TAS6417/CLN-081 Is a Pan-Mutation-Selective EGFR Tyrosine Kinase Inhibitor with a Broad Spectrum of Preclinical Activity against Clinically Relevant EGFR Mutations


Abstract

Despite the worldwide approval of three generations of EGFR tyrosine kinase inhibitors (TKI) for advanced non-small cell lung cancers with EGFR mutations, no TKI with a broad spectrum of activity against all clinically relevant mutations is currently available. In this study, we sought to evaluate a covalent mutation-specific EGFR TKI, TAS6417 (also named CLN-081), with the broadest level of activity against EGFR mutations with a prevalence of ≥1%. Lung cancer and genetically engineered cell lines, as well as murine xenograft models were used to evaluate the efficacy of TAS6417 and other approved/in-development EGFR TKIs (erlotinib, afatinib, osimertinib, and poziotinib). We demonstrate that TAS6417 is a robust inhibitor against the most common EGFR mutations (exon 19 deletions and L858R) and the most potent against cells harboring EGFR-I790M (first/second-generation TKI resistance mutation). In addition, TAS6417 has activity in cells driven by less common EGFR-G719X, L861Q, and S768I mutations. For recalcitrant EGFR exon 20 insertion mutations, selectivity indexes (wild-type EGFR/mutant EGFR ratio of inhibition) favored TAS6417 in comparison with poziotinib and osimertinib, indicating a wider therapeutic window. Taken together, we demonstrate that TAS6417 is a potent EGFR TKI with a broad spectrum of activity and a wider therapeutic window than most approved/in-development generations of EGFR inhibitors.

Implications: TAS6417/CLN-081 is a potent EGFR TKI with a wide therapeutic window and may be effective in lung cancer patients with clinically relevant EGFR mutations.

Introduction

Lung cancer is one of the most aggressive tumor types with an overall 5-year survival of patients of 18% (1). Recent discoveries of genetic alterations in oncogenic kinases and development of corresponding tyrosine kinase inhibitors (TKI) have changed treatment strategies for a subset of non–small cell lung cancer (NSCLC) with these mutations (2). Since gefitinib, a first-generation EGFR TKI, was first approved worldwide in 2002, two other generations of EGFR TKIs have been developed for treatment of advanced NSCLC with EGFR mutations. Indels around exon 19 and the exon 21 L858R are the most common mutations, and these account for >75% of all EGFR mutations in NSCLC (Table 1; ref. 3). These “classic” mutations are sensitive to first-generation (reversible: gefitinib, erlotinib) and second-generation (irreversible/covalent: afatinib, dacomitinib) EGFR TKIs. The third most common mutations are in-frame insertions in exon 20, which account for >9.3% of all EGFR mutations (Table 1), and these are insensitive to first- and second-generation EGFR TKIs (4–6) due to lack of a therapeutic window in relation to the wild-type (WT) EGFR for these TKIs (7). Except for EGFR-A763_Y764insFQA that is sensitive to approved TKIs, there are limited treatment options for other exon 20 insertions. We have previously shown that osimertinib has a relatively wider therapeutic window for EGFR exon 20 insertion than that of first- or second-generation EGFR TKIs (8), and clinical trials with this agent (NCT03191149 and NCT03414814) are ongoing. Recently, preclinical and clinical data suggest that poziotinib, a pan-ErbB TKI, is active against EGFR and Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2) insertion 20 mutations (9). Clinical trials of poziotinib for this patient population (NCT03066206 and NCT03318939) are ongoing. Another compound, TAK-788, is currently in phase I/II trial (NCT02716116) development for EGFR/ERBB2 insertion 20 mutations (4). Although both compounds have shown initial clinical responses in patients with EGFR...
or ERBB2 exon 20 insertions, they are associated with significant cutaneous plus gastrointestinal adverse events due to a small therapeutic window in relation to WT EGFR (10, 11). Poziotinib has low activity in clinical settings enriched for EGFR-T790M possibly due to its limited therapeutic window and toxicity profile (12). Other less common mutations, often called "rare mutations," include exon 18 G719X, exon 18 E709X, exon18 indels, exon 19 insertions, exon 20 S768I, exon 21 L861Q, kinase domain duplications, and EGFR rearrangements (3), with variable frequencies and only afatinib approved for G719X, S768I, and L861Q mutations (Table 1).

