Preclinical antitumor activity of S-222611, an oral reversible tyrosine kinase inhibitor of epidermal growth factor receptor and human epidermal growth factor receptor 2

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Epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) are validated molecular targets in cancer therapy. Dual blockade has been explored and one such agent, lapatinib, is in clinical practice but with modest activity. Through chemical screening, we discovered a novel EGFR and HER2 inhibitor, S-222611, that selectively inhibited both kinases with IC₅₀s below 10 nmol/L. S-222611 also inhibited intracellular kinase activity and the growth of EGFR-expressing and HER2-expressing cancer cells. In addition, S-222611 showed potent antitumor activity over lapatinib in a variety of xenograft models. In evaluations with two patient-oriented models, the intramembrane implantation model and the intracranial implantation model, S-222611 exhibited excellent activity and could be effective against bone and brain metastasis. Compared to neratinib and afatinib, irreversible EGFR/HER2 inhibitors, S-222611 showed equivalent or slightly weaker antitumor activity but a safer profile. These results indicated that S-222611 is a potent EGFR and HER2 inhibitor with substantially better antitumor activity than lapatinib at clinically relevant doses. Considering the safer profile than for irreversible inhibitors, S-222611 could be an important option in future cancer therapy.
Materials and Methods

Cells and reagents. The human cell lines used in this study were as follows: NCI-N87 (gastric cancer), BT-474, SK-BR-3, MDA-MB-453, MDA-MB-175VI, MDA-MB-361 (breast cancer), HT115 (colon cancer), Calu-3 (non-small cell lung cancer), A-431 (epidermoid carcinoma), fR2 (SV40-transformed breast epithelial cell) and MRC-5 (normal lung fibroblast). All these cells were purchased from ATCC (Manassas, VA, USA) and the European Collection of Cell Cultures (Salisbury, Wiltshire, UK). The cells were maintained in DMEM supplemented with 10–20% FBS, 100 units/mL penicillin and 100 units/mL streptomycin.

Animals. Nude mice (BALB/cAJcl-nu/nu, CLEA Japan, Tokyo, Japan) were used for most of the in vivo studies, except SCID mice (C.B-17/Kscid/scidIcl, CLEA Japan) for the HT115 model and the intrafemoral implantation model of BT-474-luc (luciferase-expressing BT-474), SCID Beige mice (CB17.Cg-PrkdcscidLys-/-/Crl; Charles River Laboratories Japan) for the BT-474 model, and hairless SCID mice (SHO-PrkdcscidHhr, Charles River Laboratories Japan, Yokohama, Kanagawa, Japan) for the intracranial implantation model of BT-474-luc. All mice were female and 6–9 weeks old. All the mice used in the experiments were killed immediately after the last measurement except the survival study. All animal studies were conducted under the approval of the Institutional Animal Care and Use Committees of Shionogi Research Laboratories.

Reagents. S-222611, lapatinib, neratinib and afatinib were chemically synthesized in Shionogi Research Laboratories (Toyonyaka, Osaka, Japan). Lapatinib, neratinib and afatinib were prepared according to the preceding references (11,21–24) Their structures were identified by comparison of their 1H NMR and mass spectrum with the reported values. In addition, their purities were confirmed by liquid chromatography mass spectrometry to be over 95%.

In in vitro experiments, these agents were dissolved with DMSO (Nacalai Tesque, Kyoto, Kyoto, Japan) and the solutions were further diluted with an assay buffer or a culture medium. In vivo experiments, these agents were suspended in 0.5% w/v/% methylcellulose 400P solution (Wako Pure Chemical Industries, Osaka, Osaka, Japan) to make dosing formulations.

In vitro kinase assay. Enzyme activities of EGFR, HER2, HER4, IGF1R, KDR, KIT, PDGFRβ and SRC were evaluated using the QSS Assist ELISA kit (Carna Bioscience, Kobe, Hyogo, Japan) following the manufacturer’s protocol. The relative inhibition rate of each data point was calculated and used to obtain the IC50 value for each drug. Each experiment was carried out thrice, each time in triplicate.

