Discovery and development of pyrotinib: A novel irreversible EGFR/HER2 dual tyrosine kinase inhibitor with favorable safety profiles for the treatment of breast cancer

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ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 13 December 2016
Accepted 19 January 2017
Available online xxxx

Keywords:
Pyrotinib
HER2-positive breast cancer
Irreversible EGFR/HER2 dual tyrosine kinase inhibitor
Preclinical
Clinical
Safety
Drug metabolism

ABSTRACT

The discovery and development of a novel irreversible EGFR/HER2 dual tyrosine kinase inhibitor SHR1258 (pyrotinib) for the treatment of HER2-positive breast cancer is presented. The structure-activity relationship of lead series and their pharmacokinetic properties were evaluated to identify the potential candidates for further in vivo efficacy studies and preclinical safety assessments. Metabolic pathway and drug-drug interaction were also investigated in preclinical settings. In particular, major metabolites in human and animal species were assessed with regard to potential toxicity or off-target side effects. Overall, the potent and selective EGFR/HER2 dual inhibitor, pyrotinib, displayed robust anti-tumor effects on HER2-overexpressing xenograft models and sufficiently safety windows in animals as well as favorable pharmacokinetic properties in human, which substantially ensures current clinical development. Finally, recent advances of pyrotinib in clinical studies are highlighted with very encouraging outcomes in patients with HER2-positive advanced breast cancer.

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1. Introduction

Epidermal growth factor receptor (EGFR) consists of a family of four different intrinsic tyrosine kinase activities including EGFR (ErbB-1; human epidermal growth factor receptor 1 [HER1]), HER2 (c-ErbB-2), HER3 (c-ErbB-3), and HER4 (c-ErbB-4). As a diagnostic and prognostic biomarker for breast cancer, HER2 overexpression or HER2 oncogene amplification demonstrates poor symptoms and poor survival, and its occurrence rate is 15–30% among women suffering from breast cancer (Ahmed et al., 2015; Ross et al., 2009). Therefore, breast cancer coupled with overexpression of HER2 is generally considered as the most severe subtype of breast cancer due to little desirable response to chemotherapy, and higher rates of recurrence and metastasis (Eroglu et al., 2014; Gonzalez-Angulo et al., 2009).

Recent advances in the anti-HER2 therapies with monoclonal antibodies and the small molecule tyrosine kinase inhibitor (TKI) significantly improved the outcome of HER2-positive breast cancer patients. Several HER2-targeted therapeutics approved by the U.S. Food and Drug Administration (FDA) for the treatment of HER2-positive breast cancer include monoclonal antibodies (trastuzumab and pertuzumab), an antibody-drug conjugate (ado-trastuzumab emtansine) and a reversible dual TKI for EGFR and HER2 (lapatinib) (Singh et al., 2014). Although trastuzumab is the first line therapy for patients with HER2-overexpressing metastatic breast cancer, a significant number of patients in the initial clinical trials of trastuzumab monotherapy showed resistance to trastuzumab-based therapy (Figueroa-Magalhaes et al., 2014; Hubalek et al., 2010; Valabrega et al., 2007). Similarly, lapatinib is effective in a subset of trastuzumab-refractory cases, but the majority of patients displayed resistance as well (Garrett and Arteaga, 2011; Sun et al., 2015). As a result, combination with chemotherapeutics is frequently adopted to maximize its therapeutic effect. However, the patients have to suffer from the painful side effects brought from systemic chemotherapeutics (Vu et al., 2014; Wu et al., 2015). More recently, great efforts have been made to develop irreversible TKIs since the covalent binding of electrophilic groups of the inhibitors with cysteine residues of the receptor could effectively overcome drug-resistance triggered by receptor mutation (Canonici et al., 2013). This new type of inhibitors, either launched or presently being developed in the stage of clinical phase III trials, include EGFR/HER2...
dual TKIs, afatinib (Hurvitz et al., 2014) also previously known as BIBW 2992 (Spicer and Rudman, 2010), and neratinib (HKI-272) (Bose and Ozer, 2009; Burstein et al., 2010; Feldinger and Kong, 2015). In clinical studies of patients with HER2-positive advanced breast cancer, an MTD of 240 mg or 320 mg was reported for neratinib (HKI-272) in different groups of patients (Io et al., 2012; Wong et al., 2009). The common neratinib-related adverse effects (AEs) including diarrhea (88%), nausea (64%), fatigue (63%) and vomiting (50%) have been reported (Wong et al., 2009). Among them, the grade III diarrhea occurred in 41% of patients in the MTD cohort (n = 39) and 83% of patients in the 400 mg cohort (n = 6), respectively (Wong et al., 2009). In addition, partial response was observed for 8 (32%) of the 25 evaluable patients with advanced breast cancer, who had previous treatment with trastuzumab, anthracyclines, and taxanes, and tumors with a baseline ErbB-2 immunohistochemical staining intensity of 2+ or 3+. Furthermore, recent clinical trials of afatinib (BIBW 2992) for patients with HER2-positive breast cancer indicated severe toxicities. Therefore, no further development of afatinib is currently planned for HER2-positive breast cancer (Cortes et al., 2015; Ring et al., 2015).

In this article, we present a novel irreversible EGFR/HER2 dual tyrosine kinase inhibitor, SHR1258 (pyrotinib), which is currently being developed for the treatment of HER2-positive breast cancer following lead optimization process and structure-activity relationship studies. The in vitro pharmacological profile, in vivo preclinical antitumor activities, pharmacokinetic characteristics and overall safety profiles of SHR1258 were also evaluated in preclinical settings. We highlight on-going clinical studies of pyrotinib exhibiting an acceptable safety profile, favorable PK properties and encouraging anti-tumor responses in patients with HER2-positive advanced breast cancer.

2. Materials and methods

2.1. Chemicals and reagents

SHR1258 (Pyrotinib) maleate (98.8%) and the reference substances of O-depicolyl metabolite (M1), O-depicolyl and pyrrolidine lactam metabolite (M2), and pyrrolidine lactam metabolite (M5) were synthesized by Hengrui Medicine Co., Ltd. (Shanghai, China). Recombinant human P450 isoenzymes (CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, and CYP4A11) and liver microsomes were purchased from BD Gentest (Woburn, MA). Purified water was generated by Milli-Q gradient water purification system (Millipore, Molsheim, France). Other commercially available reagents were of analytical grade.