Despite of dramatic response to first- and second-generation EGFR TKIs, resistance emerges over time. The EGFR-T790M mutation accounts for more than half of resistance, followed by bypass tracks, histologic (small cell) transformation, and acquired mutations in genes downstream of EGFR signaling (2). Third-generation (covalent mutation-specific: osimertinib) EGFR TKIs are effective for not only tumors with EGFR-T790M (13) but also other common mutations, which led to its approval for first-line use of advanced NSCLC with EGFR-exon 19 deletions or L858R (14).

### Table 1. Types, frequency, and EGFR TKI approval for EGFR kinase domain mutations in lung cancer (3)

<table>
<thead>
<tr>
<th>EGFR mutation</th>
<th>Approximate frequency</th>
<th>1st generation</th>
<th>2nd generation</th>
<th>3rd generation</th>
</tr>
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<tr>
<td>Sensitizing</td>
<td></td>
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<tr>
<td>Exon 19 deletion</td>
<td>45%</td>
<td>Gefitinib, erlotinib</td>
<td>Afatinib, dacomitinib</td>
<td>Osimertinib</td>
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<tr>
<td>L858R</td>
<td>35%</td>
<td>Gefitinib, erlotinib</td>
<td>Afatinib, dacomitinib</td>
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</tr>
<tr>
<td>G719X</td>
<td>3%</td>
<td>Afatinib</td>
<td>—</td>
<td>—</td>
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<tr>
<td>L861Q</td>
<td>&gt;1%</td>
<td>Afatinib</td>
<td>—</td>
<td>—</td>
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<tr>
<td>S768I</td>
<td></td>
<td>Afatinib</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Exon 18 indel/E709X</td>
<td>&lt;0.5%</td>
<td>—</td>
<td>Afatinib</td>
<td>—</td>
</tr>
<tr>
<td>Exon 19 insertion</td>
<td>&lt;0.5%</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>A763_Y764insFQEA</td>
<td>&lt;0.5%</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Exon 18-25 duplication</td>
<td>&lt;0.5%</td>
<td>—</td>
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<tr>
<td>Rearrangement</td>
<td>&lt;0.5%</td>
<td>—</td>
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<tr>
<td>Insensitizing to 1st/2nd-generation TKI</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Exon 20 insertion</td>
<td>&gt;9.5%</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>T790M inherited</td>
<td>&lt;1%</td>
<td>—</td>
<td>—</td>
<td>Osimertinib</td>
</tr>
<tr>
<td>Acquired resistance</td>
<td></td>
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<tr>
<td>T790M+sens.</td>
<td>&gt;50% (to 1st/2nd-generation TKI)</td>
<td>—</td>
<td>—</td>
<td>Osimertinib</td>
</tr>
<tr>
<td>C797X+T790M+sens.</td>
<td>&lt;40% (to osimertinib)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td>&lt;1% (each rare type)</td>
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Abbreviation: sens., sensitizing.
The aforementioned EGFR TKI approval and development scenario in 2019 highlights there is a clinical need for development of new pan-mutation–selective EGFR TKIs to maximize efficacy and simultaneously enhance the therapeutic window against WT EGFR to minimize toxicities. We have previously demonstrated that TAS6417 (also named CLN-081), a novel covalent EGFR TKI, is a potent inhibitor of EGFR insertion 20 mutations in preclinical models (7). In this study, we evaluated TAS6417 as a pan-mutation–selective EGFR TKI with a wide therapeutic window using in vitro and in vivo models.

Materials and Methods

Reagents
TAS6417 was synthesized at Taiho Pharmaceutical Co., Ltd. (7). Osimertinib, poziotinib, afatinib, and erlotinib were purchased from CHEMSCENE, LLC, MedChem Express, Selleck Chemicals, and LC Laboratories, respectively.

Cell culture
NCI-H1975 (H1975), HCC827, BEAS-2B, and NIH/3T3 cell lines were obtained from the American Type Culture Collection, and Baf3 and PC-9 cell lines were obtained from RIKEN BioResource Center. The LXF 2478L cell line was provided by Charles River Discovery Research Services, GmbH. BID007 cells were established from pleural effusion taken from a lung cancer patient with EGFR-A763_Y764insFQEA (6). BID019 cells were established from pleural effusion taken from a lung cancer patient with EGFR-N771_P772insH. NHEK-Neo cells were obtained from Lonza. All cell lines were authenticated by short tandem repeat profiling before purchase or use in this study. Cells were thawed and tested for lack of Mycoplasma contamination (Mycoplasma Detection Kit, Lonza).