Evaluation of phosphorylation of epidermal growth factor receptor and human epidermal growth factor receptor 2. Human gastric cancer cells, NCI-N87, were treated with serially diluted drug for 24 h. Total and phosphorylated EGFR and HER2 were quantitated using Human Total-EGFR, Total-ErbB2, Phospho-EGFR and Phospho-ErbB2 DuoSet IC ELISA kits (R&D Systems (Minneapolis, MN, USA)) following the manufacturer’s protocol. First, the phosphorylation ratio (phosphorylated protein/total protein) of each of triplicate sample was calculated. Subsequently, the relative phosphorylation (mean phosphorylation ratio of treated sample/mean phosphorylation ratio of control sample) for each data point was calculated and used to obtain the IC50 value.

Growth inhibition assay. Cells were seeded at 3000 cells/well in 96-well plates and incubated overnight. Serially diluted drug was added to the well and the plates were incubated for 72 h. After chromogenic reaction with WST-8 (Kishida Chemicals, Osaka, Osaka, Japan), the OD50 (with reference of OD100) was measured using an Emax microplate reader (Molecular Devices, Sunnyvale, CA, USA) and used to obtain the IC50 value. Each experiment was carried out thrice, each time in triplicate.

The following two studies were performed with NCI-N87 cells. In the study with human serum protein, 2% serum albumin (Sigma-Aldrich, St. Louis, MO, USA) or 0.08% zl-acid glycoprotein (Sigma-Aldrich) was added to the culture containing the drug. In the study with short-time pulse treatment, 6000 cells/well were seeded in 96-well crystal glass plates. At 1, 6 or 24 h after addition of serially diluted drug to the well, the culture medium was removed and each well was washed three times with DMEM with 1% FBS. The plate was then reincubated for a total of 72 h.

Evaluation of epidermal growth factor receptor and human epidermal growth factor receptor 2 expression in cell lines. Two days after cell seeding at the density of 6.0 × 105 cells/100 mm cell culture dish, the cells were lysed with lysis buffer and the amounts of EGFR and HER2 in the lysate were quantitated using Human Total-EGFR and Total-ErbB2, DuoSet IC ELISA kits (R&D Systems). The protein content of the lysates was determined using a DC protein assay kit (Bio-Rad, Hercules, CA, USA).

In vivo antitumor assay. In in vivo studies, 4 × 106 to 3 × 107 cells were implanted subcutaneously into the back of mice; however, the two breast cancer cell lines, BT-474 and MDA-MB-361, were implanted orthotopically into the mammary fat pad of mice. The cell suspensions for implantation of these two cell lines and HT115 cells included 50 v/v% Matrigel (Beckton Dickinson, Franklin Lakes, NJ, USA). After randomization, vehicle or multiple doses of S-222611 or lapatinib were administered by oral gavage daily for 10–28 days. The length and width of tumors were measured using an electronic caliper twice or thrice weekly and the tumor volume was calculated using the following formula: (length × width2)/2.

In vivo evaluation of phosphorylation of epidermal growth factor receptor and human epidermal growth factor receptor 2. NCI-N87 cells were implanted subcutaneously into the back of mice as in the in vivo antitumor assay. Tumor xenografts were excised at 6 or 24 h after administration and homogenized in the lysis buffer. The composition of the lysis buffer and the methods hereafter are the same as those of the in vitro study.

In vivo patient-oriented models. Luciferase-expressing BT-474 (BT-474-luc) cells were established by transfection of firefly luciferase expression vector into BT-474 cells and subsequent cloning. In the intrafemoral implantation model, the cell suspension containing 1.05 × 107 BT-474-luc cells and 50 v/v% Matrigel (Beckton Dickinson) were surgically implanted into the left femur of mice. In the intracranial implantation model, the cell suspension containing 2 × 106 BT-474-luc cells was surgically implanted into the brain parenchyma of mice. For measurement of bioluminescence, 0.2 mL of 10 mg/mL luciferin solution was injected intravenously into each mouse via tail vein under anesthesia. Immediately after that, the photon emitted from tumors was measured using the IVIS Imaging System 200 (Caliper Life Sciences, Hopkinton, MA, USA).

