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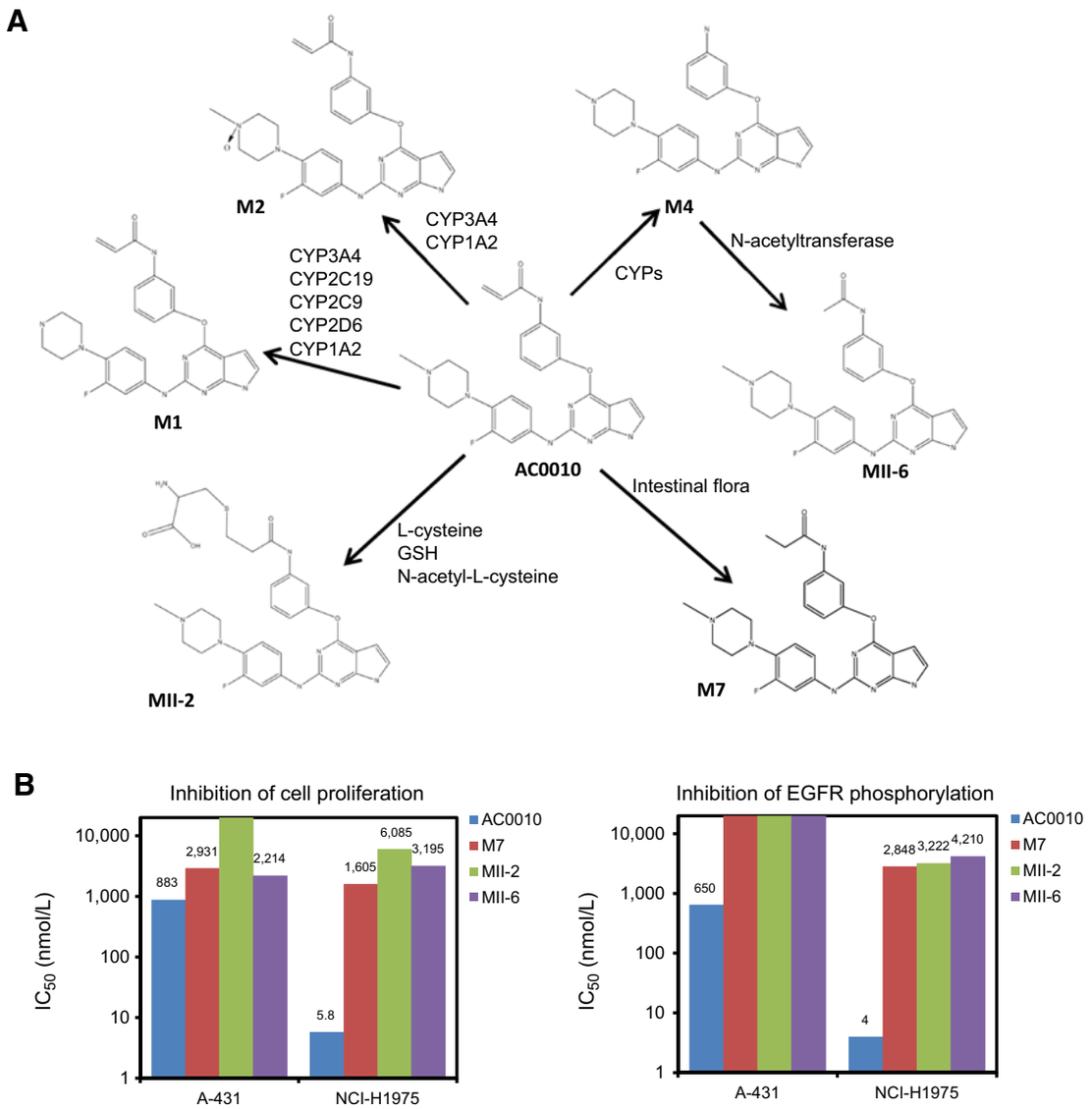

Figure 4.

Proposed AC0010 metabolic pathways and activities of its major metabolites. **A,** proposed AC0010 metabolic pathways showing the formation of metabolites through N-demethylation (M1), N-oxidation (M2), and N-dealkylation (M4) followed by acetylation (MII-6), reduction (M7), and cysteine conjugation (MII-2). **B,** inhibitory activities of AC0010 and its metabolites on cell proliferation by WST assay and EGFR phosphorylation in NCI-H1975 and A431 cells by ELSA. The IC_{50} values are indicated.

Figure 3.

