

## Original Article

# Pharmacologic characterization of CT-711, a novel dual inhibitor of ALK and c-Met

Lei Wang\*, Mingzhao Gao\*, Mengya Tong, Chengying Xie, Ye He, Li Fu, Yun Li, Haoyu Fu, Liguang Lou

*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China. \*Equal contributors.*

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**Abstract:** Anaplastic lymphoma kinase (ALK) is a validated molecular target for patients harboring ALK rearrangement, which triggers the development of ALK inhibitors. However, the activation of mesenchymal-epithelial transition factor (c-Met) has emerged as a common cause of acquired resistance induced by selective ALK inhibitors. Herein, we report the first preclinical characterization of CT-711, a novel dual inhibitor of ALK and c-Met. CT-711 demonstrates potent inhibitory activity against ALK kinase activity. Moreover, CT-711 profoundly inhibits ALK signal transduction and thereby induces G1 phase arrest and apoptosis, and results in remarkable anti-proliferative activity against ALK-driven cancer cells. Furthermore, CT-711 effectively inhibits c-Met kinase activity and potently overcomes the resistance mediated by c-Met activation. When orally administered to nude mice bearing xenografts, CT-711 exhibits favorable pharmacokinetic properties and robust antitumor activity. It is noteworthy that CT-711 is superior to crizotinib, the first-in-class ALK inhibitor, in the treatment of ALK-driven cancers in various models. The results of the current study provide a solid foundation for the clinical investigation of CT-711 in patients with tumors harboring ALK rearrangement.

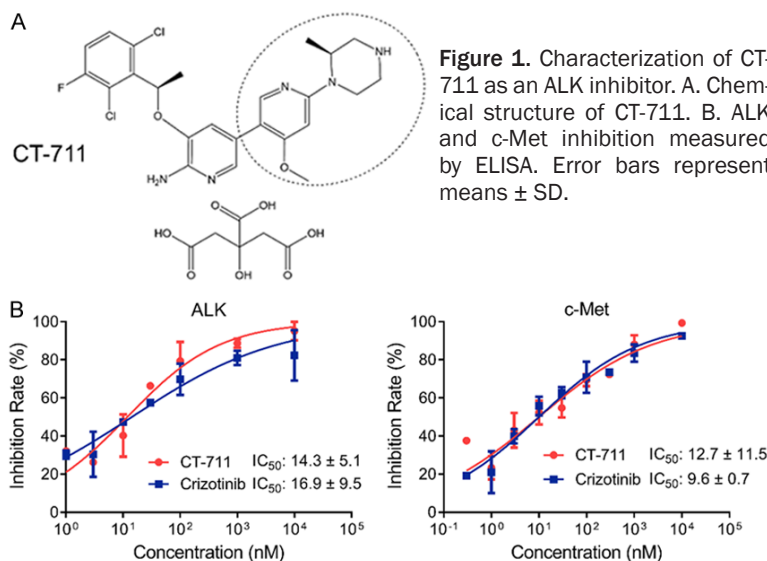
**Keywords:** CT-711, ALK, c-Met, crizotinib, antitumor activity, pharmacokinetics

## Introduction

Anaplastic lymphoma kinase (ALK) is a validated molecular target in several malignancies, including anaplastic large cell lymphoma (ALCL), inflammatory myofibroblastic tumor (IMT) and non-small-cell lung cancer (NSCLC), etc [1, 2]. ALK activates multiple pathways, including RAS/MEK/ERK and PI3K/AKT signaling cascades, which affect cell proliferation, transformation and survival signaling [3].

To date, four ALK inhibitors (crizotinib, ceritinib, alectinib and brigatinib) have been approved by the FDA for the treatment of patients with advanced NSCLC harboring ALK rearrangement, and others such as lorlatinib have shown promising results in clinical trials [4]. Crizotinib, which was developed initially as a mesenchymal-epithelial transition factor (c-Met) inhibitor, is the first-in-class ALK inhibitor with proven clinical efficacy in patients with ALK-rearranged NSCLC, ALCL and IMT [5, 6]. Ceritinib, alectinib and brigatinib are second-generation ALK inhibitors and are able to inhibit

secondary resistant mutations that are found in patients previously treated with crizotinib, but they are not efficient inhibitors of c-Met [7]. Several reports suggest that acquired resistance can be induced by the activation of c-Met signaling after the treatment with second-generation ALK inhibitors, such as alectinib, and has been shown to be overcome by crizotinib [8-10]. A phase II trial of crizotinib for alectinib-refractory patients according to HGF and MET status is ongoing (UMIN registration number 000015984). Lorlatinib is also a highly selective ALK inhibitor, which can overcome the resistance to the first- and second-generation ALK inhibitors [11]. However, a L1198F resistant mutation has been identified in an ALK-rearranged NSCLC patient after receiving lorlatinib. L1198F mutation confers resistance to lorlatinib, but restores sensitivity to crizotinib [12]. These findings suggest that the resistance to ALK inhibition is a dynamic process, highlighting the clinical value of further structural modification and optimization of crizotinib, the dual ALK/c-Met inhibitor.



**Figure 1.** Characterization of CT-711 as an ALK inhibitor. A. Chemical structure of CT-711. B. ALK and c-Met inhibition measured by ELISA. Error bars represent means  $\pm$  SD.