In the survival model, a cell suspension containing 2.5 × 106 BT-474 cells was surgically implanted into the brain parenchyma of mice. Mice were observed for any health defects once a day and the body weights were measured at least twice weekly. Mice in a moribund state or reaching any...
humane endpoint were immediately killed. In addition, all the surviving mice were killed at the end of study (106 days after implantation).

**Histopathological evaluation.** The colon and the eyeball were excised from the mice treated with S-222611, neratinib or afatinib and fixed in 10% neutral buffered formalin. After routine processing of these fixed organs, the paraffin sections were stained with H&E and examined microscopically.

**Results**

**In vitro inhibition of kinase activity by S-222611.** In search of a powerful EGFR/HER2 kinase inhibitor, we screened thousands of compounds and found S-222611. S-222611 potently inhibited EGFR, HER2 and HER4. In search of a selective kinase inhibitor, S-222611 was further confirmed in profiling with 107 kinases (Table S1). These results showed that S-222611, like lapatinib, was a selective inhibitor for EGFR, HER2 and HER4.25

Next we studied the effect of S-222611 on autophosphorylation of EGFR and HER2 in cells in order to examine whether S-222611 could be incorporated into cells and could inhibit EGFR and HER2 in intact cells. S-222611 decreased the relative phosphorylation of EGFR and HER2 in NCI-N87 cells with IC50 values below 10 nmol/L (Table 1b). These inhibitory activities of S-222611 on intracellular kinases were 2.4–3.4 times stronger than those of lapatinib.

Cancer cell growth inhibition by S-222611. Next, we evaluated the growth inhibitory activity of S-222611 for several cancer cell lines expressing EGFR and/or HER2 (Table 1c). The IC50 values for all the cancer cell lines except Calu-3 were below 1000 nmol/L, while those for SV40-transformed human breast epithelial cells, FR2, and normal human lung fibroblasts, MRC-5, were approximately 5000 nmol/L. These antiproliferative activities of S-222611 for cancer cell lines were 1.9–4.4 times higher than those of lapatinib. Thus, S-222611 can selectively inhibit the proliferation of a range of cancer cell lines expressing EGFR and/or HER2.

**Growth inhibition by S-222611 in the presence of serum protein.** The activity of a drug is commonly attenuated by the binding of serum proteins. S-222611 is highly bound (>99%) to serum albumin and α1-acid glycoprotein, as well as lapatinib. Therefore, we evaluated the growth inhibitory activity of both agents in the presence of additional serum protein. Addition of serum proteins elevated the IC50 values of both S-222611 and lapatinib (2.9–4.4-fold and 10.6–12.7-fold, respectively), but the degree of attenuation was much smaller with S-222611 (Table 1d). This refractoriness of S-222611 to serum protein binding helps explain why S-222611 can exert advantageous activity over lapatinib in a cell-based test system which intrinsically contains serum protein and, thereby, can lead to potent antitumor activity in vivo.

**In vivo antitumor activity of S-222611.** The in vivo antitumor activity of S-222611 was evaluated. Nude mice bearing NCI-N87 xenograft were treated orally with S-222611 or lapatinib. The changes of tumor volume over time are shown in Figure 1(a) (Fig. S1). S-222611 significantly inhibited the growth of both tumor models, while lapatinib failed to show antitumor activity.