In vivo antitumor efficacy and pharmacokinetics/pharmacodynamics in xenograft models. **A,** inhibition of NCI-H1975 tumor growth by AC0010 and gefitinib. Tumor growth curves are plotted as mean ± SEM ($n = 8$), and the inhibition rates in each treated group are indicated. **B,** inhibition of A431 tumor growth by AC0010 and gefitinib. Tumor growth curves are plotted as mean ± SEM ($n = 8$). **C,** AC0010 concentrations in plasma from AC0010-treated mice on days 1 and 8. The doses are indicated. p.o., oral administration. **D,** AC0010 concentrations in tumor tissues from AC0010-treated mice on days 1 and 8. Each dosing group is indicated. **E,** inhibition of EGFR phosphorylation in tumor tissues by AC0010 and gefitinib. pEGFR/EGFR values were derived from the densities of blotting bands of phosphorylated EGFR and total EGFR in NCI-H1975 tumor tissues from AC0010-treated mice after a single dose (top left) or after the last treatment of 8-day consecutive dosing (top right). The values (ratios) are indicated in the *y*-axis, and the lower number of pEGFR/EGFR indicates stronger inhibition. **F,** duration of antitumor efficacy and safety of AC0010 in subcutaneous xenograft NCI-H1975 mouse model. NCI-H1975 tumor-bearing mice were orally treated with vehicle control [0.5% methylcellulose (MC)] and AC0010 at dose levels of 12.5 and 50 mg/kg for 17 days when the tumor volume in vehicle control group reached approximately 2,000 mm^3. After 17-day dosing, the animals in vehicle control group were sacrificed, whereas animals in AC0010 groups were treated with increased dose at 500 mg/kg once per day for another 143 days. The tumor volume changes following total 160-day treatment of AC0010 are plotted as mean ± SEM.

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Table 1. AEs of evaluable-for-safety population in AC0010 dose–escalation stage ($N = 25$)

	AC0010 dose–escalation stage ($N = 25$)											
	50 mg ($n = 4$)		100 mg ($n = 5$)		200 mg ($n = 7$)		350 mg ($n = 3$)		550 mg ($n = 6$)		Total ($N = 25$)	
CTCAE grades	All	≥3	All	≥3	All	≥3	All	≥3	All	≥3	All	≥3
	Number of patients (%)											
Skin and subcutaneous tissue disorders												
Rash	0 (0)	0 (0)	0 (0)	0 (0)	2 (29)	0 (0)	1 (33)	0 (0)	2 (33)	1 (17)	5 (20)	1 (4)
Pruritus	0 (0)	0 (0)	0 (0)	0 (0)	1 (14)	0 (0)	1 (33)	0 (0)	2 (33)	0 (0)	4 (16)	0 (0)
Hyperpigmentation	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	2 (8)	0 (0)
Hand-foot syndrome	0 (0)	0 (0)	0 (0)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)
Gastrointestinal disorders												
Diarrhea	1 (25)	0 (0)	0 (0)	0 (0)	1 (14)	0 (0)	3 (100)	0 (0)	6 (100)	0 (0)	11 (44)	0 (0)
Nausea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (67)	0 (0)	2 (33)	0 (0)	4 (16)	0 (0)
Laboratory tests												
Aminotransferase increased	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)
WBC count decreased	1 (25)	0 (0)	0 (0)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	3 (12)	0 (0)
Respiratory, thoracic, and mediastinal disorders												
Cough	1 (25)	0 (0)	2 (40)	0 (0)	2 (29)	0 (0)	0 (0)	0 (0)	2 (33)	0 (0)	7 (28)	0 (0)
Hemoptysis	1 (25)	0 (0)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (12)	0 (0)
Dyspnea	0 (0)	0 (0)	0 (0)	0 (0)	2 (29)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	3 (12)	0 (0)
Nervous system disorders												
Paresthesia	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)
Dizziness	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)
AEs of interest												
Hyperglycemia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
QT prolongation	0 (0)	0 (0)	0 (0)	0 (0)	2 (29)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8)	0 (0)
Pneumonia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Abbreviation: WBC, white blood cell.

To further assess the potential skin toxicity of AC0010, a rat model was used. Rats were administrated daily with AC0010 at 300 mg/kg for 4 weeks, and in control groups, gefitinib at 50 mg/kg or vehicle control (0.5% methylcellulose) was administrated. Results showed that the skin lesions were observed in gefitinib-treated group, whereas, no apparent skin damage was found in AC0010-treated group (Supplementary Fig. S4).