We rationally designed and synthesized a series of derivatives based on the chemical structure of crizotinib. One pyrimidine derivative, CT-711, stood out in the screening and was selected for the further evaluation. CT-711 is superior to crizotinib in the treatment of ALK-driven cancers in various models and can overcome the resistance conferred by c-Met activation. Here, we present the first report of the major preclinical pharmacological results of CT-711.

## Material and methods

### Reagents and antibodies

CT-711 was provided by Jiangsu Chia-tai Tian-qing Pharmaceutical Co., Ltd (Nanjing, China). Crizotinib and afatinib were purchased from Selleckchem (Houston, TX, USA). Antibodies against p-ALK, ALK, p-ERK, ERK, p-AKT, AKT, p-c-Met, c-MET, tubulin and PARP were purchased from Cell Signaling Technology (Beverly, MA, USA). ALK was purchased from Millipore (Bedford, MA, USA). c-MET was purchased from Calbiochem (Millipore, Bedford, MA, USA).

### Enzyme-linked-immunosorbent assays (ELISA)

The inhibition on enzymatic activity was determined by ELISA as described previously [13].

### Molecular docking

The X-ray crystal structure of ALK/crizotinib complex (PDB code: 2XP2) and c-Met/crizotinib

complex (PDB code: 2WGJ) were obtained from the Protein Data Bank. Molecular docking was performed using Glide v6.9 in its SP mode. LigPrep v3.6 was applied to pre-process the compound using default parameters. The obtained docked poses were analyzed with Maestro, PyMOL and LigPlot [14].

### Cell culture

SK-N-SH, NCI-H2228, NCI-H3122, SU-DHL-1, H460, HCC827 and 32D cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured

according to instructions provided by the manufacturer. 32D EML4-ALK, 32D EML4-ALK R1275Q, 32D EML4-ALK L1152R, 32D EML4-ALK C1156Y and 32D EML4-ALK L1196M cells were constructed by transfecting EML4-ALK, EML4-ALK R1275Q, EML4-ALK L1152R, EML4-ALK C1156Y and EML4-ALK L1196M plasmids into 32D cells. Acquired afatinib-resistant cells (HCC827-A1) were established by exposing parental HCC827 cells to increasing concentrations of afatinib (from 0.1 nM to 1  $\mu$ M) for 12 mo and selecting clones through the limiting dilution method.

### Cell proliferation assay

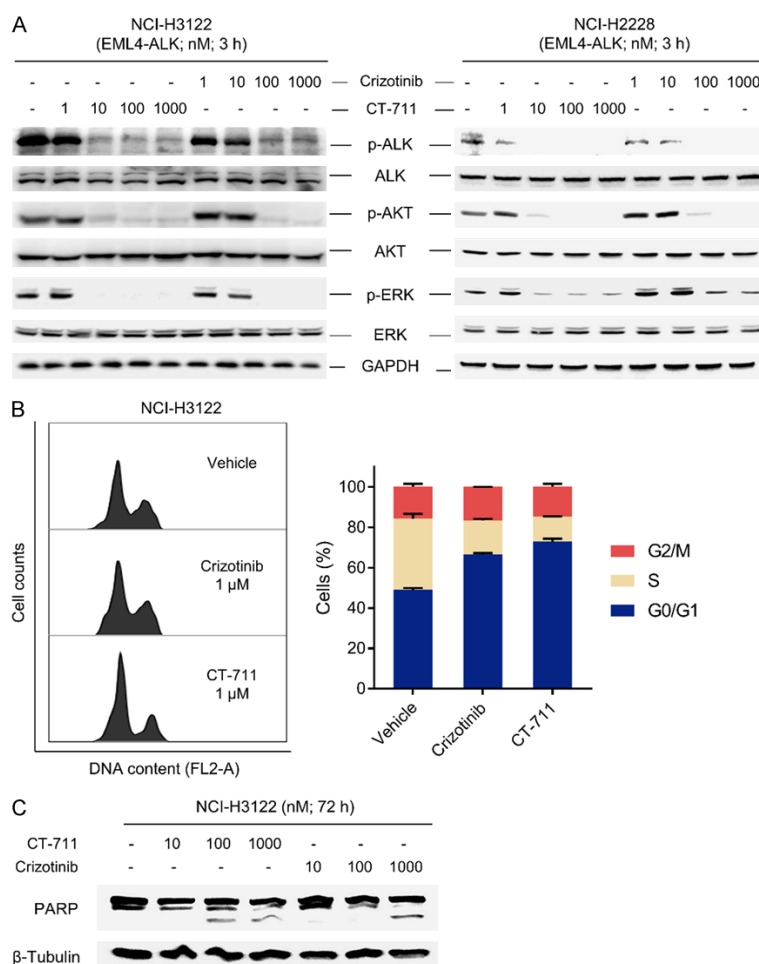
Cells were treated with serial dilutions of drugs for 72 h, and half maximal inhibitory concentration ( $IC_{50}$ ) was determined with MTT assay (for suspended cells) or sulforhodamine B assay (for adherent cells) as described previously [15].

### Western blotting

The standard Western blotting [15] was used to detect the changes in protein levels caused by the indicated treatments.