2.2. LC-MS/MS instrumentation and general conditions

For preclinical studies, LC-MS/MS for in vitro and in vivo sample bioanalysis consisted of an HPLC system (Shimadzu, Japan) equipped with a binary solvent manager, an auto sampler and an AB4000 triple quadrupole mass spectrometer (AB SCIEX) with electrospray ionization (ESI) source. MS detection was performed in positive ESI mode (scan mode MRM) with the source temperature maintaining at 500 °C. Settings for other parameters included the IonSpray voltage at 5500 V, curtain gas (nitrogen) 15 psi and collision gas 6 psi. Generally, ion source 1 and ion source 2 gases were set at 50 psi. Data acquisition and analysis were performed using Analyst software (AB SCIEX); A Hypersil Gold C8 column (30 mm × 2.1 mm i.d., 1.9 μm; Thermo, USA) thermostatted at 35 °C. The flow rate was set to 0.3 mL/min. The mobile phase consisted of water with 0.2% formic acid (A) and acetonitrile (B). Gradient elution started from 15% (B), followed by a linear gradient to 85% (B) over 6.6 min and held for another 0.6 min then zoomed to 15% (B) in the next 0.1 min, and finally re-equilibrated to 15% (B) in 0.9 min. For human PK studies and metabolite quantifications, another MS detection was employed as described previously (Zhu et al., 2016).

2.3. In vitro liver microsome stability

The liver microsomal incubations consisted of 100 mM PBS (pH 7.4) containing test compound, e.g., 1 μM SHR1258, 1 mM NADPH and 0.5 mg/mL liver microsomes (mouse, rat, dog and human, respectively). The reaction mixtures were pre-incubated for 3 min at 37 °C before the addition of corresponding liver microsomes, then terminated at 0, 5, 15, 30, 45 min by adding equal volume of ice-cold acetonitrile. The final concentration of organic solvents was <0.1% in all incubations. The samples were centrifuged at 12000 rpm for 10 min at 4 °C ready for bioanalysis.

2.4. CYP phenotyping and metabolite identification studies

In vitro CYP phenotyping for identifying metabolizing CYP enzymes and metabolite identifications in in vivo and human samples were described in the previous study (Zhu et al., 2016).

2.5. In vivo PK and human PK studies

Animals utilized for preclinical studies include nude mice, rats and dogs. All animals were treated in accordance with Institutional Guide for the Care and Use of Laboratory Animals. Nude mice (around 20 g, 9 males and 9 females) were purchased from Sino-British Sippr/BK Lab Animal Co. Ltd. (Shanghai) (SCXK 2013–0016), Sprague Dawley (SD) rats (200–250 g, 3 males and 3 females) from Shanghai SLAC Laboratory Animal Co., LTD (SYXX 2003–0029), and beagle dogs (9–13 kg, 2 males and 2 females) from Beijing Marshall Biotechnology Co., Ltd. (SCXK 2009–0002), respectively. Briefly, test compounds were administrated in both intravenous (i.v.) and intragastric (i.g.) for mice, rats and dogs in order to obtain their bioavailability. Plasma samples of nude mice, SD rats and dogs were collected at pre-dose and 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h after the IV administration, plasma samples of SD rats were collected at pre-dose and 1.0, 2.0, 3.0, 3.5, 4, 4.5, 5, 6, 8, 12, 24 h and plasma samples of beagle dogs were collected at pre-dose and 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24 h after the i.g. dose.

Human sample collection was conducted in Teda International Cardiovascular Hospital (Tianjin, China) with study protocol approved by the ethics committee of the hospital. Written informed consent was obtained from the subjects enrolled in this study. Ten healthy subjects participated in the study for human PK and metabolite identification as well as elimination study. After an overnight fast, each subject received a single oral administration of 240 mg pyrotinib maleate tablet. Plasma samples were collected at pre-dose and 0.5, 1, 2, 3, 4, 5, 6, 7, 9, 12, 24, 36, 48, 72, and 96 h post-dose. Urine samples were collected at pre-dose and 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–72, and 72–96 h post-dose. Feces samples were collected at pre-dose and 0–24, 24–48, 48–72, and 72–96 h post-dose. All the samples were preserved at −80 °C until analysis. A range of five doses (80, 160, 240, 320 and 400 mg) was conducted for Phase I dose escalation study. All PK and TK parameters were calculated throughout a non-compartmental model using Phoenix WinNonlin software (5.2) (Pharsight Corporation, USA).

2.6. Sample preparation for bioanalysis

The 50 μL aliquot of acetone (containing internal standard) and 250 μL of acetone solution were added to 50 μL of the pooled plasma, followed by vortexed and centrifugation for 5 min (12,000 rpm). The supernatant was subsequently transferred to a clean 96-well plate for injection. The urine samples were treated as same as plasma samples. Feces samples from subjects were ground and weighed for each time interval. Then 5–fold volume of methanol (1.5, g/v) was added to each 1 g feces, followed by homogenization and ultrasonication for 15 min then vortex and centrifugation for 5 min (12,000 rpm). The
supernatant was subsequently transferred to a clean 96-well plate for injection.

2.7. Preclinical toxicity and toxicokinetic (TK) studies

Prior to chronic toxicity studies on rat and dog, dose escalations were investigated in both rats and dogs to determine approximate maximum tolerated dose (MTD), respectively. Then, a rat chronic toxicity study (5, 20, 100 mg/kg) up to 182 days was conducted, followed by a dog chronic toxicity study (3, 10, 30/45 mg/kg) up to 272 days. The corresponding TK and accumulation factor data as well as major findings are discussed in preclinical toxicity section. It should be noted that the highest dose was changed from 45 mg/kg to 30 mg/kg after 15 days due to observed adverse effect during the dog chronic toxicity studies.

2.8. In vitro activity and in vivo efficacy studies

The EGFR/HER2 kinase inhibition assays were utilized to determine the in vitro activity of the compounds. The half maximal inhibitory concentration IC50 (the concentration of the tested compound showing 50% inhibition of the enzyme activity) of each compound was measured by incubating a series of concentrations of the tested compounds with a specific enzyme and substrate. The EGFR kinase assay used a human-derived recombinant protein (cell signaling technology, #7908), which reacted with the peptide substrate at different concentrations of test compounds in a buffer solution containing a mixture of 60 mM HEPES (pH 7.5), 5 mM MgCl2, 5 mM MnCl2, 3 mM Na2VO4, 1.25 M DTT and 20 μM ATP at 25 °C for 45 min. The HER2 Kinase Assay Kit was purchased from Invitrogen Corporation (#PV3366), which was reacted with the protein substrate (Tyr 87) at different concentrations of tested compounds in a buffer solution containing a mixture of 60 mM HEPES (pH 7.5), 5 mM MgCl2, 5 mM MnCl2, 3 mM Na2VO4, 1.25 M DTT and 20 μM ATP at 25 °C for 60 min. Both EGFR and HER2 kinase activities were determined by a time-resolved fluorescence method.

