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Research paper

Analysis of resistance mechanisms to abivertinib, a third-generation EGFR tyrosine kinase inhibitor, in patients with *EGFR* T790M-positive non-small cell lung cancer from a phase I trial

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ABSTRACT

Background: Resistance to third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) presents a major clinical challenge in advanced non-small cell lung cancer (NSCLC). Here, we report resistance mechanisms to abivertinib, a novel third-generation EGFR TKI, from a phase I dose-escalation/expansion study (NCT02330367).

Methods: Patients with *EGFR* T790M-positive advanced NSCLC and progression on prior EGFR TKIs received abivertinib in dose escalation (50–350 mg twice daily [BID]) or expansion (300 mg BID) cohorts. Patients enrolled at Guangdong Lung Cancer Institute who underwent next-generation sequencing (NGS)-based genomic profiling upon abivertinib progression (prior to October 30, 2018) were enrolled in this exploratory analysis.

Findings: Thirty of 73 patients enrolled were eligible for resistance analysis. Upon abivertinib progression, 27 patients provided plasma samples (six patients also provided paired samples from the progression sites) and three patients only provided tissue samples from the progression sites for NGS. A heterogeneous landscape of resistance to abivertinib was observed: 15% (4/27) experienced *EGFR* T790M loss and 13% (4/30) developing *EGFR* tertiary mutations including C797S. *EGFR* amplification was observed in 11 patients (37%), and considered a putative resistance mechanism in seven (23%) patients. Other *EGFR*-independent resistance mechanisms involved *CDKN2A*, *MET*, *PIK3CA*, *HER2*, *TP53*, *Rb1* and small-cell lung cancer transformation.

Interpretation: Our findings reveal a heterogenous pattern of resistance mechanisms to abivertinib which is distinct from that previously reported with osimertinib. *EGFR* amplification was the most common resistance mechanism in this cohort.

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1. Introduction

Third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) specifically targeting *EGFR* T790M and *EGFR*-activating mutations have demonstrated impressive activity in patients with *EGFR* T790M-positive non-small cell lung cancer (NSCLC) following acquired resistance to prior EGFR TKIs [1,2]. Unfortunately, resistance to third-generation EGFR TKIs also inevitably occurs. Resistance mechanisms to osimertinib and rociletinib have been shown to be heterogenous and involve multiple genes such as *EGFR*, *MET*, *PIK3CA*, *HER2*, *KRAS*, *RB1*, *BRAF* and histologic transformation [3–10]. In addition, the predominant resistance mechanisms to osimertinib and rociletinib appear to differ. *EGFR* C797S mutation, a frequent resistance mechanism to osimertinib [4–7], occurs infrequently (~3%–5%) in patients who progress on rociletinib [9,10]. However, despite available information on resistance mechanisms, clinical strategies to overcome resistance to these third-generation EGFR TKIs inhibitors remains largely unknown.

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Research in context

Evidence before this study

Before preparing this manuscript, we searched PubMed for studies investigating resistance mechanisms to the third-generation EGFR TKIs. The following search terms were used: "osimertinib" OR "AZD9291" OR "rociletinib" OR "CO-1686" OR "EGF-816" OR "AC0010" OR "avitinib" OR "abivertinib" OR "olumitinib" OR "HM61713" OR "ASP8273" AND "*small cell lung cancer" OR "NSCLC" (published in English language between January 1, 2013 and November 15, 2018). We identified reports of resistance mechanisms to osimertinib and rociletinib involving 346 patients and 55 patients, respectively. Together, these reports suggested a heterogenous resistance landscape for both agents involving multiple genes such as *EGFR*, *MET, PIK3CA*, *BRAF*, *HER2*, *ROS1*, *RET*, *KRAS* and histologic transformation. A limited number of studies were also identified which reported resistance mechanisms for abivertinib (one case series involving 16 patients), HM61713 (one case study involving one patient) and ASP8273 (two case studies involving two patients), and did not provide conclusive findings.