Table 1. In vitro pharmacological activity of S-222611 and lapatinib

| IC50 (nmol/L) | S-222611 | Lapatinib |
|--------------|----------|-----------|
| (a) **In vitro inhibition** | | |
| EGFR | 1.48 ± 0.06 | 1.52 ± 0.07 ns |
| HER2 | 7.15 ± 0.51 | 8.74 ± 0.24 ** |
| HER4 | 2.49 ± 0.10 | 4.62 ± 1.35 ns |
| KDR, IGF1R, SRC, KIT, PDGFRβ | >10 000 | >10 000 |
| (b) **Inhibition of relative phosphorylation of receptor kinases in NCI-N87 cells** | | |
| Phosphorylation of EGFR | 4.5 | 11.0 |
| Phosphorylation of HER2 | 1.6 | 5.4 |
| (c) **Growth inhibition of cultured cells** | | |
| EGFR | HER2 |
| NCI-N87 (stomach) | ++ | +++ |
| BT-474 (breast) | + | +++ |
| SK-BR-3 (breast) | ++ | +++ |
| MDA-MB-453 (breast) | – | ++ |
| MDA-MB-175VII (breast) | + | ++ |
| HT115 (colon) | ++ | + |
| Calu-3 (lung) | ++ | +++ |
| FR2 (breast) | + | 5366.7 ± 65.2 |
| MRC-5 (lung) | ++ | 4964.6 ± 340.3 |
| (d) **Effect of human serum protein** | | |
| Standard medium | 7.8 ± 1.4 | 35.2 ± 6.0 |
| +2% Serum albumin | 22.9 ± 0.8 | 372.7 ± 35.5 |
| +0.08% α1-Acid glycoprotein | 34.4 ± 3.2 | 448.5 ± 36.6 |

(a) Inhibition of enzyme activity. IGF1R, KDR, KIT, PDGFRβ and SRCB were not inhibited over 50% by both drugs at the highest concentration tested (10 000 nmol/L). (b) Inhibition of relative phosphorylation of EGFR and HER2 in NCI-N87 cells. (c) Inhibition of growth of cell lines. Expression levels of EGFR and HER2 for each cell line are indicated as scores (++, below 1; +, 1–10; ++, 10–100; ++++, above 100, pg EGFR or HER2/μg total cellular protein). In a and c, the results of statistical analysis (ns P ≥ 0.05, *P < 0.05, **P < 0.01, S-222611 versus lapatinib, Welch’s t-test) are presented in the right side of the date for lapatinib. (d) Inhibition of growth of NCI-N87 cells in the presence of human serum proteins. All the values are shown as mean ± SD of triplicate experiments except for inhibition of phospho-RTKs in NCI-N87 cells (b) where only IC50 values are shown.
tumor activity over lapatinib
inhibitory activity of S-222611 contributed to the superior anti-
shown to be longer than that of lapatinib. Such sustained kinase
the phosphorylation of EGFR
identical (Fig. S2a). Thus, the inhibitory activity of S-222611 on
tumor xenograft of both S-222611 and lapatinib were nearly
macokinetic analysis revealed that the drug concentrations in the
that the inhibitory activity of S-222611 persisted even at 24 h,
HER2 between 6 and 24 h after a single administration revealed
sensitively stronger than that of lapatinib at the same dose basis.
(xenograft. To confirm the mechanism of the antitumor activity
in vivo. | vol. 105 | no. 8 | 1043
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Fig. 1. S-222611 inhibits the growth of NCI-N87 tumor and phosphorylation of epidermal growth factor receptor (EGFR) and human epidermal
growth factor 2 (HER2) in the tumor. The animal model was prepared by subcutaneous implantation of human gastric cancer cells, NCI-N87 into
the back of nude mice. (a) After randomization at 3 days after implantation (n = 9), vehicle or multiple doses of S-222611 or lapatinib were
orally administered daily for 21 days. To avoid visual complexity, only two doses of lapatinib are depicted in this graph. The four full doses of
lapatinib are presented in Figure S1. Tumor volume was measured twice or thrice weekly and the mean tumor volume with SD was represented
at each data point. **P < 0.01 (versus vehicle, Dunnett’s test). (b–e) After randomization at 12 days after implantation (n = 9), vehicle or multiple
doses of S-222611 or lapatinib were administrated orally. The relative phosphorylations of EGFR (b,d) and HER2 (c,e) were calculated and the
mean with SD is presented in each bar graph. In b and c, the relative phosphorylations of EGFR (b) and HER2 (c) in tumor collected 6 and 24 h after
administration of the indicated doses of S-222611 or lapatinib are shown. In d and e, the relative phosphorylations levels of EGFR (d) and HER2
(e) in the tumor collected 6 and 24 h after administration of 50 mg/kg of S-222611 or lapatinib are shown. ns: P > 0.05, **: P < 0.01 (Tukey’s
multiple comparison). (f) The fold increase of the IC50 of each short-time pulse treatment compared to that of 72-h treatment was calculated
and the mean with SD is presented in each bar graph (n = 3).