AC0010 is safe and overcomes T790M-induced resistance in NSCLC patients

In this study, 25 patients were enrolled in a dose–escalation study (Supplementary Table S7). Only 1 DLT was observed at the dose level of 550 mg/day, where a female patient had developed a CTCAE grade 3 rash, due to the allergy to AC0010 (Table 1) but not to the inhibition of wild-type EGFR. AEs of any grade and of grade 3 or higher are summarized in Table 1. The most common AEs considered to be drug related were diarrhea (44% of patients), rash (20% of patients), and pruritus (16%). Although diarrhea and rash increased in frequency in a dose-dependent manner, the majority of them were grade 1. There was no drug discontinuation in all treated patients. No pneumonitis, hyperglycemia, or grade 3 prolongation of the corrected QT interval event was observed in these 25 patients.

Two relapsed NSCLC patients with positive EGFR T790M mutation detected in the biopsy samples after first-generation EGFR TKI treatment were treated with AC0010 at dose levels of 100 and 550 mg, and radiographic responses were confirmed with both patients (Fig. 5A and B). The first patient was a 59-year-old female with stage IV disease. The patient received 6 cycles of pemetrexed plus cisplatin as first-line chemotherapy, followed by the treatments with 2 cycles of pemetrexed, EGFR TKI (gefitinib) for 27 months, 2 cycles of pemetrexed plus Avastin, 6 cycles of pemetrexed plus cisplatin, and 1 cycle of docetaxel plus capecitabine. The patient was enrolled to receive AC0010 treatment at 100 mg dose cohort. Tumor shrinkage of 20% was detected after the first cycle treatment (4 weeks) and 31% after three cycle treatments (12 weeks; Fig. 5A). The second NSCLC patient was a 48-year-old male with stage IV disease. The patient received 3 cycles of pemetrexed plus carboplatin regimen as first-line chemotherapy, followed by gefitinib treatment for 41 months until the tumor relapsed. The patient was enrolled to receive AC0010 treatment at 550 mg dose cohort. Tumor shrinkage of 33% was detected after the first cycle treatment and 36% after three cycle treatments (Fig. 5B). Figure 5C shows the plasma pharmacokinetic profiles of the two patients after dosing at 100 and 550 mg once per day for 28 days, respectively.

Discussion

In recent clinical studies, the third-generation EGFR inhibitors, such as osimertinib and rociletinib, are capable of inhibiting mutant EGFR with T790M and produce responses in more than 50% of patients who acquired the resistance against first-generation EGFR TKIs (18, 19). Structurally, osimertinib, rociletinib, and previously reported tool compound WZ4002 are pyrimidine-based EGFR TKIs (17, 20–21). The new EGFR TKI, AC0010, reported in this study is a pyrrolopyrimidine-based third-generation EGFR TKI, which is structurally distinct from the currently reported third-generation EGFR TKIs. Using the docking model, the pyrrolopyrimidine core of AC0010 showed more stable binding than the pyrimidine core in tool compound WZ4002 in a computer-added model, and such stable binding also correlated well with the increase in the inhibitory potency of the mutant EGFR with T790M and a good selectivity over wild-type EGFR (Fig. 2). In both cell-based