Figure 1.
(Continued.) G, Western blot analysis showing the effects of increasing concentration of osimertinib, poziotinib, and TAS6417 in BEAS-2B and H1975 cells. H, Selectivity indexes showing mutation selectivity calculated as ratios of the IC_{50} values of NHEK-Neo to those of each cell line. I, Selectivity indexes calculated as ratios of the IC_{50} values Ba/F3 expressing WT EGFR to those of Ba/F3 cells expressing each mutant EGFR.
prior to experiments that were initiated within the initial five passages.

Genetically engineered cell lines
Ba/F3 cells expressing WT and mutant EGFR were generated as previously described (6, 7). The mutant EGFRs tested in this study were L858R, L858R+T790M, exon 19 deletions (delE746_A750 and delE747_P753insS), exon 19 deletions + T790M, G1796A+C790M, G1796A+C790M, L861Q, L861Q+C790M, S768I+V769L, A763_Y764insPFGA, V769_D770insASV, D770_N771insSVD, D770_N771insSVD, D770_N771insSVD, and H773_V774insNPH, and H773_V774insPH. For H1975 EGFR-D770_N771insSVD cells, H1975 N771insSVD, D770_N771insG, H773_V774insNPH, and H773_V774insPH. For H1975 EGFR-D770_N771insSVD cells, H1975 cells expressing EGFR-D770_N771insSVD were established as described in our previous report (7). After establishment, sequencing analysis was performed to confirm the integration of WT and mutant EGFR.

Cell viability assay
Cell viability was assessed using the CellTitre-Glo luminescent cell viability assay or the CellTitre 96 AQUEOUS One Solution cell proliferation assay as instructed by the manufacturer (Promega). For a panel of Ba/F3 cell lines expressing human EGFRs, the cells were plated at an optimal density onto 96-well plates, followed by 72 hours of exposure to the compound in mouse IL3 (mIL3)–free conditions. Ba/F3 parent cells and Ba/F3 EGFR WT cells were stimulated with 1 ng/mL mIL3 and 50 ng/mL EGF, respectively, during compound treatment. For a panel of human lung cancer cell lines and normal cells (NHEK-Neo and BEAS-2B cells), the cells were plated as described above and incubated for 24 hours for attachment, followed by compound treatment for 72 hours. IC50 values were determined using GraphPad Prism 7 (GraphPad Software).

Immunoblotting
Cells were treated with EGFR inhibitors as indicated. After incubation, cells were lysed in cell lysis buffer [20 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 1 mmol/L β-glycerophosphate, 1 mmol/L Na3VO4, 1 mmol/L NaF, protease inhibitor cocktail set III (EMD Millipore), and 1 mmol/L PMSF]. Protein lysates (20 μg) were loaded on 10% SDS-polyacrylamide gels and transferred on PVDF membranes (Millipore). All antibodies were purchased from Cell Signaling Technology and used at 1:1,000 dilution: Anti-EGFR antibody (Catalog number: 4267), anti-AKT antibody (9106), and anti-phosphoAKT antibody (4060), anti-phosphoERK antibody (4685), anti-ERK antibody (9102), anti-phosphoEGFR antibody from Cell Signaling Technology and used at 1:1,000 dilution: Anti-EGFR antibody (Catalog number: 4267), anti-AKT antibody (9106), and anti-phosphoAKT antibody (3777), anti-phosphoAKT antibody (4060), anti-phosphoERK antibody (4685), and anti-β-Actin antibody (4970).

Apoptosis assay
Apoptosis was assessed using Caspase-Glo 3/7 Assay System (Promega). Briefly, cells (PC-9: 5,000 cells/well, H1975: 3,000 cells/well, BID007: 5,000 cells/well, BID019: 7,000 cells/well, BEAS-2B: 3,000 cells/well) were plated on 96-well plates and incubated for 24 hours. Then cells were treated with 0.1% DMSO, 0.1 mmol/L TAS6417, or 1 mmol/L TAS6417 and incubated for 48 hours. Caspase activity was measured as instructed by the manufacturer.