### Cell cycle analysis

Cells were fixated in ethanol and stained with propidium iodide following standard methods. The cell cycle was analyzed by fluorescence-activated cell sorting using a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA).



**Figure 2.** Effects of CT-711 on signalling transduction, cell cycle and apoptosis in ALK-driven cells. **A.** NCI-H3122 and NCI-H2228 cells were treated with CT-711 or crizotinib for 3 h. Whole cell lysates were detected by Western blotting. **B.** NCI-H3122 cells were treated with CT-711 or crizotinib for 24 h. Cell cycle was analyzed by flow cytometry. Left: representative images; Right: data from three separate experiments expressed as mean  $\pm$  SD. **C.** NCI-H3122 cells were treated with CT-711 or crizotinib for 72 h. Whole cell lysates were detected by Western blotting.

### In vivo study

Female Balb/cA nude mice (5-6 weeks old) were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). Tumor models were established by subcutaneously inoculating nude mice with NCI-H2228, NCI-H3122, SK-N-SH, SU-DHL-1, 32D EML4-ALK, 32D EML4-ALK L1196M and 32D EML4-ALK C1156Y cells. When tumors reached a volume of 100-300 mm<sup>3</sup>, mice were randomized into control (n = 12) or treatment (n = 6) groups. Control groups were given vehicle alone, and treatment

groups received CT-711 or crizotinib via intragastric daily for a total of 14 or 21 d. Tumor volume was calculated as (length  $\times$  width<sup>2</sup>)/2.

Pharmacokinetic/pharmacodynamic studies were carried out as described previously [16]. Mice bearing NCI-H2228 tumors received a single i.g. of 25 mg/kg CT-711 or crizotinib, and then tumor tissue and blood were collected at multiple time points (0, 0.5, 1, 2, 4, 8, 10, 24 h) post-dosing. Concentrations of CT-711 or crizotinib in plasma and tissue were determined by HPLC/tandem mass spectrometry. Tumor samples were lysed with RIPA buffer and analyzed by Western blotting.

All animal experiments were carried out in accordance with guidelines of the Institutional Animal Care and Use Committee at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

### Statistical analysis

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

### Results

#### CT-711 is a potent inhibitor of ALK and c-Met kinases

CT-711 (**Figure 1A**) significantly inhibited ALK in a cell-free enzymatic assay, exhibiting an IC<sub>50</sub> value of 14.3  $\pm$  5.1 nM (**Figure 1B**). Besides, CT-711 also inhibited the activity of c-Met, exhibiting an IC<sub>50</sub> value of 12.7  $\pm$  11.5 nM (**Figure 1B**). The IC<sub>50</sub>s of crizotinib against ALK and c-Met were 16.9  $\pm$  9.5 nM and 9.6  $\pm$  0.7 nM, respectively (**Figure 1B**). Based on these results, we conclude that CT-711 is slightly

**Table 1.** Anti-proliferative activity of CT-711 against ALK-driven and c-Met-driven cells

Cell lines	Types	Characteristic	IC <sub>50</sub> (nM, Mean ± SD)		IC <sub>(Cri)</sub> /IC <sub>50 (TQ)</sub> <sup>a</sup>
			CT-711	Crizotinib	
NCI-H3122	Non-Small Cell Lung Cancer	EML4-ALK fusion	46.5 ± 2.6	173.2 ± 13.9	3.7
NCI-H2228	Non-Small Cell Lung Cancer	EML4-ALK fusion	314.9 ± 255.1	468.1 ± 275.7	1.5
SU-DHL-1	Large Cell Lymphoma	NPM1-ALK fusion	15.9 ± 0.1	44.3 ± 1.6	2.8
SK-N-SH	Neuroblastoma	ALK F1174L	506.9 ± 37.3	626.6 ± 56.8	1.2
NCI-H460	Large Cell Lung Cancer	ALK WT	2456.5 ± 74.3	1914.5 ± 98.3	0.78
HCC827	Non-small cell lung cancer	ALK WT	4457.0 ± 155.6	3286.0 ± 312.5	0.74

<sup>a</sup>Cri, crizotinib; TQ, CT-711. Cells were treated with different concentrations of drugs for 72 h, and cell proliferation was measured using sulforhodamine B assays. Data shown represent means ± SD of three independent experiments.

**Table 2.** Anti-proliferative activity of CT-711 against 32D cell lines transfected with EML4-ALK or EML4-ALK mutations

Cell lines	Drug	IC <sub>50</sub> (nM, Mean ± SD)	IC <sub>50 (Cri)</sub> /IC <sub>50 (TQ)</sub> <sup>a</sup>
32D EML4-ALK	Crizotinib	108.6 ± 7.1	4.2
	CT-711	25.8 ± 0.2	
32D EML4-ALK R1275Q	Crizotinib	101.2 ± 0.5	4.8
	CT-711	22.6 ± 1.1	
32D EML4-ALK L1152R	Crizotinib	227.8 ± 6.7	3.7
	CT-711	60.9 ± 2.2	
32D EML4-ALK C1156Y	Crizotinib	926.5 ± 76.4	3.3
	CT-711	281.2 ± 0.5	
32D EML4-ALK L1196M	Crizotinib	433.4 ± 15.5	2.5
	CT-711	175.4 ± 0.6	

<sup>a</sup>Cri, crizotinib; TQ, CT-711. Cells were treated with different concentrations of drugs for 72 h, and cell proliferation was measured using MTT assays. Data shown represent means ± SD of three independent experiments.

nib and CT-711, respectively (**Figure 2B**), indicating a stronger cell cycle arrest induced by CT-711. Apoptosis was also assessed by the appearance of PARP intermediate cleavage product. CT-711 was significantly more potent than crizotinib, which paralleled the inhibition of ALK signaling pathway (**Figure 2C**). These data further confirmed that CT-711 is an ALK inhibitor with improved ALK inhibitory activity compared with crizotinib.