Added value of this study

This exploratory analysis involved 30 patients with *EGFR* T790M-positive advanced NSCLC and disease progression on abivertinib, a third-generation EGFR TKI, in a phase I dose-escalation/expansion study. To our knowledge, this study provides the largest cohort of resistance data for abivertinib, with comprehensive genomic profiling performed by a Clinical Laboratory Improvement Amendments (CLIA)-certified next-generation sequencing centre. We observed a heterogenous resistance landscape for abivertinib involving multiple genes such as *EGFR*, *MET*, *TP53*, *RB1*, *HER2*, *PIK3CA* and *CDKN2A*, as well as histologic transformation. *EGFR* tertiary mutations including C797S were also observed upon abivertinib resistance, which have not been reported previously. We also provide the first report of differences between abivertinib and osimertinib in terms of predominant resistance mechanisms and *EGFR* T790M status post-treatment.

Implications of all the available evidence

Our study indicates that the resistance pattern to abivertinib appears to be unique. This observation underscores the need to further evaluate resistance mechanisms associated with each third-generation EGFR TKI and suggests a potential of rational sequence with different third-generation EGFR TKIs based on the distinct resistance profiles. Importantly, our findings indicate the need for *EGFR* amplification to be evaluated upon abivertinib resistance. Future studies are needed to investigate potential strategies to overcome resistance to abivertinib by combination treatments targeting specific resistance mechanisms.

To date, limited case reports have indicated the feasibility of treating patients with resistance to osimertinib mediated by *EGFR* C797S [11,12], *MET* amplification [13,14], *ROS1* fusion [15], *RET* fusion [16].

Abivertinib is a pyrrolopyrimidine-based, irreversible, EGFR TKI, structurally distinct from pyrimidine-based osimertinib and rociletinib [17]. Abivertinib selectively inhibits *EGFR*-activating and T790M mutations and has demonstrated promising efficacy and good tolerability in phase I/II studies, conducted in *EGFR* T790M-positive patients who progressed on prior first- or second-generation EGFR TKIs [2,18]. In a phase I dose escalation/expansion study, recommended phase II dose (RP2D) of abivertinib was determined at 300 mg BID based on a median progression-free survival of 6·9 months and objective response rate of 40% in *EGFR* T790M-positive patients progressed from prior EGFR TKIs (data not published). While the heterogeneity and specificity of the resistance landscapes reported for osimertinib and rociletinib underscores the need to elucidate resistance mechanisms of other third-generation EGFR TKIs, resistance to abivertinib is poorly elucidated. Initial findings from a small cohort of patients indicated potential resistance mechanisms for abivertinib may include *BRAF* V600E mutation (1/16 patients), *ROS1* fusion (1/16), *MET* amplification (1/16) and *HER2* amplification (2/16), with no *EGFR* C797S mutation detected [2].

To investigate resistance mechanisms to this novel third-generation EGFR TKI, we performed next-generation sequencing (NGS)-based genomic profiling of patients who enrolled at the Guangdong Lung Cancer Institute in a phase I dose escalation/expansion study of abivertinib and experienced progression.

2. Materials and methods

2.1. Patient enrolment and sample collection

Patients with *EGFR* T790M-positive NSCLC (based on central laboratory testing of tumour biopsies following disease progression on first- or second-generation EGFR TKIs) received oral abivertinib, administered in 28-day cycles in escalating dose cohorts (50, 100, 150, 200, 250, 300 and 350 mg BID) or 300 mg BID in an expansion cohort in a phase I trial (NCT02330367). The compound structure of abivertinib has been reported by Xu et al. [17] Patients enrolled at Guangdong Lung Cancer Institute who provided plasma and/or samples from the progression sites upon disease progression (PD) with abivertinib were included in this exploratory analysis of potential mechanisms of resistance to abivertinib. For patients who provided plasma samples, their paired peripheral white blood cells were also collected. As a pre-planned exploratory analysis, longitudinal plasma samples were also obtained from a subgroup of patients at each radiographic evaluation timepoint (baseline, month 1 and every 2 months thereafter until PD or until end of abivertinib treatment for patients who continued abivertinib beyond PD). This study was approved by the ethics committee at Guangdong Provincial People's Hospital, and all patients provided written, informed consent. The study adhered to the principles of Declaration of Helsinki and the Good Clinical Practice guidelines.