The general procedures of the in vitro cell proliferation inhibition assays were performed on cancer cells (A431, SK-BR-3 and NCI-N87) at a suitable concentration (e.g., 5000 cells/mL medium). Then the cells were incubated in a carbon dioxide (5% CO₂) incubator until they reached 85% confluency, subsequently, cell culture medium was replaced by fresh one with test compounds added in a series of concentrations (generally 6 to 7 concentrations). The cells were then put back to the incubator and cultured continuously. After 72 h, the activity of the test compounds for inhibiting the cell proliferation was determined by a colorimetric assay (Cell counting kit-8).

In vivo efficacy studies were performed on BALB/Ca-nude mice (6 to 7 weeks, female) from SLAC. Nude mice were hypodermic inoculated BT-474 human breast cancer cell or SK-OV-3 ovarian cancer cell. After tumor grew to 150 to 250 mm³, mice were randomly divided into groups and dosed once daily. The volume of tumors and the weight of the mice were measured and recorded for 2–3 times per week. The volume of tumor (V) was calculated as V = 1/2 axxb² (a: length of tumors, b: width of tumors). Tumor growth inhibition (TGI) was calculated as: TGI (%) = 100 – (VT – VTD) / (VC – VCD) × 100%; where VTD and VT are the tumor volumes of the beginning and finish days of dosed groups, respectively; and VCD and VC are the tumor volumes of the beginning and finish days for the control group, respectively. In the case of tumor regression, TGI was calculated as: TGI (%) = (VT – VTD) / VTD × 100.

2.9. Synthesis of SHR1258 (pyrotinib)

2.9.1. Synthesis of [(2R)-1-methylpyrrolidin-2-yl]methanol (12b)

Lithium aluminium hydride (230 mg, 6 mmol) and N tert-butoxycarbonyl-l-prolinal (400 mg, 2 mmol) were dissolved in 10 mL of dry tetrahydrofuran in an ice-water bath. After 20 min, the reaction mixture was heated to reflux for 2 h. Then, 5 mL of methanol was added dropwise to the reaction mixture in an ice-water bath, followed by addition of 5 mL of water, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to obtain compound (12R)-1-methylpyrrolidin-2-yl)methanol 12b (221 mg, yield 77.0%) as a colorless oil, which has a 116 [M + 1] in MS m/z (ESI).

2.9.2. Synthesis of (2R)-1-methylpyrrolidine-2-carbaldehyde (12c)

Dimethyl sulfoxide (820 μL, 11.46 mmol) was dissolved in 5 mL of dichloromethane in a dry ice-acetone bath, followed by dropwise slow addition of oxalyl chloride (968 mg, 7.64 mmol). After stirring for 45 min, a solution of [(2R)-1-methylpyrrolidin-2-yl)methanol 12b (220 mg, 1.91 mmol) in 2 mL of dichloromethane was added to the solution. The reaction mixture was stirred for 45 min, and triethylamine (1.9 mL, 13.37 mmol) were added. The reaction mixture was stirred for 10 min, then warmed up to room temperature and stirred for 1 h. The reaction mixture was washed with water (20 mL) and saturated brine (10 mL) successively. The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure, and the resulting residue was purified by alumina column chromatography to obtain (2R)-1-methylpyrrolidine-2-carbaldehyde 12c (300 mg) as a yellow solid, which was directly used in the next step without purification.

2.9.3. Synthesis of compound 12d

N-[[3-Chloro-4-(2-pyridylmethoxy)phenyl]amino]-3-cyano-7-ethoxy-6-quinolyl]-3-(2-methylpyrrolidin-2-yl)prop-2-enamide, was according to following procedure.

**Please cite this article as:** Li, X., et al., Discovery and development of pyrotinib: A novel irreversible EGFR/HER2 dual tyrosine kinase inhibitor with favorable safety profile, European Journal of Pharmaceutical Sciences (2017), http://dx.doi.org/10.1016/j.ejps.2017.01.021
(624 mg, yield 99.9%) as a light yellow solid, which has a 624 [M + 1] in MS m/z (ESI).

2.9.4. Synthesis of compound 12

(E)-N-[4-[[3-Chloro-4-(2-pyridylmethoxy)phenyl]amino]-3-cyano-7-ethoxy-6-quinolyl]-2-diethoxyphosphoryl-acetamide (compound 12).

N-[4-[[3-Chloro-4-(2-pyridylmethoxy)phenyl]amino]-3-cyano-7-ethoxy-6-quinolyl]-2-diethoxyphosphoryl-acetamide 12d (250 mg, 0.40 mmol) was dissolved in 10 mL of dry tetrahydrofuran in a dry ice-acetone bath, followed by dropwise addition of a solution of lithium bis (trimethylsilyl)amide (1 M) in toluene (440 μL, 0.44 mmol). The reaction mixture was stirred for 30 min, added dropwise with a solution of (2R)-1-methylpyrrolidine-2-carbaldehyde 12c (90 mg, 0.80 mmol) in 5 mL of tetrahydrofuran, and stirred for 30 min, then warmed up to room temperature and stirred for 12 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography to obtain compound 12 (46 mg, yield 19.7%) as a yellow solid, which has a 583.4 [M + 1] in MS m/z (ESI).

1H NMR (400 MHz, DMSO-d6): δ 9.16 (s, 1H), 8.63 (d, 1H, J = 4.4 Hz), 8.56 (s, 1H), 8.26 (s, 1H), 7.83 (t, 1H, J = 9.2 Hz), 7.76 (m, 2H), 7.57 (m, 1H), 7.40 (d, 2H, J = 10.8 Hz), 7.19 (d, 1H, J = 8.8 Hz), 7.06 (m, 2H), 6.34 (d, 1H, J = 15.2 Hz), 5.53 (s, 2H), 4.39 (m, 2H), 3.32 (m, 1H), 3.10 (m, 2H), 2.73 (s, 3H), 2.37 (m, 2H), 2.08 (m, 2H), 1.64 (t, 3H, J = 6.8 Hz).