Temporal evaluation of kinase inhibition and growth inhibitory activity of S-222611. To examine the time stability of kinase inhibition by S-222611, the kinetics of the enzyme inhibition
was studied. Lapatinib was reported to bind to an inactive conformation of enzyme and showed a slower off-rate of dissociation from EGFR than erlotinib.\(^{27}\) We compared the
dissociation rates from EGFR and HER2 between S-222611 and lapatinib and found that the rates of dissociation of S-222611 were significantly slower than those of lapatinib
(Fig. S3a,b). Importantly, these results showed that S-222611 was a reversible inhibitor like lapatinib.
Next we evaluated the growth inhibitory activity for NCI-
N87 cells with short-time pulse treatment, a pattern which resembles the drug exposure in vivo. Generally, the shorter the pulse treatment time, the higher the concentration of drug
needed to inhibit the growth. If the kinase inhibition by drug is more sustainable, it is reasoned that the increase of IC50 in
the short-time pulse treatment would be smaller. The IC50 values of S-222611 and lapatinib were calculated and the increase
of the IC50 in short-time pulse treatment is represented in Figure 1(f). Compared to 72-h treatment, the IC50 of lapatinib increased \(44.9, 33.7\) and \(14.1\)-fold with \(1, 6\) and \(24\)-h treat-
ment, respectively. However, the IC50 value of S-222611 increased only \(13.1, 6.2\) and \(3.1\)-fold with \(1, 6\) and \(24\)-h treatment, respectively. In addition, the differences of IC50
between S-222611 and lapatinib were \(17.0, 27.0\) and \(22.6\) with 1, 6 and 24-h treatment, respectively. Thus, S-222611 was
clearly superior to lapatinib with short-time pulse exposure. Considering these data along with sustained inhibition of phosphorylated EGFR and HER2 in the tumor in vivo, the slower dissociation rate of S-222611 should contribute to its superior in vivo antitumor activity over lapatinib.

In vivo antitumor activity in various types of cancer. As EGFR and HER2 are expressed in many types of cancer, S-222611 was tested for its antitumor activity in various cancer models in vivo (Fig. 2a–d, Table S2). In HER2-overexpressing human breast cancer models, S-222611 showed remarkable antitumor activity in the BT474 tumor model (Fig. 2a) and complete growth inhibition at 50 mg/kg even in the relatively less sensitive MDA-MB-361 tumor model (Fig. 2b). The antitumor activity of lapatinib for EGFR-dominant cancer was limited compared with that for HER2-expressing cancer. S-222611 showed potent antitumor activity even in EGFR-dominant tumor models (Fig. 2c,d and Table S2). Moreover, the treatment with a higher dose of S-222611 (>50 mg/kg) completely inhibited the growth of tumors or caused marked tumor regression in most of the models tested. These results indicate that S-222611 should be an effective treatment in a variety of tumor types.