2.2. Genomic analysis

NGS was performed by a Clinical Laboratory Improvement Amendments (CLIA)-certified testing centre (Burning Rock Biotech, Guangzhou, China). Samples provided by all patients upon abivertinib PD and available pre-treatment samples were analysed using panels of 295 or 168 cancer-related genes (Burning Rock Biotech, Guangzhou, China). For patients who provided longitudinal plasma samples, we tested samples obtained at both RECIST and clinical PD. For plasma genotyping, NGS of peripheral white blood cells was also performed to avoid false positive plasma genotyping due to clonal haematopoiesis. Sequencing data were mapped using Burrows-Wheeler aligner 0.7.10 (average sequencing depth of 10,000×). Local alignment optimization, variant calling and annotation (minimum loci depth of 100) were performed using GATK 3·2, MuTect (Broad Institute, Cambridge, MA, USA) and VarScan (Genome Institute, Washington University, USA) software. A full description of genomic analysis procedures is provided in the Appendix.

2.3. FISH analysis of EGFR gene copy number

For patients with *EGFR* amplification identified by NGS, *EGFR* gene copy number were further analysed by fluorescence *in situ* hybridization (FISH) in available tumour tissue as previously described using Vysis LSI EGFR/CEP7 FISH Probe (Abbott) [19]. *EGFR* gene amplification was defined as gene-to-chromosome ratio ≥2 or presence of gene cluster or ≥15 gene copies in ≥10% of the cells, according to the Colorado system [19,20]. Two observers independently scored a minimum of 50 tumour cells. All FISH analyses were performed in a blinded fashion without access to patients' clinical information or treatment outcome.

2.4. Statistical analysis

Duration of abivertinib treatment (time from the first to the last dose of abivertinib) was calculated using Kaplan-Meier analysis. Log-rank test was used to compare subgroups. Statistical analysis was performed using R software.

3. Results

3.1. Clinical and pathological characteristics of patients enrolled

Seventy-three patients were enrolled in the phase I dose-escalation and expansion study of abivertinib at Guangdong Lung Cancer Institute. Of the 57 patients who developed PD by October 30, 2018, samples were obtained at progression and underwent NGS in 32 individuals. Two patients with CSF NGS only were excluded and 30 were included in this analysis (Fig. 1). At the time of abivertinib progression, twenty-seven (90%) patients underwent plasma NGS including 21 (70%) with single plasma NGS, four (13%) with paired plasma/tissue NGS and two (7%) with paired plasma/CSF NGS (Table 1). Three (10%) patients had NGS of tissue biopsies from the progression sites only. Further details of the patients' characteristics and sample types provided are in Supplementary Table S1.

The clinicopathological features of the 30 patients are summarized in Table 1. Patients had received a median (range) of 2 (1–5) lines of treatment prior to abivertinib and 37% had a prior history of central nervous system metastases. All patients were positive for *EGFR* T790M mutation in tumour tissues obtained prior to abivertinib treatment. Twenty-one patients were *EGFR* 19 deletion-positive, eight were *EGFR* 21 L858R mutation-positive and in one patient only *EGFR* T790M mutation (without *EGFR*-activating mutation) was detected prior to abivertinib.

Table 1
Clinical characteristics of patients.

	Patients included in the resistance analysis ($n = 30$)
Gender, *n* (%)	
Men	11 (37)
Women	19 (63)
Age (years)	54 (34–71)
Histological type, *n* (%)	
Adenocarcinoma	28 (93)
Squamous-cell carcinoma	2 (7)
Prior lines of treatment, median (range)	2 (1–5)
Prior history of CNS metastases, *n* (%)	
Yes	11 (37)
No	19 (63)
EGFR-activating mutation, *n* (%)	
Exon 19 deletion	21 (70)
Exon 21L858R mutation	8 (27)
Undetected[a]	1 (3)
EGFR T790M status, *n* (%)	
Positive	30 (100)
Negative	0
Abivertinib dose, *n* (%)	
100 mg–350 mg, BID (effective dose)	29 (97)
50 mg, BID	1 (3)
NGS sample type upon abivertinib PD, *n* (%)	
Plasma only	21 (70)
Plasma and tissue	4 (13)
Plasma and CSF	2 (7)
Tissue only	3 (10)

CNS, central nervous system.
[a] Tissue biopsy obtained upon progression with gefitinib showed *EGFR* T790M mutation, only (no *EGFR*-activating mutation).