3. Results and discussion

3.1. Lead optimization and structure-activity relationship

Based on the structure of HKI-272, the first set of chemical screening indicated that retention of the upper right warhead of scaffold was necessary for potency, while the change of down left warhead seemed insensitive to activity. We focused mainly on modifications in the down left warhead as exemplified in R-X in Fig. 1. Three series of compounds were thus explored and structure-activities of selected compounds with their in vivo PK properties are given in Table 1. Many compounds showed high potency in the EGFR- and HER2-dependent biochemical and cellular assays. As highlighted in Table 1, although compounds 3 and 4 showed good potencies, the oral plasma exposures (AUC) of these two compounds were too low in rats. The compounds 1 and 2 showed favorable rat PK profiles, however, compound 2 seemed to have extremely high clearance-to-bioavailability ratio in dogs (CL/F = 1983 mL/min/kg, data not shown in Table 1). In another sulfone series such as compounds 5, 6, 7 and 8, all turned out to be fast metabolized in in vivo rat PK. In the third series X = none (compounds 9 and 10), it was observed that the potency was decreased dramatically. Excitingly, the single five membered-ring compounds (11, 12) showed high potency and adequate PK profiles, which were selected for further in vivo efficacy study. The de-methyl compound 13 is more potent than others but the plasma exposure in rats was too low. Finally, compound 12 named as SHR1258 showed overall advantages in terms of in vitro potency, favorable PK profiles and in vivo antitumor efficacies as well as safety profiles, which was thus selected as a preclinical candidate (PCC) for further development. Overall PK profiles and safety assessments of SHR1258 are discussed in details in Sections 3.2, 3.5 and 3.6.

In addition, Fig. 1 shows the binding model of SHR1258 in the catalytic region of HER2 kinase. In this model, the N1 atom of the 3-cyanoquinoline forms a hydrogen bond with the hinge region Met-801. Meanwhile, the double bond of the inhibitor SHR1258 connected with Cys-805 through the Michael-addition reaction, which can form the covalent binding with target enzymes as an irreversible inhibitor (Ahmed et al., 2015; Ross et al., 2009; Wisser and Mansour, 2008). Based on the high similarity of SHR1258’s structure to HKI-272 (Fig. 1) as well as the same hinge area and binding sites, SHR1258 was presumed to form covalent binding with target enzymes as reported in reference (Wisser and Mansour, 2008).

The potential risk of covalent binding forming reactive metabolites is further discussed in metabolite assessment Section 3.4.

3.2. Overall pharmacokinetic properties of pyrotinib

Table 2 summarizes the physicochemical properties and overall PK properties of pyrotinib in preclinical animal species with acceptable bioavailability of 20.6%, 43.5% and 13.5% in nude mice, rats and dogs, respectively. Pyrotinib had favorable drug-like physicochemical...
properties with an LogD<sub>7.4</sub> of 2.68 and a solubility of 13.2 µM in fasten state simulated intestinal fluid (FaSSIF, pH = 6.5). In particular, the data showed relatively higher oral exposure of pyrotinib in human subjects with a much longer half-life than that of preclinical animal species such as mouse, rat and dog (Table 2). Good correlations between the predicted hepatic clearance (CL<sub>hep</sub>) from in vitro mouse and dog

Table 1

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liver microsomal stability and the observed in vivo clearance in mice (37.2 mL/min/kg) and in dogs (28 mL/min/kg), respectively. However, a poor correlation of in vitro and in vivo metabolized was observed in rats. In addition, mass balance studies in rats suggested that fecal excretion was the major route of elimination for pyrotinib. It should be pointed out that formulations used for animal PK studies were commonly used suspensions without further optimization. Despite predicted high clearance in human, pyrotinib exhibited a notable exposure in human used as a reference drug to validate the efficacy model. All the compounds were dosed once daily (QD) over a 21-day period. The volume of tumors and the weight of the mice were measured and recorded for 2–3 times per week. The TGI% (tumor growth inhibition) of SHR1258 on day 21 is 109%, 157%, 159% at the doses of 5 mg/kg, 10 mg/kg, 20 mg/kg respectively, while HKI-272 is 152% at 20 mg/kg. It was apparent that SHR1258 and HKI-272 were well tolerated at all the doses tested without mortality or significant body weight loss (<5% relative to the vehicle controls) observed during the treatment (Fig. 2, A and B). In addition, as shown in Fig. 2 (C and D), SHR1258 in the secondary SK-OV-3 ovarian xenograft model showed the result (TGI% on day 21 is 2%, 12%, 83% at the doses of 2.5 mg/kg, 5 mg/kg, 10 mg/kg respectively), which further confirmed its robust in vivo antitumor efficacy at 10 mg/kg. Therefore, it was concluded that SHR1258 exhibited an effective inhibition or antitumor efficacies with statistical significance (P < 0.005) based upon two relevant tumor growth models relative to the vehicle controls.

### 3.4. Metabolism and drug–drug interaction (DDI) evaluation

As shown in Table 2, in vitro liver microsomal stability data of pyrotinib indicates relatively short half-lives and predicted moderate to high hepatic clearance cross species in mouse, rat, dog and human using a simplified prediction model (Wan et al., 2010). Phase I CYP enzymes were believed to play an important role in pyrotinib’s metabolism. Reaction–phenotyping data turned out that the pyrotinib was hardly metabolized in recombinant human isoenzymes such as CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP4A11. In contrast, pyrotinib was observed to undergo extensive metabolism in the incubation with CYP3A4 with >75% of the parent drug consumed. Furthermore, CYP3A5 was also observed to contribute to the metabolism of pyrotinib but only with a decrease of approximately 15% of the parent drug after 1 h incubation. Given that the abundance and metabolism rate of CYP3A5 is much lower than CYP3A4 in vivo, the CYP3A4 was identified as the most active enzyme catalyzing the metabolism of pyrotinib, and to a lesser extent by CYP1B1, CYP2C8, CYP2C19, CYP2D6, and CYP3A5. It should also be mentioned that most of the phase I metabolites characterized in vitro after 1 h incubation with liver microsomes and recombinant CYP3A4 were also detected in vivo in humans as well as in animal species, whose major metabolites are discussed in more details in the following metabolite section. Based on in vitro CYP inhibition (IC50) and human Cmax as shown in Table 4, it can be estimated that CYP3A4 shows large safety margins (>81-fold human using the highest dose 400 mg), when using their total Cmax for DDI potential evaluations. Considering that the metabolism of pyrotinib was predominantly catalyzed by CYP3A4 which has the highest abundance in humans and broad substrate specificity, and in particular, cancer patients are often on multiple medications with a number of market drugs mainly metabolized by CYP3A4 (Niwa et al., 2008; Zhou et al., 2005), it is worthwhile to monitor the effects of co-administered CYP3A4 inhibitors or inducers on pharmacokinetics and metabolism of pyrotinib in clinic, since the change of plasma concentration or metabolic behaviours is directly relevant to drug safety and efficacy due to DDIs (Dresser et al., 2000). A drug metabolized by a polymorphic enzyme and/or a primary enzyme (e.g. >25 of the total metabolic clearance) has an increased relative risk of drug–drug interactions and/or individual variations. As a notable example of neratinib metabolized mainly by CYP3A4 (Lopez-Tarruella et al., 2012), coadministration with the potent CYP3A4 inhibitor, ketoconazole, significantly increased the exposure of neratinib in humans, with the Cmax and AUC raised by 3.2- and 4.8-fold, respectively. It was thus recommended for neratinib to adopt dose adjustment when administered with CYP3A4 inhibitors (Abbas et al., 2011). As pyrotinib proved a sensitive substrate of CYP3A4 by phenotyping study, it is likely that pyrotinib is also susceptible to the interaction with CYP3A4 inhibitors due to its similar structure and metabolic pathway to neratinib. As a result, further evaluation of the pyrotinib for clinical DDIs with potent CYP3A4 inhibitors or inducers would be highly recommended (FDA, 2012).

