
</tr>
<tr>
<td>Pictilisib</td>
<td>1</td>
<td>7.77 ± 0.26</td>
<td>0.16 ± 0.11</td>
<td>0.021 ± 0.014</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.26 ± 0.33</td>
<td>0.0669 ± 0.0069</td>
<td>0.0127 ± 0.0011</td>
</tr>
</tbody>
</table>

Results reported as mean ± S.D. (n = 3).
v/v). A stable-labeled internal standard, [D8]GDC-0941, was spiked into the MALDI matrix solution at 2 \( \mu \text{M} \) before deposition onto the tissue sections. Matrix solution was homogenously spray-coated onto the tissue using a HTX TM-Sprayer (HTX Technologies, Chapel Hill, NC). Matrix-coated tissue sections were transferred to the MALDI mass spectrometer (MS) (SolariX 7T FT-ICR, Bruker Daltonics, Bremen, Germany) for imaging analysis. Imaging data were collected at 100 \( \mu \text{m} \) pixel resolution in positive ionization mode, under continuous accumulation of selected ions (CASI) windows optimized for each analyte (50–125 Da). Laser intensity and the number of shots were optimized for sensitivity of each analyte (500–700 shots) with ion detection collected over the mass range of \( m/z \) 150–900. Drug images were generated based on accurate mass of each compound (pictilisib \( m/z \) 514.1690; GNE-317 \( m/z \) 415.1547) using FlexImaging v4.0 64-bit (Bruker Daltonics, Billerica, MA) with a mass tolerance of \( \pm 4 \) mDa and normalized to internal standard response.

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time (h)</th>
<th>Plasma ( \mu \text{M} )</th>
<th>Normal Brain Brain ( \mu \text{M} )</th>
<th>Brain-to-Plasma Ratio</th>
<th>Free Brain-to-Free Plasma Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNE-317</td>
<td>1</td>
<td>2.45 ± 0.45</td>
<td>3.79 ± 1.09</td>
<td>1.53 ± 0.21</td>
<td>0.555 ± 0.076</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.61 ± 0.35</td>
<td>1.87 ± 0.38</td>
<td>1.17 ± 0.02</td>
<td>0.422 ± 0.007</td>
</tr>
<tr>
<td>Pictilisib</td>
<td>1</td>
<td>5.51 ± 1.21</td>
<td>0.150 ± 0.057</td>
<td>0.0271 ± 0.0089</td>
<td>0.00752 ± 0.00245</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.25 ± 0.73</td>
<td>0.178 ± 0.061</td>
<td>0.0247 ± 0.0082</td>
<td>0.00684 ± 0.00229</td>
</tr>
</tbody>
</table>

**Fig. 2.** Brain distribution of pictilisib and GNE-317 in the U87 orthotopic model of GBM. Distribution of pictilisib (A) and GNE-317 (B) in U87 tumors after oral administration of 150 mg/kg and 50 mg/kg, respectively. Localization of the tumors by cresyl violet staining and drug distribution in MALDI MS images are presented. For animals 1 and 4, T2-weighted images of U87 intracranial tumors are displayed to provide the location of the tumor regions in MR images. Dynamic contrast-enhanced MR imaging (DCE-MRI; 5 minutes after Gd injection) showing intense contrast in the tumor confirm the disruption of the BBB in this model.
Threshold intensities were set according to average internal standard response across the two imaging experiments, which allowed for the direct comparison of the images obtained with the two compounds.

**Histology.** Upon completion of the imaging experiments, the matrix coating was removed by rinsing the glass slide in 100% methanol for 30 seconds or until the entire matrix was visibly removed. Tissue sections were stained using a freshly prepared 0.5% cresyl violet staining solution (Chaurand et al., 2004) by submerging the glass slide for 30 seconds, then they were rinsed for an additional 30 seconds in two cycles of 100% ethanol. Microscope images were obtained on an Olympus BX51 (Olympus, Tokyo, Japan) at 10× magnification and stitched using MicroSuite Analytical v3.0 software (Olympus). Subsequently, stained images were coregistered to the optical images in FlexImaging for visualization and annotation of tumor and nontumor regions for the drug images.

**Intratumor Distribution.** To assess intratumor distribution, imaging MALDI MS data from the U87 and GS2 tumor models were processed in FlexImaging v4.0 64 bit (Bruker Daltonics) across the mass range of m/z 400–525. The cresyl violet stained microscope images were coregistered to the extracted ion images corresponding to the exact mass of each drug, with a mass tolerance of ±4 mDa. Image threshold values were independently adjusted to obtain the best visualization of dynamic range for each drug signal as it was detected across the brain tissue. Regions of interest (ROIs) were selected based on the MALDI MS ion image to assess the variation of drug distribution within the tumor tissue. Average drug signal from ROIs representing areas of the highest and lowest drug intensities within the tumor tissue were compared with the average drug signal from an arbitrarily selected nontumor region (NT) of the brain tissue.