#### *CT-711 inhibits the proliferation of ALK-driven cells*

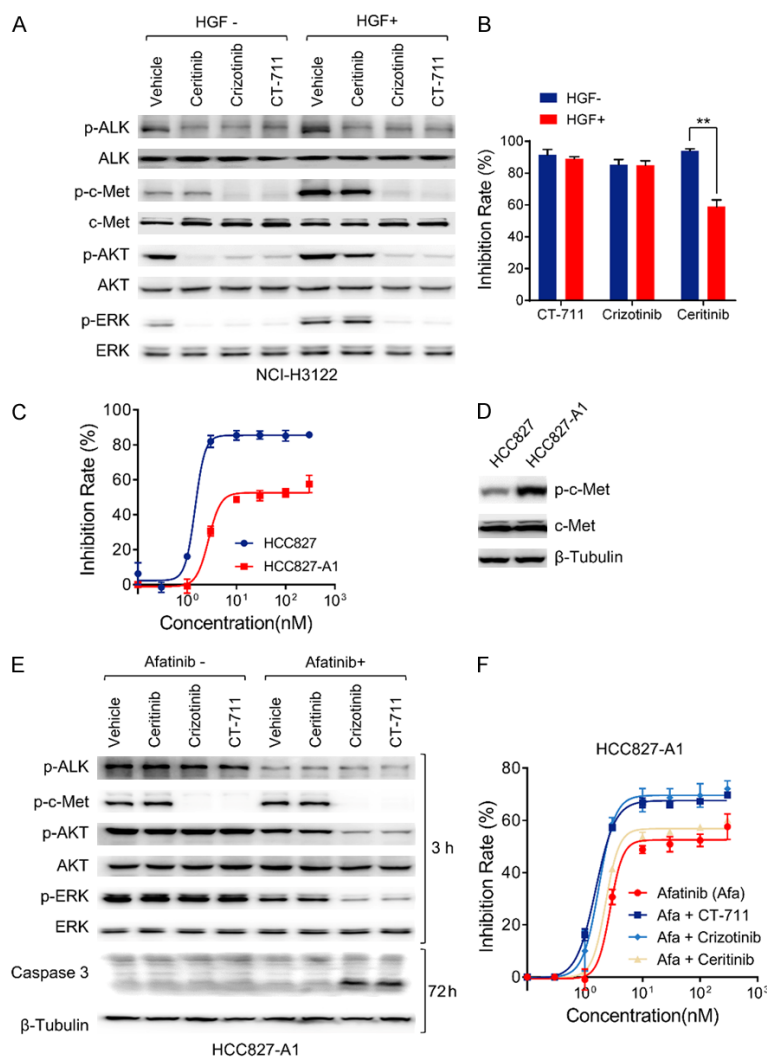
more potent than crizotinib in the inhibition of ALK and reserves the inhibitory activity against c-Met.

#### *CT-711 inhibits ALK signaling pathway and induces G1 arrest and apoptosis*

We next evaluated the target suppression activity of CT-711 against ALK in cancer cells. NCI-H3122 and NCI-H2228 cells harboring EML4-ALK fusion genes were exposed to CT-711 and the signaling pathway proteins were determined by Western blotting. CT-711 dose-dependently and markedly inhibited the phosphorylation of ALK and the downstream AKT and ERK (**Figure 2A**). It is noteworthy that CT-711 eliminated the signaling at 10 nM, whereas crizotinib needed 100 nM to do this (**Figure 2A**). Then, the cell cycle profile was evaluated in NCI-H3122 cells. The proportion of G1-phase cells was increased from the control level of 48.9% to 66.5% and 72.8% by crizoti-

Since it has been well demonstrated that ALK rearrangement contributes to the induction of tumor cell proliferation [17], we next evaluated the anti-proliferative effects of CT-711 against a panel of human cancer cell lines with distinct genotypes. CT-711 was preferentially efficacious against cells expressing EML4-ALK (NCI-H3122, NCI-H2228), NPM1-ALK (SU-DHL-1) and ALK activating F1174L point mutation (SK-N-SH), but not ALK wild-type cells (NCI-H460, HCC827) (**Table 1**). Notably, compared with crizotinib, CT-711 was more potent in the ALK-driven cancer cells and less potent in the ALK WT cancer cells, indicating that CT-711 shows much more selective targeting of ALK-driven cancer cells than crizotinib.