### Table 2

Overall physicochemical properties and pharmacokinetic properties of pyrotinib in animal species and human.

<table>
<thead>
<tr>
<th>PK</th>
<th>Dose (mg/kg)</th>
<th>t1/2 (h) in vitro</th>
<th>Cmax (μL/min/kg)</th>
<th>CL (μL/min/kg)</th>
<th>Vd (μL/kg)</th>
<th>Cmax (ng/mL)</th>
<th>t1/2 (h) in vivo</th>
<th>AUC0-24h (ng-h/mL)</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (i.v.)</td>
<td>0.5</td>
<td>0.93</td>
<td>56</td>
<td>37.2</td>
<td>5027</td>
<td>181*</td>
<td>1.56</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>Mouse (i.g.)</td>
<td>1</td>
<td>3.6</td>
<td>181*</td>
<td>39,413</td>
<td>15.6</td>
<td>2.52</td>
<td>92.2</td>
<td>20.6</td>
<td></td>
</tr>
<tr>
<td>Rat (i.v.)</td>
<td>3</td>
<td>3.6</td>
<td>54.3</td>
<td>3.5</td>
<td>1350</td>
<td>3.5</td>
<td>4.42</td>
<td>15,012</td>
<td></td>
</tr>
<tr>
<td>Rat (i.g.)</td>
<td>3</td>
<td>3.5</td>
<td>NA</td>
<td>NA</td>
<td>730</td>
<td>3.38</td>
<td>6530</td>
<td>43.5</td>
<td></td>
</tr>
<tr>
<td>Dog (i.v.)</td>
<td>1</td>
<td>0.35</td>
<td>21.2</td>
<td>28</td>
<td>8157</td>
<td>3.41</td>
<td>603</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dog (i.g.)</td>
<td>3</td>
<td>0.35</td>
<td>NA</td>
<td>NA</td>
<td>40.2</td>
<td>3.06</td>
<td>243</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Human (oral)</td>
<td>1.3</td>
<td>0.31</td>
<td>18.5</td>
<td>45.3*</td>
<td>57,000</td>
<td>32.3</td>
<td>15.0</td>
<td>510</td>
<td></td>
</tr>
</tbody>
</table>

Physicochemical properties

- LogD = 2.68, Solubility = 13.2 μM in FaSSIF (pH 6.5)

Mean values of male and female at the lowest dose. Human dose 80 mg and taking 60 kg as the average body weight. i.v. formulations for mouse, rat and dog: normal saline; i.g. formulations for animal PK studies were commonly used suspensions without further optimization. Despite predicted high clearance in human, pyrotinib exhibited a notable exposure in human used as a reference drug to validate the efficacy model. All the compounds were dosed once daily (QD) over a 21-day period. The volume of tumors and the weight of the mice were measured and recorded for 2–3 times per week. The TGI% (tumor growth inhibition) of SHR1258 on day 21 is 109%, 157%, 159% at the doses of 5 mg/kg, 10 mg/kg, 20 mg/kg respectively, while HKI-272 is 152% at 20 mg/kg. It was apparent that SHR1258 and HKI-272 were well tolerated at all the doses tested without mortality or significant body weight loss (<5% relative to the vehicle controls) observed during the treatment (Fig. 2, A and B). In addition, as shown in Fig. 2 (C and D), SHR1258 in the secondary SK-OV-3 ovarian xenograft model showed the result (TGI% on day 21 is 2%, 12%, 83% at the doses of 2.5 mg/kg, 5 mg/kg, 10 mg/kg respectively), which further confirmed its robust in vivo antitumor efficacy at 10 mg/kg. Therefore, it was concluded that SHR1258 exhibited an effective inhibition or antitumor efficacies with statistical significance (P < 0.005) based upon two relevant tumor growth models relative to the vehicle controls.

### Table 3

Inhibition of cell proliferation for SHR1258.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tumor type</th>
<th>HER2 expression</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR1258</td>
<td>H2K-272</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT474</td>
<td>Breast</td>
<td>++ + +</td>
<td>5.1</td>
</tr>
<tr>
<td>SK-OV-3</td>
<td>Ovarian</td>
<td>++ + +</td>
<td>43.0</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast</td>
<td>–</td>
<td>3500</td>
</tr>
</tbody>
</table>

Please cite this article as: Li, X., et al., Discovery and development of pyrotinib: A novel irreversible EGFR/HER2 dual tyrosine kinase inhibitor with favorable safety profile, European Journal of Pharmaceutical Sciences (2017), http://dx.doi.org/10.1016/j.ejps.2017.01.021
In addition to favorable CYP safety margins, the estimated hERG safety windows of pyrotinib are sufficiently enough (>16-fold) in human as estimated in Table 4, based on in vitro hERG data and total human plasma Cmax in a conservative manner. It should be emphasized that the CYP and hERG safety windows can be considerably greater using free Cmax instead of total Cmax for evaluations when taking into consideration the plasma protein binding of pyrotinib (fraction unbound = 0.75% in human). It was suggested that a 30-fold margin between hERG IC50 and free Cmax may suffice for drugs currently undergoing clinical evaluations (Redfern et al., 2003).