In vivo efficacy experiments
All animal protocols were reviewed and approved according to regional Institutional Animal Care and Use Committees.
hypothesized that it has a wide therapeutic window for "classic" EGFR mutations. Western blot analysis demonstrated that phosphorylation of EGFR was suppressed in a dose-dependent manner, and completely inhibited by poziotinib at 10 nmol/L and afatinib at 100 nmol/L, whereas TAS6417 or osimertinib failed to inhibit EGFR phosphorylation even at 1,000 nmol/L in the NHEK-Neo cell line (Supplementary Fig. S1; ref. 17). These results were well correlated with IC_{50} values calculated by the viability assay (Fig. 1F), suggesting that WT EGFR is implicated in the growth and survival. Therefore, we used IC_{50} values for the NHEK-Neo cell line to calculate selectivity indexes (SI: WT EGFR/mutant EGFR ratio) to determine how specific each compound is against EGFR mutants. TAS6417 and osimertinib had similar SI in H820 cells (12 and 13, respectively), and TAS6417 had highest SI in other cell lines (Fig. 1H). In HCC827 cells, even though TAS6417, afatinib, and poziotinib had similar IC_{50}s, SI of TAS6417 was 6-fold and 124-fold higher than that of afatinib and poziotinib, respectively (Fig. 1H).

Of note, enzymatic analysis demonstrated that TAS6417 concentrations showing the same levels of remaining kinase activity of a preformed WT EGFR enzyme–inhibitor complex were higher than osimertinib (Supplementary Fig. S2, left plots; e.g., level of phosphorylated substrate at final measurement point was 22% for 1 nmol/L of TAS6417, and 0.3 nmol/L of osimertinib), whereas those for D770_N771insNPG were similar (Supplementary Fig. S2, right plots). These results suggest that TAS6417 may show faster dissociation from WT EGFR compared with osimertinib, which could explain mutation-selective characteristics of TAS6417.

We were able to validate these results using isogenic Ba/F3 cells expressing EGFR harboring L858R, L858R+T790M, delL747_P753insS, or delL747_P753insS+T790M (6, 7). As expected, IC_{50}s of all inhibitors showed below 100 nmol/L for Ba/F3 cells expressing L858R and exon 19 deletion, whereas only osimertinib, poziotinib, and TAS6417 showed IC_{50}s below 100 nmol/L in Ba/F3 expressing L858R+T790M or exon 19 deletion+T790M (Supplementary Table S1). TAS6417 showed highest SI in all Ba/F3 lines (Fig. 1I).

These results taken together indicate that TAS6417 is a potent inhibitor against the most common EGFR-sensitizing mutations in the presence or absence of the EGFR-T790M mutation with a wider therapeutic window than erlotinib, afatinib, osimertinib, or poziotinib (Fig. 1H and I).

TAS6417 inhibits uncommon EGFR mutations in the presence or absence of T790M.

We next questioned whether TAS6417 has an activity against other EGFR mutations with a clinical prevalence that exceeds 1% (Table 1), including EGFR-G719X, S768I, and L861Q for which

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**Figure 2.**

TAS6417 has an in vitro activity and selectivity to inhibit EGFR with exon 18/21 uncommon mutations. A–E, Dose inhibition curves and SDs of three independent experiments for erlotinib (blue), afatinib (red), osimertinib (green), poziotinib (purple), and TAS6417 (orange) in Ba/F3 cells expressing EGFR G719A (A), S768I+V769I (B), L861Q (C), G719A+T790M (D), and L861Q+T790M (E). F, Selectivity indexes of TAS6417 and other EGFR TKIs tested were calculated as the WT/mut ratio of the IC_{50} values.
only afatinib is approved with lesser degree of clinical activity reported for first- and third-generation EGFR TKIs (18, 19).