Abivertinib was started at 50 mg BID in one patient, 100 mg BID in five, 150 mg BID in five, 200 mg BID in five, 250 mg BID in seven, 300 mg BID in six, 350 mg BID in one, respectively (Supplementary Table S1). According to the previous report on this phase I study, the effective doses of abivertinib ranged from 100 to 350 mg BID [18]. Twenty-nine (97%) patients in the current cohort received doses of abivertinib within the effective range and one (3%) patient received a lower dose of abivertinib. Best responses to abivertinib of partial

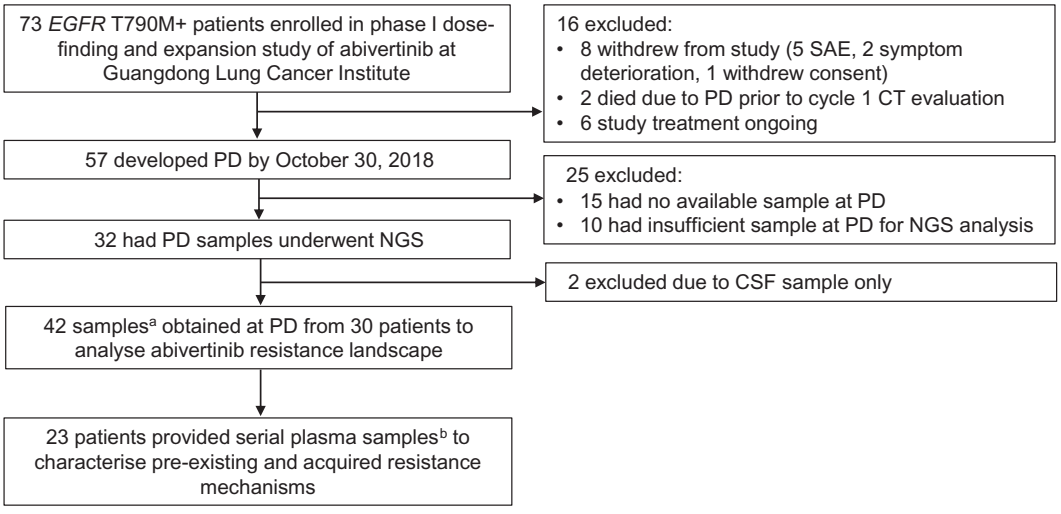

Fig. 1. Study profile. [a]All 30 patients provided samples upon abivertinib progression. Of these patients, six had plasma samples obtained at both RECIST PD and clinical PD, and another six patients had samples from the progression site and paired plasma. Therefore, a total of 42 samples were obtained. [b]Serial plasma samples were collected at study baseline (prior to abivertinib treatment) and at each radiologic evaluation until PD and beyond PD (if treatment was continued). CT, computed tomography; NGS, next-generation sequencing; PD, progressive disease; SAE, serious adverse events.

response (PR), stable disease (SD) and PD were achieved in 13 (43%), 14 (47%) and three (10%) patients, respectively. Median (range) duration of abivertinib treatment was 4·9 (0·9–27·1) months. Thirteen patients (43%) continued to receive abivertinib for >1 month beyond PD for a median (range) of 3·5 (1·4–10·5) months.

3.2. EGFR T790M status upon abivertinib progression

Previous studies have suggested that only patients with *EGFR* activating mutations detectable upon osimertinib PD were eligible for *EGFR* T790M status analysis [6,21]. By this criterion, 27 of the 30 patients who had detectable *EGFR*-activating mutations upon abivertinib progression were eligible for *EGFR* T790M status analysis. While the remaining three were excluded for T790M status analysis due to no *EGFR* activating mutations detected upon abivertinib progression. *EGFR* T790M was preserved in 23 (85%) patients and lost in four (15%) patients. Of the four patients with paired plasma and tissue samples upon abivertinib progression, there was discrepancy in *EGFR* T790M status between tissue and plasma in two individuals. In one patient, *EGFR* T790M was found in tissue only, while it was found only in plasma in the other patient (Fig. 2).

3.3. Heterogeneous resistance mechanisms to abivertinib

We observed a heterogeneous resistance landscape for abivertinib. In these 30 patients, recurrent alterations (alterations occurred in ≥2 patients) were observed in 22 genes, including *EGFR*, *MET*, *CDKN2A*, *PIK3CA*, *HER2*, *Rb1*, and *TP53*, as shown in the heatmap (Fig. 2). Allelic fraction and copy number information are available in Supplementary Table S2.