**Quantitation of Drugs in Brain and Plasma**

At time of brain harvesting for MALDI imaging analysis, blood samples (0.2 ml) were also collected via cardiac puncture from each mouse and deposited into tubes containing dipotassium ethylenediaminetetraacetic acid (K2 EDTA) as an anticoagulant. After mixing the blood with K2 EDTA, we stored the samples on ice; within 1 hour of collection, they were centrifuged for 5 minutes at 2000g and 2–8°C. Plasma was collected and stored at −80°C until analysis.

Total concentrations of pictilisib and GNE-317 in both plasma and brain tissue (from nontumor frontal cortex) were determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Brains were processed and analyzed by LC-MS/MS after completion of the MALDI analyses. Mobile phases were prepared in 0.1% formic acid in water (v/v; mobile phase A) and 0.1% formic acid in acetonitrile (v/v; mobile phase B). Weights from isolated brain tissue were recorded before homogenization in 4 volumes of water. After a protein precipitation with 100% acetonitrile, supernatants from brain and plasma extractions were injected onto a Phenomenex (Torrance, CA)

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**A Pictilisib**

- **Animal 7**
- **Animal 8**
- **Animal 9**

**B GNE-317**

- **Animal 10**
- **Animal 11**
- **Animal 12**

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**Fig. 3.** Brain distribution of pictilisib and GNE-317 in the GS2 orthotopic model of GBM. Distribution of pictilisib (A) and GNE-317 (B) in GS2 tumors after oral administration of 150 mg/kg and 50 mg/kg, respectively. Localization of the tumors by cresyl violet staining and drug distribution in MALDI MS images are presented. For animals 7 and 10, T2-weighted images of GS2 intracranial tumors are displayed to provide the location of the tumor regions in MR images. DCE-MRI (5 minutes after Gd injection) showing no contrast enhancement indicate that the BBB is intact in this model.
Kinetex Phenyl-Hexyl column (30 × 2.1 mm, 2.6 μm particle size) using a Nexera SIL 30ACMP autosampler (Shimadzu Corp., Kyoto, Japan) coupled to a 1290 Infinity pump (Agilent Technologies, Santa Clara, CA) running a 48-second gradient of 3% to 97% mobile phase B at 1.2 ml/min.

Analysis was performed on an ABSciex QTrap 5500 mass spectrometer (Applied Biosystems, Foster City, CA) in positive ion mode monitoring the transitions m/z 514.1→338.1 and m/z 415.1→385.1 for pictilisib and GNE-317, respectively. Plasma and brain concentrations were determined against a 1/x weighted quadratic fit curve range of 0.005 μM to 40 μM. Free brain concentrations of pictilisib and GNE-317 were determined as previously described (Salphati et al., 2010).

**Results and Discussion**

Pictilisib, a PI3K inhibitor currently being evaluated in phase II clinical trials, is a substrate for the efflux transporters Pgp and BCRP, which limit its brain penetration (Salphati et al., 2010). In contrast, GNE-317, a PI3K/mTor inhibitor, is able to cross the BBB (Heffron et al., 2012). In our present study, we evaluated by MALDI imaging the distribution of these two compounds in brains of mice bearing intracranial tumors. Two GBM models were selected: U87 and GS2 glioma cells. The U87 cell line is a well-characterized and widely used glioma model (Weller et al., 1998; Clark et al., 2010). These cells grow adherently in tissue culture and, when implanted as xenografts, develop well-delineated tumors with impaired BBB. In contrast, the GS2 cells grow in vitro as neurospheres and recapitulate the diffusive infiltration and mostly intact BBB of the invading regions of human GBM (Salphati et al., 2012).

Pictilisib and GNE-317 were administered as a single oral dose, at 150 and 50 mg/kg, respectively, to mice bearing either tumor. Doses were selected based on previous pharmacokinetic and efficacy studies. Plasma concentrations of the two compounds measured 1 and 6 hours after the dose (Tables 1 and 2) were consistent with our previous studies (Salphati et al., 2010; Salphati et al., 2012). Brain levels obtained from the nontumored hemisphere after tissue homogenization confirmed the brain penetration potential of each compound, with total brain-to-plasma ratios lower than 0.03 for pictilisib and greater than 1 for GNE-317 (Tables 1 and 2). These ratios did not markedly differ between 1 and 6 hours, indicating that the distribution was not time dependent and suggesting that analysis of intratumor distribution 1 hour after the dose would be representative of later time points.

Although they are informative, these data do not provide insight into the spatial distribution of the compounds within the brain or tumor, specifically. Further understanding of brain drug delivery was obtained with MALDI imaging, which clearly showed the distribution of the compounds within the brain and/or the tumors. In the U87 model, pictilisib had levels close to the limit of detection in most of the brain whereas it was more readily detected specifically in the tumor (especially in animal 2; Fig. 2A and Fig. 4A), as annotated by cresyl violet staining and MRI (Fig. 2A).