Cell viability assays demonstrated that Ba/F3 cells expressing G719A had IC50 less than 20 nmol/L for afatinib, poziotinib, and TAS6417 (Fig. 2A; Supplementary Table S2), whereas only poziotinib and TAS6417 showed IC50 less than 20 nmol/L in Ba/F3 cells expressing G719A +T790M (Fig. 2D; Supplementary Table S2). The SIs of TAS6417 were 4.3- and 3.7-fold higher for G719A and 83- and 54-fold higher for G719A +T790M than those of afatinib and poziotinib, respectively (Fig. 2F; Supplementary Table S2). In a compound EGFR-S768I mutation, S768I + V769L, the IC50s of afatinib (42 nmol/L) and poziotinib (7 nmol/L) were lower than that of TAS6417 (100 nmol/L; Fig. 2B; Supplementary Table S2), but the SI of TAS6417 was 6.1- and 7.7-fold higher than those of afatinib and poziotinib, respectively (Fig. 2F). Ba/F3 cells expressing L861Q alone were slightly less sensitive to TAS6417 (Fig. 2C; Supplementary Table S2); however, the SI of TAS6417 was higher than that of afatinib (Fig. 2F). Interestingly, Ba/F3 cells expressing L861Q + T790M double mutations were sensitive to both osimertinib and TAS6417 determined by both IC50 and SI (Fig. 2E and F). These in vitro data suggest that TAS6417 may be effective for other EGFR mutations with ≥1% clinical prevalence alone, compound with other mutations and in association with EGFR-T790M.

Potency of TAS6417 against cells harboring EGFR exon 20 insertion

We also evaluated the potency and selectivity of TAS6417 and poziotinib in cells harboring heterogeneous EGFR exon 20 insertions. We had previously shown that TAS6417 inhibits activity of some EGFR exon 20 insertions, suppresses cell growth, and induces apoptosis in cells harboring these mutants (7). Another group has reported that poziotinib, a pan-ErbB TKI, shows selective activity against EGFR exon 20 insertions in preclinical models and partial responses at recommended clinical doses (albeit significant side effects) in some patients with these mutations (9).

To this end, we used two cell lines isolated and established from lung cancer patients with EGFR exon 20 insertions: BID007 (EGFR-A763_Y764insFQEA; ref. 6) and BID019 (EGFR-N771_H772insH). We also used engineered H1975 cells expressing EGFR-D770_N771insSVD in which the EGFR-L858R +T790M was deleted (H1975-insSVD) and LXF2478L cells that were established from a patient-derived xenograft (PDX) tumor with EGFR-V769_D770insASV (7). In BID007, poziotinib

Figure 3. TAS6417 is more selective for EGFR exon 20 insertion mutations than other EGFR TKIs. A–D, Dose inhibition curves and SDs of three independent experiments for erlotinib (blue), afatinib (red), osimertinib (green), poziotinib (purple), and TAS6417 (orange) in BID007 (A), BID019 (B), H1975-InsSVD (C), and LXF2478L cells (D). (Continued on the following page.)
showed the lowest IC50 of 1 nmol/L, followed by afatinib (3 nmol/L), TAS6417 (3 nmol/L), erlotinib (53 nmol/L), and osimertinib (87 nmol/L). In BID019 cells, poziotinib showed the lowest IC50 (0.9 nmol/L) followed by TAS6417 (21 nmol/L) and afatinib (221 nmol/L). Consistent with these results, inhibition of EGFR phosphorylation was achieved at 1 and 10 nmol/L for poziotinib, 100 and 1,000 nmol/L for osimertinib, and 10 and 100 nmol/L for TAS6417 in BID007 cells and BID019 cells, respectively (Fig. 3E). However, IC50 of TAS6417 was 16-fold lower, but SI of TAS 6417 was 22-fold higher compared with those of erlotinib in BID007 cell lines (Fig. 3A and F), suggesting that TAS6417 is more potent and selective against EGFR-A763_Y764insFQEA. In BID019 cells, TAS6417 showed the highest SI, which was 5.7- and 75-fold higher than those of poziotinib and afatinib, respectively (Fig. 3B and F). Essentially similar results were obtained from H1975-insSVD (Fig. 3C) and LXF2478L cells (Fig. 3D), demonstrating that TAS6417 showed the highest SI in all Ba/F3 lines (Fig. 3G).

These experiments suggest that TAS6417 is a more selective inhibitor against EGFR exon 20 insertion mutations than poziotinib in vitro.

**TAS6417 induces apoptosis in EGFR-mutant cell lines**

Next, we investigated the mechanism by which TAS6417 inhibits cell viability. We and others have shown that EGFR inhibition induces apoptosis in EGFR-mutant cells by activating the caspase cascade (20, 21). When EGFR-mutant cells (PC-9, H1975, BID007, and BID019 cells) were treated with TAS6417, caspase 3/7 (executors of cell death) were activated; whereas no activity was detected in BEAS-2B cells (Supplementary Fig. S3). These results suggest that TAS6417 leads to apoptosis via inhibition of mutant EGFR.