To further investigate the inhibitory activity of CT-711 against cells with clinically relevant ALK mutations, we expressed the EML4-ALK WT and four mutated forms of EML4-ALK in the 32D cell line. CT-711 effectively inhibited the



**Figure 3.** Effects of CT-711 on c-Met-mediated resistance. (A) NCI-H3122 cells were treated with CT-711 (1  $\mu$ M), crizotinib (1  $\mu$ M) and ceritinib (1  $\mu$ M) for 3 h, then treated with HGF (50 ng/mL) for 30 min. Whole cell lysates were detected by Western blotting. (B) NCI-H3122 cells were treated with CT-711 (1  $\mu$ M), crizotinib (1  $\mu$ M) and ceritinib (1  $\mu$ M) in the presence or absence of HGF (50 ng/mL) for 72 h. (C) HCC827 and HCC827-A1 cells were treated with afatinib for 72 h. Cell proliferation (in B and C) was measured using sulforhodamine B assays. Data shown represent means  $\pm$  SD of three independent experiments.  $^{**}P < 0.001$ . (D) Whole cell lysates of HCC827 and HCC827-A1 cells were detected by Western blotting. (E) HCC827-A1 cells were treated with afatinib (100 nM), CT-711 (1  $\mu$ M), crizotinib (1  $\mu$ M) and ceritinib (1  $\mu$ M) alone or in combination for 3 h. Whole cell lysates were detected by Western blotting. (F) HCC827-A1 cells were treated with afatinib alone or in combination with CT-711 (100 nM), crizotinib (100 nM) and ceritinib (100 nM) for 72 h. Cell growth was determined using the sulforhodamine B assay. Data shown represent means  $\pm$  SD of three independent experiments.

proliferation of 32D cells harboring EML4-ALK WT and R1275Q, and had relatively weaker effects on cells carrying EML4-ALK L1152R, C1156Y and L1196M. Notably, the inhibitory activity of CT-711 was more potent than that of

crizotinib in all the examined ALK-positive 32D cell lines (Table 2).

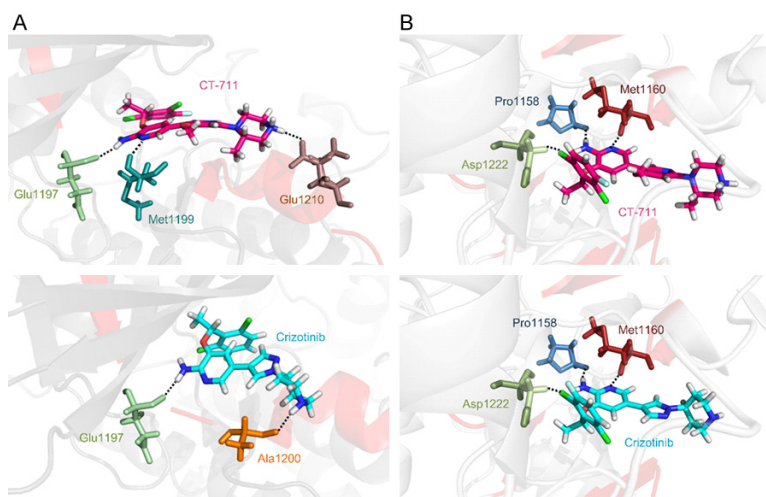
Collectively, the data suggested that CT-711 selectively inhibited ALK-driven cell proliferation with high potency.

*CT-711 inhibits c-Met and overcomes resistance conferred by c-Met activation*

As c-Met activation was a common cause of acquired resistance induced by selective ALK inhibitors [8-10], we next explored the capacity of CT-711 against c-Met induced resistance. HGF, a c-Met ligand, activated c-Met signaling in NCI-H3122 cells (Figure 3A). Ceritinib, a selective ALK inhibitor, failed to eliminate the activation of AKT and ERK in the presence of c-Met activation, despite its significant inhibitory activity on ALK. However, CT-711 revealed profound inhibition on the activation of both ALK and c-Met, as well as the downstream AKT and ERK (Figure 3A). Consistently, extrinsic HGF conferred resistance to ceritinib, but not to CT-711 in NCI-H3122 cells (Figure 3B).

We further evaluated the inhibition of CT-711 against resistant cells with c-Met overactivation. HCC827-A1, an afatinib (an EGFR inhibitor)-resistant cell line (Figure 3C) with aberrant c-Met activation (Figure 3D), was derived from the parental HCC827 cells by long-term exposure to afatinib. CT-711 significantly inhibited c-Met activation and restored the inhibition of afatinib on AKT and ERK phosphorylation in HCC827-A1 cells (Figure 3E). Moreover, CT-711 notably induced apoptosis when combined with afatinib (Figure 3E) and reversed





**Figure 4.** Structural modeling of CT-711 with ALK and c-Met. A. Molecular modeling of the ALK-crizotinib/CT-711 complex. B. Molecular modeling of the c-Met-crizotinib/CT-711 complex. Key residues of ALK were shown as sticks. Hydrogen bonds were shown as dashed lines.

the resistance of afatinib in HCC827-A1 cells (**Figure 3F**). In the contrast, the selective ALK inhibitor crizotinib had no such effects.

Together, these results suggest that CT-711 can inhibit c-Met and overcome the resistance conferred by c-Met activation, highlighting the advantage in the treatment of ALK-positive cancer accompanied with c-Met activation.