### 3.5. Metabolite safety assessments

In the previous study (Zhu et al., 2016), metabolic pathway of pyrotinib has been detailed with a total of 24 metabolites identified based on chromatographic retention, accurate mass measurement and MS2 spectra in human plasma, feces, and urine, respectively. The structures of three major metabolites M1, M2, and M5 were also confirmed using synthetic reference standards. As highlighted in Fig. 3, three key metabolites were likely biotransformed via the dealkylation, oxidation, dehydrogenation and carbonylation of phase I metabolic pathway to form O-depicolyl (M1), O-depicolyl and pyrrolidine lactam (M2) and pyrrolidine lactam (M5). The metabolites M1 and M2 exhibit strong activities at EGFR but not at HER2, and M5 has very weak potency at EGFR and no activity at HER2, which are advantageous from the viewpoints of PK/PD modeling and safety evaluation. Herein, the safety of major metabolites (i.e. M1, M2 and M5) circulating in human was evaluated according to FDA’s guidance on metabolite in safety testing (MIST) (FDA, 2008). Table 5 summarizes the exposures of three major metabolites and the corresponding percentages of each metabolite to the parent drug pyrotinib in human as well as preclinical animal species from rats and dogs. In comparison with the exposures of major metabolites in human with those of animal species, it is apparent that the exposure level of key metabolite M5 (>10%) circulating in human has been covered in rat animal species, where the exposure is also close to that of dog animal species tested. The exposure level (AUC) of M1 in human is close to that of rat animal species, and the exposure level (AUC) of M2 in human is covered by rat animal species and is similar to that of

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**Fig. 2.** In vivo efficacies of SHR1258 in mouse BT-474 (A, B) and SK-OV-3 (C, D) xenograft models. (A): Relative tumor growth versus treatment days and (B): Average body weight versus treatment days in BT-474 model; (C): Relative tumor growth versus treatment days and (D): Average body weight versus treatment days in SK-OV-3 model.

### Table 4

Estimated safety margins of CYP inhibition and hERG based total human Cmax.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>CYP1A2, 2C9, 2D6, 3A4 (μM)</th>
<th>CYP2C19 (μM)</th>
<th>hERG (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>43 0.07</td>
<td>160</td>
<td>264 70</td>
</tr>
<tr>
<td>160</td>
<td>102 0.17</td>
<td>240</td>
<td>109 29</td>
</tr>
<tr>
<td>240</td>
<td>156 0.27</td>
<td>320</td>
<td>240 18</td>
</tr>
<tr>
<td>320</td>
<td>175 0.31</td>
<td>400</td>
<td>60 16</td>
</tr>
<tr>
<td>400</td>
<td>170 0.29</td>
<td></td>
<td>64 17</td>
</tr>
</tbody>
</table>

CSS-max: steady state plasma concentration; CYP safety margins calculated by in vitro CYP inhibition IC50/day28 CSS-max; hERG safety margins calculated by in vitro hERG IC50/day28 CSS-max, based on a 28 day repeat dose (QD). Last day Cmax is the geometric mean values of several subjects.

* No hERG inhibition detected at a concentration of 25 μM.
dog animal species. In addition, the circulating metabolites may contribute to inhibitory DDIs (Isoherrannen et al., 2009). However, our additional study confirmed that these major circulating metabolites M1, M2 and M5 have no liabilities in CYP inhibition with all IC50 values of major CYPs over 5 μM. It is also noteworthy that pyrotinib as an irreversible TKI possesses α,β-unsaturated carbonyl moiety as a Michael acceptor for possible covalent binding with the target enzymes from bioactivation to reactive metabolite formation, which likely introduce side-effect or idiosyncratic adverse drug reactions (IADRs) (Kalgutkar and Didiuk, 2009; Park et al., 2006). As a result, stable phase II metabolic reactions including cysteine and N-acetylcysteine conjugates were also evaluated. The metabolites resulting from cysteine and N-acetylcysteine conjugation with the electrophilic group of pyrotinib were detected in humans, but only in low levels in vivo (data not shown), which was similar to the metabolic behaviours of afatinib (Stopfer et al., 2012). In order to improve the targeting effect as well as selectivity and minimize the potential of off-target binding (e.g., glutathione and cysteine throughout the body), the reactivity of the electrophilic group should be relatively low, and irreversible inhibition must occur after proximity to their target proteins via a first non-covalent interaction to the active sites of the target enzymes (Carmi et al., 2012; Singh et al., 2011). Therefore, large amounts of glutathione or cysteine conjugates should be avoided when evaluating in vivo metabolic behaviours of TCIs (Shibata and Chiba, 2015). It was reported that acrylamide or substituted acrylamide proved to be the good choice for TCIs to provide balanced reactivity and selectivity (Fry, 2000). Our study confirmed that pyrotinib only generated a negligible amount of cysteine and N-acetylcysteine conjugates, suggesting minor off-target binding. In other words, only a limited propensity to form reactive metabolites with cysteine and N-acetylcysteine ensured the absorbed drug to impart therapeutic effect by efficient covalent binding with the target enzymes. Overall, based on preclinical metabolite assessments from animal species in comparison with human metabolite profiles, it can be concluded that testing of additional human major metabolites for toxicity is not necessary.

3.6. Preclinical safety studies

As summarized in Table 6, preclinical chronic toxicity studies in rats with repeated dose (QD) for 182 days resulted in an MTD > 100 mg/kg with corresponding exposures 202,260 (ng·h/mL), while chronic toxicity studies in dogs with repeated dose (QD) for 272 days gave an MTD > 30 mg/kg with relatively lower exposures around 2462 (ng·h/mL). Toxicokinetic data of daily gavage exposures show a good linear dose-exposure relationship in rat, dog as well as on human except for a saturated absorption observed at the highest dose of 400 mg for human (Table 6). As mentioned above, the exposure level of in vivo efficacy (5 mg/kg) on nude mouse xenograft model was approximately 460 ng·h/mL from the nude mouse PK exposure. Consequently, the estimated preclinical safety margins of pyrotinib, based on the rat and dog MTD exposures and the efficacy exposure, would be 440-fold and 5.4-fold, respectively, which indicates favorable safety profiles in preclinical animal species.