**In vivo efficacy of TAS6417 against EGFR mutations**

To evaluate whether TAS6417 is effective against EGFR mutations in vivo, we used mouse xenograft models. Preliminary experiments showed that H1975, NIH/3T3 expressing G719A, and NIH/3T3 expressing G719A-T790M, BID007, H1975-insSVD formed tumors in nude mice. All three doses (50, 100, and 200 mg/kg/day) of TAS6417 administration...
achieved a significant tumor reduction of H1975 xenografts driven by EGFR-L858R/T790M in a comparable fashion with osimertinib (Fig. 4A). Both afatinib and TAS6417 inhibited tumor growth in mice implanted with NIH/3T3 cells expressing either EGFR-G719A mutation (Fig. 4B). Interestingly, TAS6417 was more potent against EGFR G719+T790M with treatment/control (T/C) ratios of 24.3%, 14.5%, and 8.9% at 50, 100, and 200 mg/kg/day, respectively, compared with those of 48.9% of afatinib (20 mg/kg/day; Supplementary Fig. S4). As we have previously shown, TAS6417 was effective in mice implanted with H1975-InsSVD (7). As poziotinib has shown to be effective in some patients with EGFR Ins20 but its toxicity results in dose reduction in the majority of clinical cases, we wanted to compare TS6417 with poziotinib to evaluate their bioavailability and safety in mice. Poziotinib at 1 mg/kg/day suppressed growth of xenograft tumors similarly to that of TAS6417 at 100 mg/kg/day in mice implanted with either BID007 (Fig. 4C) or H1975-InsSVD (Fig. 4D). However, poziotinib at this dose led to body weight loss in all mice, which indicates a dose-limiting toxicity of this EGFR TKI. In contrast, TAS6417 at 200 mg/kg/day showed no body weight loss (Fig. 4E). Indeed, one mouse implanted with BID007 in the poziotinib arm died during the course of treatment due to extensive skin rash and body weight loss, and we had to reduce the dose of poziotinib from 1 to 0.5 mg/kg/day on day 11 due to extensive skin rash, and body weight loss, and we had to reduce the dose of poziotinib at 1 mg/kg/day to 0.5 mg/kg/day as 2 mice showed body weight loss greater than 20%.

Figure 4. TAS6417 suppresses growth of tumors driven by common, uncommon, and ins20 EGFR mutations in vivo. A, Nude mice bearing H1975 cells (n = 6 per group) were treated with either vehicle, TAS6417 at 50, 100, or 200, or osimertinib at 25 mg/kg. The administration was orally once daily for 15 consecutive days. B, Nude mice bearing NIH/3T3 EGFR G719A allografts (n = 6 per group) were treated with either vehicle, TAS6417 at 50, 100, or 200 mg/kg, or afatinib at 20 mg/kg. One mouse of control group was euthanized on day 11 due to overgrowth of tumor. C, Nude mice bearing BID007 (n = 6 per group) were treated with either vehicle, TAS6417 at 50, 100, or 200 mg/kg, or poziotinib at 1 mg/kg. One mouse treated with poziotinib died on day 10 due to extensive skin rash, and the dose was reduced to 0.5 mg/kg/day as 2 mice showed body weight loss greater than 20%. D, Nude mice bearing H1975 EGFR D770_N771InsSVD xenografts (n = 6 per group) were treated with either vehicle, TAS6417 at 100 or 200 mg/kg, or poziotinib at 0.5 or 1 mg/kg. E, Body weight changes in mice described in D. Data are presented as mean tumor volume ± SEM or mean body weight change ± SEM in each group.

Discussion

Herein, we show that the TAS6417 is a unique EGFR TKI with activity against a wide spectrum of cells driven by clinically relevant EGFR mutations, including the majority of mutations that have a prevalence of ≥1% in EGFR-mutated NSCLC: classic...
mutations (exon 19 deletions and L858R), less common mutation (G719X, S768I, and L861Q), the first/second-generation TKI resistance T790M, and exon 20 insertion mutations. Not only was TAS6417 active in a more robust number of mutants than other select EGFR TKIs that are approved (erlotinib, afatinib, osimertinib) or in-development (poziotinib), but it also had a wider therapeutic window (lower inhibition of the WT EGFR). The later quality may indicate that it can reach higher serum and tissue concentrations prior to dose-limiting cutaneous and gastrointestinal toxicities that can plague other potent EGFR TKIs. As indicated in Fig. 5, TAS6417 is the most selective EGFR inhibitor among others tested in this study.