#### *Structural basis for the inhibition of ALK and c-Met by CT-711*

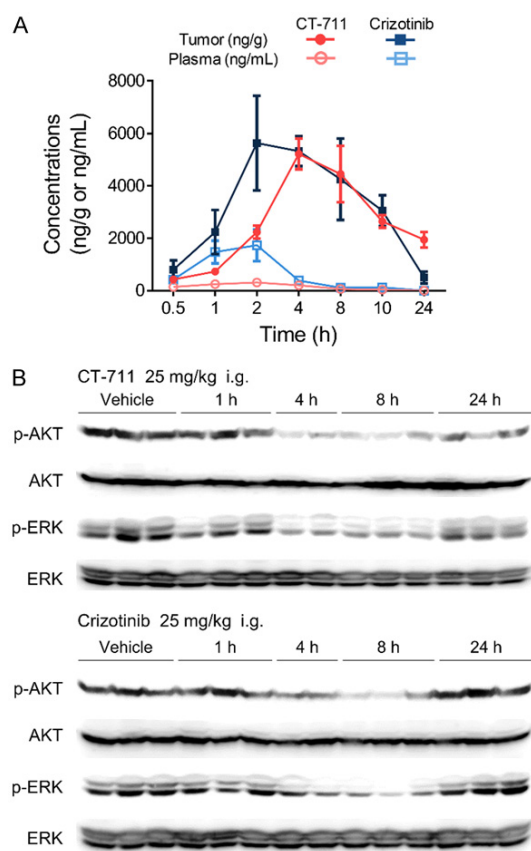
Next, we explored the binding sites of CT-711 in ALK using structural modeling. The ALK

structure from a crystal of ALK-crizotinib complex [18] was used as the template structure. CT-711 was found to occupy the same active pocket of ALK with crizotinib, but bind to ALK in a slightly different manner (**Figure 4A**). The nitrogen atom of piperazine in CT-711 formed a hydrogen bond with Glu1210, whereas the nitrogen atom of piperidine in crizotinib formed a hydrogen bond with Ala1200. Much more interestingly, the amino group and nitrogen atom in pyridine ring of CT-711 formed two hydrogen bonds with Glu1197 and Met1199, but the pyridine moiety in crizotinib formed only one hydrogen bond with Glu1197 (**Figure 4A**). The extra hydrogen bond in ALK-CT-711 complex may result in a closer binding of the compound to ALK and the stronger inhibitory activity against ALK kinase.

We also explored the binding mode of CT-711 in c-Met. The c-Met structure from a crystal of c-Met-crizotinib complex [18] was used as the template structure. We found that CT-711 occupied the same active pocket of c-Met with the same binding mode with crizotinib (**Figure 4B**).

#### *Pharmacokinetic characteristics of CT-711*

Given the superior activity of CT-711 against ALK *in vitro*, we next analyzed its pharmacokinetic profiles in the NCI-H2228 xenograft model. Following i.g. treatment at a dose of 25 mg/kg, the  $C_{max}$  of CT-711 in plasma and tumor tissue were 311.0 ng/mL and 5218.0 ng/g,



**Figure 5.** Pharmacokinetic/pharmacodynamic characteristics of CT-711 in NCI-H2228 tumor-bearing mice. NCI-H2228 tumor-bearing mice were intragastric administered a single 25 mg/kg dose of CT-711 or crizotinib and sacrificed at the indicated times. A. Concentrations of CT-711 and crizotinib in plasma and tumor were determined. B. Tumor extracts were analyzed by Western blotting.

**Table 3.** Pharmacokinetic parameters of CT-711 in NCI-H2228 tumor-bearing mice

		T <sub>max</sub> (h)	C <sub>max</sub> (ng/mL)	AUC <sub>all</sub> (ng*h/mL or ng*h/g)	AUC <sub>INF_obs</sub> (ng*h/mL or ng*h/g)	MRT <sub>last</sub> (h)	t <sub>1/2</sub> (h)
CT-711	Plasma	2.0	311.0	1906.2	1925.3	5.0	3.8
	Tumor	4.0	5218.0	67890.3	109023.9	23.6	14.6
Crizotinib	Plasma	2.0	1733.0	6472.4	6488.2	3.9	3.0
	Tumor	2.0	5635.0	67230.8	71083.3	8.9	5.3

Mice bearing NCI-H2228 tumors received a single i.g. of 25 mg/kg CT-711 or crizotinib. Concentrations of CT-711 or crizotinib in plasma and tissue were determined by HPLC/tandem mass spectrometry.

respectively (**Figure 5A** and **Table 3**). The C<sub>max</sub> in tumor was 32.7-fold higher than the *in vitro* IC<sub>50</sub> (314.9 nM or 159.5 ng/mL, **Table 1**) for inhibition of NCI-H2228 cell proliferation. Moreover, tumor concentrations were still higher than the IC<sub>50</sub> value (about 12.2-fold) at 24 h post administration. The exposure (AUC<sub>0-∞</sub>) of CT-711 in tumor (67890.3 h-ng/g) was 35.6-fold higher than that in plasma (1906.2 h-ng/mL) with a 3.8-fold longer half-life (14.6 vs 3.8 h). On the other hand, the exposure of crizotinib was 10.4-fold higher in tumor (67230.8 h-ng/g) than plasma (6472.4 h-ng/mL) with a 1.8-fold longer half-life (5.3 vs 3.0 h). CT-711 displays a higher distribution in tumor and longer half-life compared with crizotinib.