Table 5
Exposures of three major metabolites and corresponding percentages of each metabolite to pyrotinib.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>M1% (AUCM1/AUC0)</th>
<th>M2% (AUCM2/AUC0)</th>
<th>M5% (AUCM5/AUC0)</th>
<th>M1 (AUC, ng·h/mL)</th>
<th>M2 (AUC, ng·h/mL)</th>
<th>M5 (AUC, ng·h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>80 mg</td>
<td>7.70</td>
<td>7.10</td>
<td>15.10</td>
<td>60.7</td>
<td>55.3</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>160 mg</td>
<td>5.20</td>
<td>3.90</td>
<td>14.80</td>
<td>85.3</td>
<td>63.3</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>240 mg</td>
<td>5.80</td>
<td>4.50</td>
<td>15.40</td>
<td>162</td>
<td>126</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>320 mg</td>
<td>7.60</td>
<td>6.20</td>
<td>13.20</td>
<td>278</td>
<td>228</td>
<td>483</td>
</tr>
<tr>
<td></td>
<td>400 mg</td>
<td>7.70</td>
<td>7.10</td>
<td>15.10</td>
<td>251</td>
<td>191</td>
<td>500</td>
</tr>
<tr>
<td>Rat</td>
<td>5 mg/kg</td>
<td>0.03</td>
<td>0.03</td>
<td>32.63</td>
<td>7.15</td>
<td>7.07</td>
<td>6943</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg</td>
<td>0.07</td>
<td>0.12</td>
<td>42.03</td>
<td>53.2</td>
<td>106</td>
<td>34,179</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
<td>0.06</td>
<td>0.15</td>
<td>40.96</td>
<td>190</td>
<td>493</td>
<td>132,492</td>
</tr>
<tr>
<td>Dog</td>
<td>3 mg/kg</td>
<td>0.92</td>
<td>3.06</td>
<td>17.08</td>
<td>2.98</td>
<td>10.1</td>
<td>59.0</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
<td>1.27</td>
<td>4.25</td>
<td>11.79</td>
<td>21.9</td>
<td>69.5</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>1.88</td>
<td>6.41</td>
<td>9.60</td>
<td>58.4</td>
<td>184</td>
<td>281</td>
</tr>
</tbody>
</table>

Last dose in human (400 mg), in rat (100 mg/kg) and dog (30 mg/kg) indicates the MTD. M% is the percentage of metabolite AUC to parent pyrotinib AUC.
In addition, as shown in Table 6, the data shows acceptable or consistently low level of pyrotinib accumulations in rat (repeated dose up to 182 days), in dog (repeated dose up to 272 days) as well as in human (repeated dose up to 28 days) in comparison with the exposure level of last day to that of the first day administration. This is also line with the observed tissue distribution in rats, where tissue concentrations dramatically declined with time from $t_{\text{max}}$ (around 1 h) to 8 h, although the concentrations of some tissue such as stomach, small intestine (SI), spleen, fat and lung were relatively higher than plasma concentrations (Fig. 4). Although it was surprising to notice some adverse effects in dogs after administrating 45 mg/kg for 15 days at a relatively lower level of exposure than that of rat toxicity study, neither apparent accumulations nor specific metabolites were found in dog species.

Table 6
Toxicokinetics of pyrotinib in rats, dogs and human subjects.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC$_{24}$ (ng·h/mL) (Day 1)</th>
<th>AUC$_{24}$ (ng·h/mL) (Last day)</th>
<th>Accumulation factor (AF)</th>
<th>Note</th>
<th>Dose regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat(a)</td>
<td>5 mg/kg</td>
<td>11,573</td>
<td>14,746</td>
<td>1.27</td>
<td></td>
<td>NOAEL = 5 mg/kg</td>
</tr>
<tr>
<td></td>
<td>QD, 182 day</td>
<td>20 mg/kg</td>
<td>35,287</td>
<td>47,696</td>
<td>1.35</td>
<td>MTD &gt; 100 mg/kg</td>
</tr>
<tr>
<td></td>
<td>repeat dose</td>
<td>100 mg/kg</td>
<td>132,211</td>
<td>202,260</td>
<td>1.53</td>
<td>NOAEL = 3 mg/kg</td>
</tr>
<tr>
<td>Dog(a)</td>
<td>3 mg/kg</td>
<td>241</td>
<td>262</td>
<td>1.09</td>
<td></td>
<td>NOAEL = 3 mg/kg</td>
</tr>
<tr>
<td></td>
<td>QD, 272 day</td>
<td>10 mg/kg</td>
<td>859</td>
<td>1454</td>
<td>1.69</td>
<td>MTD &gt; 30 mg/kg</td>
</tr>
<tr>
<td></td>
<td>repeat dose</td>
<td>45/30 mg/kg(b)</td>
<td>4009</td>
<td>2462</td>
<td>0.61</td>
<td>QD, 28 day repeat dose</td>
</tr>
<tr>
<td>Human(d)</td>
<td>80 mg</td>
<td>415</td>
<td>549</td>
<td>1.32</td>
<td></td>
<td>MTD</td>
</tr>
<tr>
<td></td>
<td>160 mg</td>
<td>926</td>
<td>1260</td>
<td>1.35</td>
<td></td>
<td>MTD</td>
</tr>
<tr>
<td></td>
<td>240 mg</td>
<td>1320</td>
<td>2080</td>
<td>1.57</td>
<td></td>
<td>MTD</td>
</tr>
<tr>
<td></td>
<td>320 mg</td>
<td>1970</td>
<td>2660</td>
<td>1.35</td>
<td></td>
<td>MTD</td>
</tr>
<tr>
<td></td>
<td>400 mg</td>
<td>1860</td>
<td>2270</td>
<td>1.22</td>
<td></td>
<td>MTD</td>
</tr>
</tbody>
</table>

a AUC data presented are mean values of male and females.
b The highest dose was changed from 45 mg/kg to 30 mg/kg after 15 days due to observed adverse effect.
c AF calculated by AUCLast Day/AUCDay1.
d AUC data presented are geometric mean values of human subjects. NOAEL: no observed adverse effect level; MTD: maximal tolerated dose.

In addition, as shown in Table 6, the data shows acceptable or consistently low level of pyrotinib accumulations in rat (repeated dose up to 182 days), in dog (repeated dose up to 272 days) as well as in human (repeated dose up to 28 days) in comparison with the exposure level of last day to that of the first day administration. This is also line with the observed tissue distribution in rats, where tissue concentrations dramatically declined with time from $t_{\text{max}}$ (around 1 h) to 8 h, although the concentrations of some tissue such as stomach, small intestine (SI), spleen, fat and lung were relatively higher than plasma concentrations (Fig. 4). Although it was surprising to notice some adverse effects in dogs after administrating 45 mg/kg for 15 days at a relatively lower level of exposure than that of rat toxicity study, neither apparent accumulations nor specific metabolites were found in dog species.