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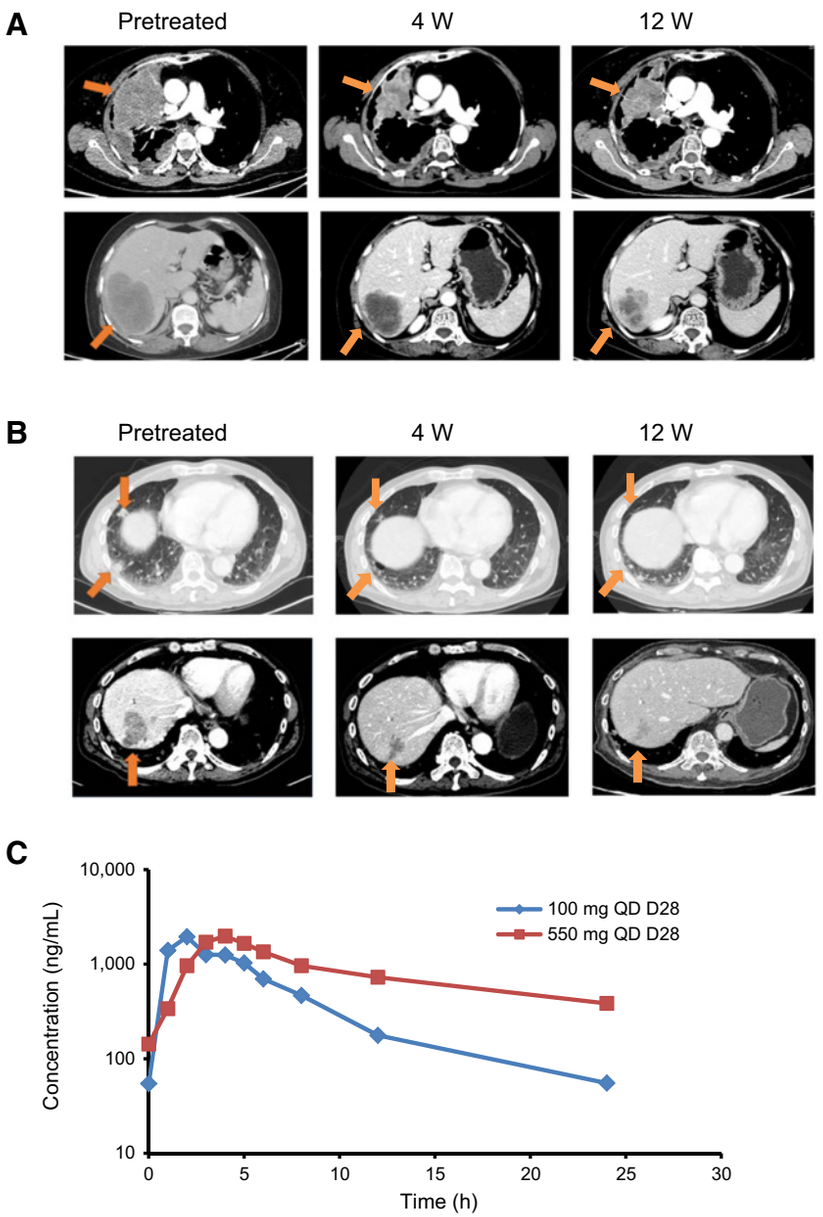

Figure 5.

Response of NSCLC patients with T790M-acquired mutations to AC0010 treatment and their AC0010 plasma concentrations. **A,** NCSLC patients received AC0010 treatment at a dose of 100 mg once per day for three cycles (28 days/cycle). The lesions indicated by arrows are shown in the CT images of lung (top) and liver (bottom). **B,** NCSLC patients received AC0010 treatment at a dose of 550 mg once per day for three cycles. The lesions indicated by arrows are shown in the CT images of lung (top) and liver (bottom). **C,** AC0010 from patients treated at doses of 100 and 550 mg per day. AUC_{0-24h} values were 10,800 and 19,500 ng·h/mL for patients receiving 100 mg per day (**A**) and for patients receiving 550 mg per day (**B**), respectively. QD, every day.

assays and animal models, AC0010 revealed the unique features of the third-generation EGFR TKI previously reported in other third-generation EGFR TKIs (17, 20–21), which include (i) irreversibly binding EGFR by forming a covalent bound with Cys 797 in the ATP-binding pocket (Supplementary Fig. S1); (ii) sparing wild-type EGFR; and (iii) overcoming T790M-induced resistance (Figs. 2 and 3). In *in vivo* duration study, 14 mice bearing the NCI-H1975 tumors were treated with AC0010 daily for 160 days, and tumors in 12 of 14 mice were inhibited during the 160-day treatment, suggesting the durable inhibition activity of AC0010. However, tumor relapses were detected in two mice at days 106 and 135, indicating acquisition of resistance against AC0010 may have developed in these two mice. Acquired drug resistance was also seen in the clinical studies of pyrimidine-based irreversible EGFR inhibitors, such as osimertinib and rociletinib (23–26). The resistance against the newly developed third-generation EGFR TKIs will significantly limit the long-term clinical success of third-generation EGFR TKIs. Results from nonclinical study models indicated that mechanisms by which the third-generation EGFR TKIs, including WZ4002, osimertinib, and rociletinib, induce resistance were similar and drugs are cross-resistant (27). Emerging clinical data also revealed that the C797S mutation is detected in approximately 40% of EGFR-mutant NSCLC patients with T790M who developed acquired resistance to osimertinib (23). Interestingly, some EGFR-resistant mutations induced by pyrimidine-based irreversible EGFR inhibitors, such as exon19 Del/C797S, are still sensitive to quinazoline-based EGFR inhibitors gefitinib and afatinib (27, 28). Therefore, irreversible EGFR inhibitors with different chemical core structure may reveal different resistant mechanisms. Using resistant cells derived from AC0010 long-term treated xenograft tumors and

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drug-induced resistant clones from cell cultures, we found that AC0010 showed cross-resistance with other third-generation compounds, such as osimertinib and rociletinib. However, our preliminary data showed that the resistance level is different between AC0010 and osimertinib (Supplementary Table S4). Studies to further understand the underlying mechanisms of acquired resistance against third-generation EGFR TKIs with different chemical structures are warranted to help us design better clinical treatment strategies for patients to gain maximum benefits from EGFR-TKI–based target therapy.