Based on the observations that EGFR are overexpressed in a variety of solid tumors including lung cancer, first and second generations of EGFR TKIs were originally developed to target WT EGFR prior to discovery of EGFR kinase domain mutations in NSCLC. Subsequent studies identified that classic EGFR mutations lower ATP affinity and provide tighter binding to first/second-generation EGFR TKIs (22), which creates a favorable therapeutic window over the WT EGFR. Currently, multiple EGFR TKIs (including gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib) are approved for tumors harboring EGFR-exon 19 deletion and L858R, afatinib approved for tumor with EGFR-G719X, S768I, or L861Q, and osimertinib for those harboring EGFR-T790M. Notwithstanding, no currently approved TKI has a broad spectrum of activity against all clinically relevant mutations that have a prevalence of ≥1% (Table 1). TAS6417 is unique in preclinical studies due to its favorably wide therapeutic window due to its lower inhibitory potency against the WT EGFR in multiple preclinical models tested. We believe that this pattern of enhanced activity against mutant versions of EGFR versus WT EGFR is more important than the pure potency alone (IC_{50} in proliferation assays) of an EGFR TKI. As an example, poziotinib had lower inhibitory concentrations against the majority of mutations tested, but it also had potent inhibition of WT EGFR, data that may explain the severe cutaneous and gastrointestinal toxicity profile observed in the clinic that hampered the activity of poziotinib against EGFR-T790M–enriched tumors (12).

TAS6417 also displayed a favorable therapeutic window in preclinical models driven by EGFR-G719X, S768I, and L861Q mutations. The SI of TAS6417 was more than 4-fold higher than those of afatinib in these mutants. As only afatinib is approved in this clinical setting, TAS6417 may be a future candidate to be developed in these less common EGFR-mutated NSCLCs. Interestingly, TAS6417 was more potent for these mutants when together with EGFR-T790M (Fig. 2D and E) highlights the possible efficacy in EGFR-G719X–, S768I–, and L861Q–mutated tumors that become resistant to first/second-generation EGFR TKIs if EGFR-T790M is the acquired resistance mechanism. Indeed, T790M can emerge when tumors harboring G719A or L861Q become resistant to gefitinib (23). Use of TAS6417 may also delay emergence of resistance.

Most notably, we again confirm in this study that TAS6417 is a potent EGFR TKI for EGFR exon 20 insertion—expanding our prior work (7). TAS6417 had a more favorable therapeutic window against all EGFR exon 20 insertion mutations tested than afatinib in cell lines models, and equal to more activity with lesser toxicities in PDX in vivo studies. Poziotinib is one EGFR TKI alongside TAK-788 that are undergoing clinical development as EGFR/ERBB2 exon 20 insertion specific TKIs. The clinical activity of both drugs has been reported in initial studies (9–11), but severe toxicities were noted in the majority of cases treated with poziotinib highlighting its narrow therapeutic window in relation to EGFR WT. Even though TAS6417 has 13.6–32- and 10–30-fold lower IC_{50} than those of poziotinib in our BaF3 cell models expressing EGFR-exon 20 insertion mutations and in EGFR exon 20 insertion–mutated NSCLCs (BID007 and BID019), the selectivity indexes of TAS6417 were 32- and 6-fold higher than those of poziotinib, respectively (as shown by Figs. 3F and 5). Therefore, these mutants may be prime candidates for a potent and selective EGFR TKI such as TAS6417. The reason behind the wider mutation spectrum with mutation selectivity of TAS6417 is unclear. However, given TAS6417 has a unique scaffold differing from other representative EGFR TKIs, it may be partly responsible for its distinctive profile. Indeed, some interim analyses suggest that TAS6417 may occupy a peculiar space of ATP-binding site (manuscript in preparation).