In terms of common adverse effects observed during toxicity studies in both rats and dogs, major findings of rat toxicity studies at MTD included slower bodyweight gain, slightly decreased food consumption, partial hair lost on the head, neck, back and/or abdomen, slight or mild atrophy/blunting of small intestine villi on ileum and duodenum. Significantly decreased triglyceride (TG), total protein (TP) and albumin (ALB) ($P > 0.001$) were observed at MTD dose in rat, but these blood chemistry parameters (TG, TP and ALB) seemed to be normal at the NOAEL dose as compared to the control group. While major findings of dog toxicity testing appeared somewhat different from rat species, in addition to reduced motor activity and decreased body weight and food consumption, some emesis or fluid feces, mild degeneration of seminiferous tubules and epididymal ducts associated with epididymal oligospermia on the histopathological examination were observed. Although some mild and apparently reversible toxicities were seen in the MTD dose groups compared to the control groups, no obvious accumulations and metabolites-related toxicities were observed in either species, and no deaths were seen in all dose groups for either species.

3.7. Current clinical development
At present, pyrotinib is under clinical investigations in both China (Xu et al., 2015a, b) and USA, which was originally being developed for targeting solid tumors with overexpression of HER2 patients. Up to date, six phase I and one phase I/II clinical studies of pyrotinib are being undertaken in China (6 trials) and United States (1 trial). The first-in-human study of tolerability, safety and PK of pyrotinib, and another phase I study of the food’s impact on PK conducted in healthy subjects have been completed. The single administration of pyrotinib in an increasing dose of 80–400 mg demonstrated a desirable PK profile of pyrotinib with the $t_{\text{max}}$ of 4.0–5.5 h, $t_{1/2}$ of 15.0–20.9 h, C$_{\text{max}}$ of 32.3–179 ng/mL and AUC$_{0-24}$ of 510–3260 ng·h/mL, respectively, and its half-life supported once daily dosing regimen. Our analysis on the
dose-exposure (C_{max} and AUC_{0–1}) relationship showed good linear PK characteristics. In addition, PK parameters of T_{max}, C_{max}, and AUC were not statistically significant (P > 0.05) between genders at all doses. With regard to safety, pyrotinib was well tolerated with only grade 1 AEs reported, and no dose-limiting toxicity (DLT) was observed up to 400 mg when administered to healthy subjects. In the second study performed in healthy subjects, it was noticed that food enhanced its bioavailability with increases of 45.5% in AUC_{0–1} and of 80.6% in C_{max} following the 320 mg administration comparing fed (n = 11) with fasted (n = 11) conditions, recommending to administer the drug under fed condition.

In the first-in-patient study (NCT01937689), a total of 38 patients with HER2-positive metastatic breast cancer orally received 80 mg (n = 3), 160 mg (n = 8), 240 mg (n = 8), 320 mg (n = 9), 400 mg (n = 8) or 480 mg (n = 2) of pyrotinib once daily. Since the first 2 patients in the 480 mg cohort experienced DLT (grade III diarrhea), the MTD of pyrotinib was 400 mg per day as determined in this study (Xu et al., 2015b). The AUC_{0–24} and C_{max} parameters indicated high exposure levels without significant accumulation after repeated dosing of 28 days in Chinese patients. The most common AEs were grade 1/II gastrointestinal symptoms such as diarrhea. The incidence and severity of diarrhea were dose-dependent and clinically manageable up to 400 mg. The majority of incidences occurred in the first cycle was transient. Furthermore, pharmacodynamic profile of the antitumor activity was notable for pyrotinib, which were with the best ORR of 55.6% and 87.5% for the 320 mg and 400 mg dose cohorts, and the median PFS was corresponding 31.9 and 59.7 weeks, respectively, in Chinese patients with HER2-positive metastatic breast cancers who previously treated with trastuzumab or trastuzumab na"ive. The data from pyrotinib mono-therapy supports further investigations in the phase I dose-escalation study of pyrotinib in combination with capecitabine (NCT02361112) as well as phase I/II study of pyrotinib in combination with capecitabine (NCT02422199) in breast cancer patients with HER2-positive metastatic disease. The recruitments of these studies in Chinese patients with HER2-positive metastatic breast cancer have been completed, and these studies are ongoing to collect the long-term safety and efficacy data. The preliminary results indicated that the pyrotinib at 400 mg once daily in combination with capecitabine was also generally safe and well-tolerated with encouraging antitumor response when administrated to HER2-positive breast cancer patients. In summary, preliminary clinical findings indicate that pyrotinib may achieve an excellent balance between efficacy and toxicity. As a novel promising therapeutic agent with potent irreversible inhibition to both HER2 and EGFR, the pivotal phase III studies for the treatment of HER2-positive breast cancer will be undertaken in the near future.

4. Conclusions

Through in vitro activity screening and PK optimization approach, we discovered a novel and proprietary EGF/HER2 dual inhibitor, pyrotinib, which displayed excellent in vitro potency and selectivity as well as robust in vivo efficacy in HER2-dependent mouse xenograft models. Metabolic pathway data indicated that the pyrotinib was primarily metabolized by CYP3A4 and to a lesser extent by CYP1B1, CYP2C8, CYP2C19, CYP2D6 and CYP3A5, suggesting the necessity of monitoring potential DDIs when co-administered with other potent CYP3A4 inhibitors or inducers. Moreover, the quantitative assessment of pyrotinib metabolism confirmed that not only the major metabolites circulating in human have been identified but also the exposure level of major metabolites in human has been covered in preclinical toxicological studies in animal species. Overall, as an orally irreversible inhibitor, pyrotinib demonstrated desirable pharmacokinetic properties and safety profiles. Our interim analysis of pyrotinib phase I and preliminary phase II data highlights that healthy volunteers and patients are safe and well-tolerated in the dose escalation studies at a dose up to 400 mg with encouraging antitumor effect in patients with HER2-positive advanced breast cancer. Current on-going clinical trials will be continuously validating its therapeutic value as an important option for this new molecular entity in the treatment of HER2+ breast cancer.

Disclosure

The authors declare no competing financial interest.

Acknowledgments

This project has been supported by National New Drug Key Program (2013ZX09102010) in China. We would like to thank Dr. Peng Cho Tang, Mr. Bin Wang, Mrs. Fangfang Jin and the whole project team members who had made contributions to this project. We are also grateful for Mi Tang for her assistance in data collection for preparing this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejps.2017.01.021.

References