Table 2
Characteristics of patients with detected *EGFR* amplification.

Patient	Sample type	*EGFR* activating mutation	T790M status	Pre-existing/acquired *EGFR* amp	Other potential concomitant resistance mechanisms
2	P	21L858R	+	Acquired	—
9	P	Del19	+	Pre-existing	*AXL* amp#
16	P	Del19	+	Pre-existing	*MET* amp, *EGFR* C797S
18	P	Del19	+	Acquired	*CDKN2A/2B* del
20	P	21L858R	+	Pre-existing	*PIK3CA* E545K & SCLC
23	T[a] & P	Del19	+	Pre-existing	—
24	CSF[a] & P	Del19	+	Unknown	*CDKN2A* del
26	T	Del19	+	Unknown	*EGFR* L718V
28	T[a] & P	Del19	−	Unknown	*MET* amp
29	T	Del19	+	Acquired	—
30	T & P	Del19	+	Acquired	*EGFR* C797S#

21L858R, *EGFR* 21L858R mutation; CSF, cerebrospinal fluid; amp, amplification; Del19, *EGFR* 19 deletion; P, plasma; T, tissue; *AXL* amp#, *EGFR* copy number gain was observed upon RECIST disease progression, while *AXL* amp was detected upon clinical disease progression with further copy number gain of *EGFR*; *EGFR* C797S #, *EGFR* C797S mutation was detected only in plasma, while *EGFR* amp was detected in both tissue and plasma upon abivertinib progression in P30.

[a] *EGFR* amp was identified in P23, P24, P28 by tissue or CSF, but not in plasma.

3.4. EGFR-dependent resistance mechanisms

We observed for the first time of abivertinib resistance mediated by *EGFR* tertiary mutations in four (13%) patients upon abivertinib progression. *EGFR* C797S mutations were detected in three (10%) patients; all