In addition, we also evaluated the effects of CT-711 on the ALK signaling pathway in NCI-H2228 xenografts. CT-711 exhibited a strong inhibition on p-AKT and p-ERK at 4 h and 8 h, and still showed some inhibition at 24 h (**Figure 5B**). However, crizotinib only decreased p-AKT and p-ERK at 4 h and 8 h, and signaling was completely recovered at 24 h (**Figure 5B**). The more durable inhibition of signaling by CT-711 may be resulted from its longer half-life in tumor.

#### CT-711 inhibits the growth of ALK-driven tumors *in vivo*

The antitumor potential of CT-711 was further investigated in multiple human tumor xenograft models *in vivo*. Tumor regression was observed in NCI-H2228, NCI-H3122 and SU-DHL-1 xenografts at a dose of 50 mg/kg (**Figure 6A**). However, the tumor shrinkage extent of crizotinib-treated groups was not as remarkable as that of CT-711 at the same dosage. Superior antitumor activity was also observed in SK-N-SH xenografts model, where CT-711 at a dose of 25 mg/kg and crizotinib at a dose of 50 mg/

kg caused similar growth inhibition effects (**Figure 6A**).

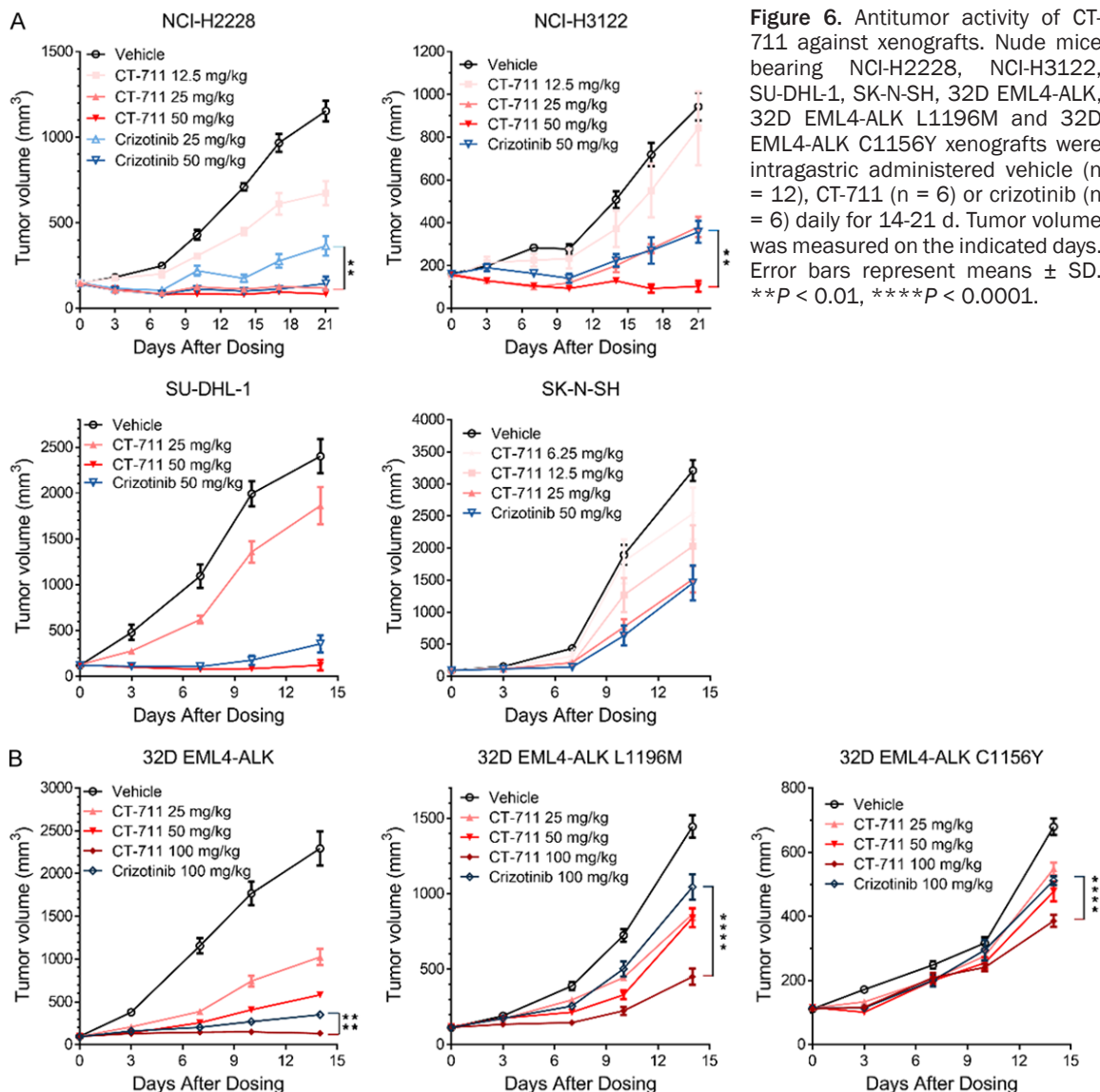
We also assessed the *in vivo* antitumor activity of CT-711 against 32D harboring EML4-ALK L1196M and EML4-ALK C1156Y mutations. Although CT-711 in 32D expressing resistant mutations was not as effective as that in 32D expressing EML4-ALK WT, the efficacy of CT-711 was significantly superior to that of crizotinib in all the models (**Figure 6B**). These results indicated that CT-711 might be more effective than crizotinib in patients with secondary mutations within EML4-ALK kinase.