In vivo activities of S-222611 on the cancer cell growth in bone marrow and brain and on the survival in intracranial implantation model. We evaluated S-222611 in patient-oriented models. Because bone metastasis is observed in more than 80% of advanced breast cancer patients with significant morbidity, we examined S-222611 using a model of luciferase-expressing human breast cancer cells implanted into the femur of nude mice. S-222611 showed approximately four times more potent activity than lapatinib and completely inhibited the growth of cancer cells at 50 mg/kg (Fig. 3a,b). Next, we tested S-222611 in the intracranial implantation model of breast cancer cells, because brain metastasis occurs in 10–16% of breast cancer patients. In this model, 25 mg/kg S-222611 significantly inhibited the growth of cancer cells in the brain to the same degree as 200 mg/kg lapatinib (Fig. 3c,d). This strong activity against intracranial tumor was further confirmed in survival assessments. S-222611 significantly prolonged survival at doses over 40 mg/kg, although the activity of lapatinib was not significant even at 200 mg/kg (Fig. 3e). All mice treated with 80 mg/kg S-222611 survived until the last observation (106 days after implantation). These results support the potential clinical value of S-222611 in the treatment of metastatic disease to the brain.

Comparison of S-222611 with irreversible inhibitors. Recently, several irreversible EGFR/HER2 inhibitors have been evaluated in clinical trials and shown promising efficacy. We compared the antitumor activity of S-222611 with irreversible inhibitors neratinib and afatinib (Fig. 4a). If we simply compared these drugs at the same dose, the efficacy of S-2322611 was weaker than those of both irreversible inhibitors, although statistical significance was observed only between 25 mg/kg S-222611 and 25 mg/kg afatinib. Generally, irreversible inhibitors are more potent in kinase inhibition and antitumor activity than reversible inhibitors, but the clinical trials of these irreversible inhibitors revealed that the frequency...
and degree of the adverse effect are also significantly increased.\(^{(10-15,32,33)}\) To assess the toxicity profile, we conducted a histopathological examination with mice used in the antitumor efficacy studies and observed the pivotal toxicity in the colon and eyeball in neratinib-treated and afatinib-treated mice. In the colon, mucosal regeneration was observed in \(\frac{2}{9}\) mice of the 25 mg kg\(^{-1}\) neratinib-treated group and \(\frac{4}{9}\) mice of the 12.5 mg kg\(^{-1}\) afatinib-treated group (Fig. 4b). These findings indicated prior injuries in the colon intestine. In the eyeball, atrophy in the corneal epithelium was observed in \(\frac{1}{9}\) mouse of the 6.25 and 25 mg kg\(^{-1}\) neratinib-treated groups, and \(\frac{5}{9}\) mice of the 6.25 and 12.5 mg kg\(^{-1}\) afatinib-treated groups (Fig. 4c). Neither of these toxicological findings was observed with 12.5 and 25 mg kg\(^{-1}\) S-222611-treated mice. These results indicated that S-222611 should be more effective than irreversible inhibitors when compared at doses that are not associated with toxic changes.

**Discussion**

Our initial observations documented that S-222611 is a potent kinase inhibitor with an IC\(_{50}\) lower than 10 nmol/L for both EGFR and HER2. Its inhibitory activity was comparable with that of lapatinib by simple comparison of the IC\(_{50}\) value (Table 1a). Subsequently, cell-based evaluation in vitro and antitumor efficacy evaluation in vivo showed S-222611 to be significantly superior to lapatinib (Tables 1a,c,S2 and Figs 1,2a–d). Extensive characterization of S-222611 revealed relative insensitivity to attenuation by serum protein binding (Table 1d) and sustained kinase-inhibitory activity (Fig. 2c), both of which contribute to the superiority of S-222611 in the in vivo evaluation.

Nonclinical examination and clinical trials to date have established the antitumor efficacy of lapatinib against HER2-expressing cancer while that against EGFR-expressing cancer...
is limited.\(^{7,8,28,29,34–36}\) Although the ED\(_{50}\) for highly sensitive HER2-dominant cancer, NCI-N87 and BT-474, were smaller than those for other cancers, the ED\(_{50}\) for EGFR-dominant cancer (A431, NCI-H292, SW48, HT115, LoVo and A498) were comparable to those for less-sensitive HER2-dominant cancer (MDA-MB-361 and Calu-3), which indicated that both EGFR inhibition and HER2 inhibition contributed to antitumor activity of S-222611 (Table S2). In addition, we have shown that the \textit{in vivo} antitumor efficacy of S-222611 was superior to that of lapatinib in all the models tested, including EGFR-dominant cancers (Fig. 2c,d, Table S2).