We should also highlight possible limitations of TAS6417 when it enters the clinical development phase. Firstly, TAS6417 seems to be an EGFR-mutant–selective inhibitor with minimal to no activity against other ErbB members and ERBB2 insertion 20 mutations (7). Secondly, it is unknown if pharmacokinetic and pharmacodynamic profiles of TAS6417 in patients will allow serum/tissue levels for activity against all EGFR mutations tested in our preclinical studies. Thirdly, it is still unclear

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Figure 5. Summary of the in vitro sensitivity of Ba/F3 cells expressing each EGFR mutation to EGFR TKIs tested in this study. IC_{50} values above 500 nmol/L are shown in red (resistant), IC_{50} values equal or below 500 nmol/L and SI under 3 are shown yellow (low mutation-selective), and IC_{50} values equal or below 500 nmol/L and SI equal or above 3 are shown in green (high mutation-selective).
whether TAS6417 is effective for tumors harboring acquired resistant mutations to the third-generation EGFR TKI osimertinib and what the on- and off-target mechanisms of acquired resistance to TAS6417 are. Among possible osimertinib-resistant mutations, one can identify EGFR-C797S as the most common, which accounts for more than 15% but less than 30% of all resistant cases (4, 24–26). Because TAS6417 covalently binds to EGFR-C797 in EGFR (7), it is likely that C797X mutations would be a vulnerability of TAS6417 and other EGFR-C797 covalent binding TKIs.

In summary, our preclinical studies demonstrate that TAS6417 is a pan-mutation–selective EGFR TKI with a broad spectrum of activity against clinically relevant EGFR mutations. In addition, TAS6417 has a wider therapeutic window in relation to WT EGFR compared with other representative approved and in-development EGFR TKIs (Fig. 5). Clinical evaluation of TAS6417 is planned in the near future to confirm our preclinical results and provide more options for patients with EGFR-mutated NSCLC.

Disclosure of Potential Conflicts of Interest
H. Udagawa reports receiving commercial research grants from AbbVie, Daiichi Sankyo, MSD, and Amgen; and has received honoraria from the speakers’ bureau of AstraZeneca, Chugai Pharma, AbbVie, MSD, Taiho Pharmaceutical, Ono Pharmaceutical, Bristol-Myers Squibb, Amgen, Boehringer Ingelheim, and Daiichi Sankyo. N. Abe is an employee of, and has an ownership interest (including patents) in Taiho Pharmaceutical T. Haruma and M. Terasaka are employees of Taiho Pharmaceutical K. Miyadera is Deputy Head at Taiho Pharmaceutical K. Goto reports receiving commercial research grants from Taiho Pharmaceutical, Chugai Pharmaceutical, Kyowa Hakko Kirin, Novartis Pharma K.K., Takeda Pharmaceutical, Eli Lilly Japan K.K., Merck Serono, Bristol-Myers Squibb, Lexo Oncology, Janssen Pharmaceutical K.K., Ignyta, Inc., Medical & Biological Laboratories, Ono Pharmaceutical, Sumitomo Dainippon Pharma, Riken Genesis, Life Technologies Japan, OncDev Development LP., Xoce, Inc., AbbVie GK, AstraZeneca K.K., Boehringer Ingelheim Japan, Inc., MSD K.K., Astellas Pharma Inc., Eisai, Daiichi Sankyo, and Pfizer; and has received honoraria from the speakers’ bureau of Bristol-Myers Squibb, Eli Lilly Japan K.K., Riken Genesis, Ono Pharmaceutical, Taiho Pharmaceutical, Daiichi Sankyo, Boehringer Ingelheim Japan, Inc., Nippon Kayaku, MSD K.K., Takeda Pharmaceutical, Pfizer Inc., Chugai Pharmaceutical, F. Hoffmann-La Roche Ltd., AstraZeneca K.K., Life Technologies Japan, Novartis Pharma K.K., Merck Serono, and SRL, Inc. D.B. Costa is a consultant at AstraZeneca, Takeda/Millennium, and Pfizer; reports receiving other commercial research support from Taike/Genentech, AstaZeneca, Pfizer, Merck, Merrimack, Bristol-Myers Squibb, Clovis Oncology, and Spectrum; and has received honoraria from the speakers’ bureau of Takeda/Millennium and Pfizer. S.S. Kobayashi is an advisory board member for Pfizer; is consultant at Ono; reports receiving commercial research grants from MiNA Therapeutics and Taiho; has received honoraria from the speakers’ bureau from Chugai, Boehringer Ingelheim, and Roche Diagnostic. No potential conflicts of interest were disclosed by the other authors.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.B. Costa, S.S. Kobayashi

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References


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