Taken together, the superior *in vivo* antitumor efficacy of CT-711 underscores the potential use of CT-711 to treat ALK-driven cancer.

#### Discussion

We here present CT-711 as a novel dual ALK and c-Met inhibitor with potent anticancer activities. A remarkable characteristic of CT-711 was that it was superior to crizotinib in the treatment of ALK-driven cancers in various models, which included cells harboring EML4-ALK fusion gene (NCI-H3122 and NCI-H2228), NPM1-ALK fusion gene (SU-DHL-1) and the most aggressive mutation ALK F1174L (SK-N-SH), both *in vitro* and *in vivo*. Secondary mutations within the ALK kinase domain have emerged as a major resistance mechanism to ALK inhibitors [19]. CT-711 exhibited an advantage over crizotinib in models with resistant mutation, both *in vitro* and *in vivo*. The remarkable anti-tumor activity of CT-711 may result from two aspects:

First, CT-711 was significantly more potent than crizotinib at inhibiting ALK mediated process. The inhibitory activity of CT-711 against ALK was slightly greater than that of crizotinib in a cell-free enzyme assay. However, the differenc-



es of the signaling inhibitory activities between the two drugs were much bigger than their differences in inhibiting the enzymatic activities. CT-711 markedly inhibited the phosphorylation of ALK and the downstream AKT and ERK with about 10-fold higher efficiency than crizotinib. These effects led to apparent G1 arrest, apoptosis and thus selective cell killing in ALK-driven cells. CT-711 was superior over crizotinib in all these processes, which provided a basis for the significant anti-tumor activity.

Second, CT-711 had favorable pharmacokinetic and drug-like properties. In the NCI-H2228 xenograft model,  $C_{max}$  of CT-711 remained above the *in vitro*  $IC_{50}$  for more than 24 h, caus-

ing sustained inhibition of ALK signaling pathways. Notably, the distribution of CT-711 was more concentrated in tumor and the residence time in tumor was longer when compared with crizotinib. The exact reason why CT-711 provided these beneficial pharmacokinetic properties was unknown, but it might be attributable to the structure of the compound. The oil-water partition coefficients (Log P) of CT-711 (4.72) was higher than that of crizotinib (3.39), which indicated that the lipid solubility of CT-711 was better than crizotinib. The better lipid solubility of CT-711 might make it easier to enter xenografts. Meanwhile, it was assumable that CT-711 might be easier to cross the blood-brain barrier, indicating the potential



advantage in the treatment of ALK-driven brain tumor and brain metastasis tumor. Besides, the carbon at the ortho-position of nitrogen in piperazine ring is likely to be oxidized *in vivo*, and the methyl group in the piperazine ring of CT-711 might contribute to block this potential metabolic site. These features may result in a longer half-life of CT-711.

c-Met is the tyrosine kinase growth factor receptor for hepatocyte growth factor, which plays an essential role to mediate cell proliferation, invasion and metastasis [20]. Crizotinib showed significant clinical efficacy in c-Met-positive patients in clinic [21]. Moreover, crizotinib could overcome c-Met-mediated acquired resistance induced by second-generation ALK inhibitors [8-10]. CT-711 also possessed potent inhibitory activity against c-Met. In NCI-H3122 cells, c-Met activation conferred resistance to ceritinib, a selective ALK inhibitor. CT-711 potently inhibited c-Met activation and overcome the resistance. In addition, CT-711 reversed the resistance to afatinib in an afatinib-resistant cell line with c-Met overactivation.

Together, our study showed that CT-711, characterized as a novel dual ALK and c-Met inhibitor, had potent antitumor activity in preclinical models. CT-711 displayed improved ALK inhibition compared with crizotinib and potently suppressed the proliferation of the ALK-driven cancer cells. CT-711 had potent inhibitory activity against c-Met and could overcome the resistance conferred by c-Met activation. In xenografts of human tumor driven by ALK rearrangement, CT-711 exhibited good pharmacokinetic properties and robust antitumor activity. All these features supported its clinical investigation.

## Acknowledgements

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## Disclosure of conflict of interest

None.

## Abbreviations

ALK, Anaplastic lymphoma kinase; ALCL, anaplastic large cell lymphoma; IMT, inflammatory myofibroblastic tumor; NSCLC, non-small-cell lung cancer; c-Met, mesenchymal-epithelial transition factor.

**Address correspondence to:** Liguang Lou, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China. Tel: +86-21-50806056; Fax: +86-21-50806056; E-mail: lgilou@mail.shcnc.ac.cn

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