Human plasma AUC of lapatinib at a clinical dose (1200–1250 mg, once daily) is 14.3–36.2 h \(\mu\)g/mL.\(^{37,38}\) For S-222611, it is 16.4 h \(\mu\)g/mL at the recommended phase 2 dose, 800 mg.\(^{19,20}\) With the pharmacokinetic parameters of lapatinib and S-222611 in nude mice (Table S4), the clinically relevant doses in mice are assumed to be approximately 50 and 50–100 mg/kg for lapatinib and S-222611, respectively. Considering clinical relevancy, the ED\(_{50}\) of S-222611 in all tested models was well below the clinically relevant dose (Table S2). For lapatinib, the clinically relevant dose barely reached the ED\(_{50}\) in only three out of 10 models. Thus, the antitumor activity of S-222611 is at the level expected for clinical efficacy against both EGFR-dominant and HER2-dominant cancers. The potential clinical relevance of antitumor activity of S-222611 was confirmed with two important patient-oriented models: the “bone metastasis” model and “brain metastasis” model (Fig. 3a–e). Excellent antitumor activity of S-222611 was achieved at clinical relevant doses in both models. Lapatinib has been extensively studied as a clinical therapeutic approach in central nervous system metastasis,\(^{39–41}\) but it is of interest that S-222611 was eight times more potent in growth inhibition (Fig. 3b) and resulted in prolonged survival (Fig. 3e) in the “brain metastasis” model. Relatively higher tissue concentrations of S-222611 (Fig. S2b,c) should contribute to these excellent antitumor activities.

Two irreversible EGFR/HER2 inhibitors, neratinib and afatinib, have been developed in clinical trials for breast and nonsmall lung cancer and the latter has been approved worldwide.\(^{41}\) The clinical studies have shown efficacy, but the frequency and severity of the adverse effects have been apparently higher than those observed with reversible inhibitors.\(^{32,33}\) As shown in Figure 4(a), the antitumor activity of S-222611 was slightly weaker than those of neratinib and afatinib at the same dose. However, in histopathological study, mucosal regeneration of the colon and corneal atrophy of the eyeball were observed in neratinib-treated and afatinib-treated mice. No remarkable changes were observed in vehicle-treated and S-222611-treated mice. Stained with H&E. Bar: 100 \(\mu\)m.
The corneal abnormality is considered to have resulted from the intervention of EGFR signals in the cornea. (43,44) This adverse effect was less commonly reported with EGFR inhibitors, (43,45) but rarely led to serious consequences like persistent corneal epithelial defect and perforating corneal ulceration. (46,47) As these histopathological findings have clinical relevance, S-222611 should have a better safety profile than irreversible inhibitors.

Our findings suggest that S-222611 can achieve an excellent balance between efficacy and toxicity. Phase I trials of S-222611 have shown very promising results, (19,20) and phase II trials are in progress.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Dose-dependent antitumor efficacy of lapatinib in the NCI-N87 model.

Fig. S2. Tumor, brain and bone marrow concentrations of S-222611 and lapatinib in tumor-bearing nude mice.

Fig. S3. Slow off-rate dissociation of S-222611 from epidermal growth factor receptor and human epidermal growth factor receptor 2.

Table S1. Kinase inhibition profile of S-222611.

Table S2. Comparison of ED50 values between S-222611 and lapatinib in various tumor models.

Table S3. Pharmacokinetics of S-222611 and lapatinib in nude mice.

Data S1. Legends for supplementary figures and tables.

Data S2. Materials and methods for supplementary figures and tables.