The preclinical pharmacokinetics/pharmacodynamics study demonstrated that the tumor inhibition of AC0010 is well correlated with duration of inhibition of EGFR phosphorylation in NCI-H1975 tumors (Fig. 3). At the dose of 200 mg/kg, the double mutant EGFR phosphorylation can be completely inhibited for 24 hours, resulting in 98% tumor growth inhibition, which suggests that the persistent exposure of AC0010 is required to gain the best therapeutic advantage in NSCLC patients. On the basis of the efficacy data of three EGFR-mutant xenograft mouse models at 50 and 200 mg/kg, which resulted in stable and regressive xenograft tumors, respectively, the human efficacious dose projected based on body surface area conversion factor (12.3) can be predicated to be in the range of 244 and 976 mg per day for a 60-kg human. Notably, in the phase I clinical trial (NCT02274337), a patient was responsive to AC0010 at the dose of 100 mg once per day (Fig. 5A). Interestingly, for this responsive case, AC0010 blood exposure was high, reaching to AUC_{0-24h} value of 10,800 ng·h/mL, which is above blood drug exposure at the effective doses in the mouse model, and close to the drug exposure of the patient at the dose of 550 mg per day (ACU_{0-24h} value, 19,500 ng·h/mL), who was also responsive to AC0010 (Fig. 5B). Detailed clinical pharmacokinetics study will be reported in separate reports.

The selective inhibition of the mutant EGFR by third-generation EGFR inhibitors greatly improves the on-target AEs that resulted from the equal inhibition of both wild-type and mutant EGFR by first-generation EGFR TKIs and second-generation EGFR TKIs, such as afatinib (29–31). In the rat model, AC0010 showed no skin lesion after 28-day treatment at a dose as high as 300 mg/kg (Supplementary Fig. S4). In clinical study, although the patient number is still small, much less occurrence of rash (24%) was seen in the patients treated with AC0010 and most of them were grade 1. Both in animal safety studies (data not shown) and in clinical trials, no severe off-target effects induced by AC0010 parent drug and its metabolites were seen, which indeed is consistent with weak to no binding of 55 safety-related target screening (Supplementary Table S2) and results from the biological analysis on AC0010 metabolite profile (Fig. 4). Off-target–related AEs were reported in patients who received the treatment of rociletinib (19). The predominant AE of rociletinib is hyperglycemia, which occurred in 47% of tested patients and 22% of them are grade 3. Hyperglycemia is caused by a rociletinib metabolite that inhibits IGF-1R (Supplementary Table S6; refs. 19, 22). AC0010 parent compound and three major metabolites do not inhibit IGF-1R (Supplementary Table S6). As a consequence, no hyperglycemia was detected in rats and monkeys in the long-term animal safety studies (data not shown) and in patients enrolled in the phase I dose–escalation study (Table 1). Furthermore, the three major metabolites of AC0010 revealed no inhibitory activity against either wild-type EGFR or mutant EGFR in contrast to a metabolite of osimertinib (AZ5104). Indeed, in patients, osimertinib showed frequent wild-type EGFR inhibition-related AEs, such as skin damage (40%), which might also have been resulted from the very potent activities of AZ5104 (21). Minimal effects on IGF1-R, on the other hand, were reported for osimertinib (32). The different safety profile of AC0010 and its major metabolites strongly suggests that AC0010 is distinct from the other two third-generation EGFR inhibitors, osimertinib and rociletinib.

AC0010 is a new third-generation EGFR inhibitor and showed potent activity against EGFR T790M mutation. Because of its distinct structure and metabolite profile, AC0010 might demonstrate unique therapeutic property in future clinical trials and provide another option for patients who develop resistance against first-generation EGFR inhibitors or for combination therapy with other anticancer agents.

Disclosure of Potential Conflicts of Interest

X. Xu has ownership interest (including patents) in ACEA Biosciences. C. Fang has ownership interest in ACEA stock. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Xu, W. Xu, W. Tang, X. Zhang, B. Xi, R. Xu, X. Fang, C. Fang, L. Zhao, L. Zhang

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Molecular Cancer Therapeutics

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