The highly localized distribution of pictilisib in the U87 tumor, with a signal approximately 13-fold higher than in the healthy part of the brain (Fig. 4A), can be explained by the impairment of the BBB in this GBM model, highlighted by the marked contrast enhancement after gadolinium injection (Fig. 2A, animal 1; Fig. 2B, animal 4), which allows even poorly brain-penetrant compounds such as pictilisib to leave the blood capillaries and distribute in the tumor. In addition, differences in the signal among the three animals bearing the U87 tumors (Fig. 2A) underscore the variability in exposure in the same...
tumor model. It is worth also noting that differences in intensity, corresponding to up to 6-fold changes in pictilisib signal within the U87 tumor, can be observed (Fig. 4A). This range of intratumor signal is most likely reflective of the variable degrees of BBB disruption within the tumor, leading to heterogeneous distribution.

Such variability in concentrations with efflux transporter substrates can also be expected between and within patients, depending on the degree and localization of the BBB disruption. Thus, optimal compound concentrations may not be achieved throughout the tumor, which is likely to limit efficacy. This finding is consistent with the variability observed with radiolabeled paclitaxel, doxorubicin (Lockman et al., 2010), and lapatinib (Taskar et al., 2012) in a model of brain metastasis of breast cancer, with up to a 10-fold range in concentrations between and within the tumor metastases. The U87 model, with its compromised BBB, represents what may occur in the contrast-enhancing region(s) of a human brain tumor. Indeed, specific localization of erlotinib in the contrast-enhancing area of a brain metastasis in a patient with non-small cell lung cancer was demonstrated by Weber et al. (2011). Similarly, high concentrations of compounds, even those known to be substrates of efflux transporters, have been reported in resected human brain tumors (primary or metastases), with lower levels in surrounding tissues (Fine et al., 2006; Pitz et al., 2011).

The lack of meaning long-term clinical benefit even in cases when a high compound concentration was measured in the tumor can be explained, at least in part, by the absence of compound distribution beyond the regions with disrupted BBB. In preclinical models, lower concentrations of erlotinib (Agarwal et al., 2013) have been measured in the tissues surrounding and in areas distant from the core of the glioma. These findings relied on dissecting the tumors and adjacent tissues from the rest of the brain, which did not allow the assessment of intratumor heterogeneity or whole-brain distribution.

In brains implanted with the GS2 tumors, which present an intact BBB, as shown by the absence of contrast-enhancement (Fig. 3), post-contrast, pictilisib was not detected in any area (Fig. 3A), in agreement with the poor brain penetration of this agent. The brain distribution of GNE-317 was strikingly different from that of pictilisib. Regardless of the tumor model, and the BBB status (as assessed by gadolinium enhancement), GNE-317 was uniformly distributed throughout the brain and tumors (Fig. 2B, and Fig. 3B). Distribution appeared consistent between mice (Fig. 2B, and Fig. 3B) and more homogenous than that of pictilisib in the U87 tumor (Fig. 4B), with similar average signal in the tumor and the normal brain regions selected (Fig. 4B), and a 2.7-fold range in signal among the regions assessed within the tumor (Fig. 4B). In the GS2 model, the GNE-317 average tumor signal appeared 2.5-fold lower than in the nontumor region selected whereas the intensity varied by about 4-fold within the tumor (Fig. 4C). The weaker signal in the GS2 tumor versus the healthy brain as well as the higher variability in distribution within the tumor compared with the U87 tumor may be explained by differences in tumor vascularization, which could impact GNE-317 distribution. Images suggest also different compound levels in white versus gray matter as previously reported in resected human brain tumors (primary or metastases), with lower concentrations in the invasive areas in addition to the core of the tumor.

In summary, our studies provide visualization of the difference in distribution for brain-penetrant or nonpenetrant compounds in two intracranial GBM models that represent different regions and features of human GBM. Our findings also support what had been hypothesized regarding the regional drug delivery in glioma (Agarwal et al., 2011) and confirm that meaningful clinical benefit, and reliable assessment of pharmacodynamics (biological) hypotheses, will most likely be achieved in GBM (or brain metastases) only with compounds able to distribute throughout the whole brain.

Authorship Contributions
Participated in research design: Salphati, Shahidi-Latham, Quaisson, Nishimura, Aliche, Pang, Olivero, Phillips.
Conducted experiments: Barck, Nishimura, Aliche.
Contributed new reagents or analytic tools: Shahidi-Latham, Quaisson, Barck, Carano.
Performed data analysis: Salphati, Shahidi-Latham, Quaisson, Barck, Pang, Carano, Phillips.
Wrote or contributed to the writing of the manuscript: Salphati, Shahidi-Latham, Barck, Nishimura, Phillips.

References


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