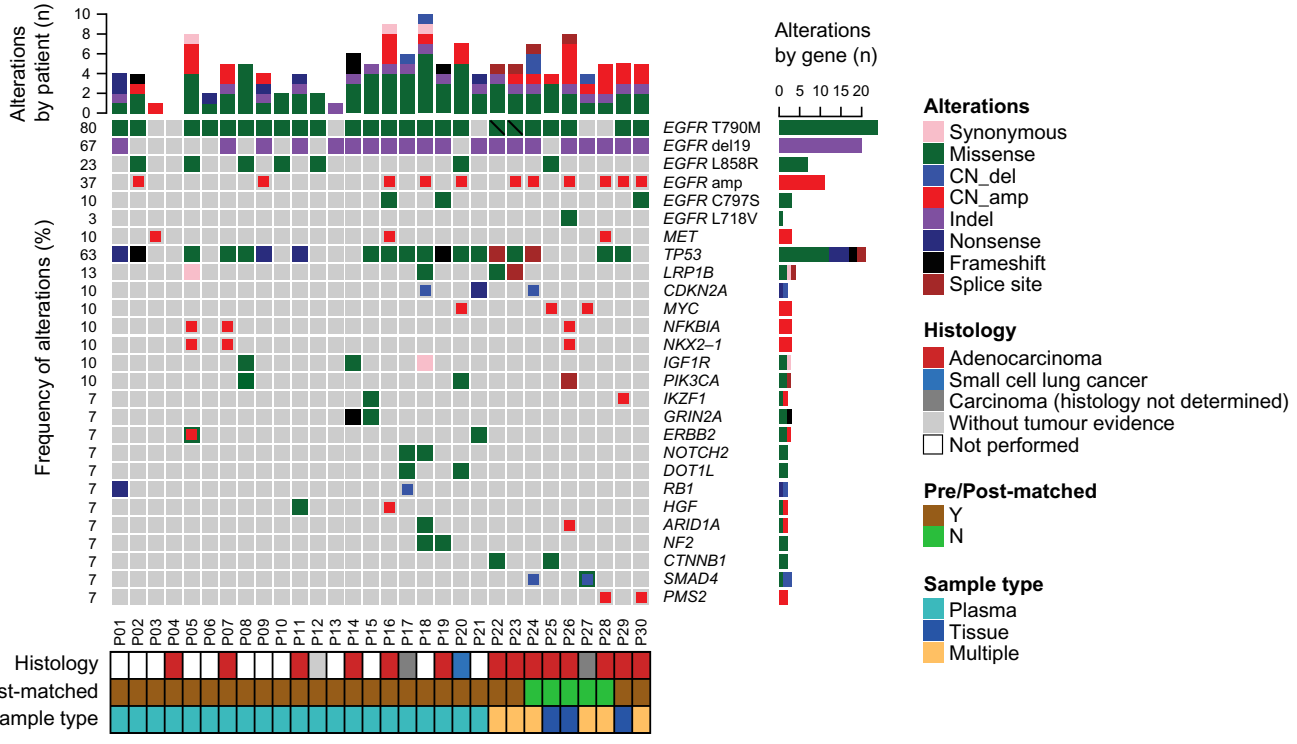

Fig. 2. Mutation spectrum upon disease progression with abivertinib. Each column represents one patient. All detected alterations in *EGFR* are captured; for other genes, only recurrent genomic alterations (≥2 patients) are shown. CN_amp, copy number amplification; CN_del, copy number deletion; *EGFR* DEL19, *EGFR* 19 deletion; *EGFR* L858R, *EGFR* 21L858R mutation; Multiple, paired plasma and tissue/cerebrospinal fluid obtained upon abivertinib progression; Not performed, without pathological evaluation; Pre-/Post-matched, patients with matched samples before abivertinib initiation and upon disease progression. "\" indicates inconsistent T790M status between paired plasma and tissue upon abivertinib progression, either plasma-negative but tissue-positive or *vice versa*. Carcinoma (histology not determined), pathological evaluation identified tumour cells in 2 patient samples obtained upon progression (1 cerebrospinal fluid and 1 hydrothorax) but could not distinguish specific histologic subtype due to limited sample. Without tumour evidence, no tumour was identified in 1 patient lung biopsy obtained upon abivertinib progression.

occurred *in* cis with *EGFR* T790M mutation. *EGFR* L718V mutation was detected in one *EGFR* T790M-positive (3%) patient at disease progression.

In addition to *EGFR* tertiary mutations, *EGFR* amplification was observed in 11 (37%) patients upon abivertinib progression (Table 2). Of these patients, eight had matched pre- and post-treatment samples for NGS analysis. *EGFR* amplification emerged post-abivertinib treatment in four patients. While three patients harboured *EGFR* amplification prior to treatment, *EGFR* copy number was elevated at PD. Therefore, *EGFR* amplification was considered a putative resistance mechanism for abivertinib in seven (23%) patients. The last patient with matched pre- and post-treatment samples harboured *EGFR* amplification prior to the treatment without copy number gain post-abivertinib treatment and showed evidence of small-cell lung cancer transformation upon abivertinib progression. Consequently, *EGFR* amplification was not considered as putative resistance mechanism for this patient. *EGFR* amplification was detected in three further patients upon abivertinib progression. However, as matched pre-treatment samples were not available, we were unable to determine whether *EGFR* amplification served as a resistance mechanism in these individuals (Fig. 3, Table 2).

Interestingly, concurrent resistance mechanisms were observed in patients with *EGFR* amplification, including *EGFR* C797S mutation ($n = 2$), *MET* amplification ($n = 2$), in one case this also co-occurred with *EGFR* C797S mutation), *EGFR* L718V mutation ($n = 1$), *CDKN2A* del ($n = 2$), and *AXL* amplification ($n = 1$) (Table 2). Data from patients with matched pre- and post-treatment samples indicated *EGFR* amplification may have functioned as a co-driver in three patients who developed other well-characterized resistance mechanisms. In one patient, *EGFR* amplification was acquired post-abivertinib treatment and detected both in plasma and tissue biopsy from the progression site, while *EGFR* C797S mutation was only identified in plasma. In another patient with *EGFR* amplification (copy number, CN = 5·87) and *MET* amplification (CN = 2·73) in pre-treatment plasma, *EGFR* C797S mutation was only detected at allelic fraction (AF) of 0·52% in post-treatment plasma, while *EGFR* activating mutation was detected with an AF of 61·8% together with the copy number gain in *EGFR* (CN = 7·14) and *MET* (CN = 4·34), suggesting that distinct clones may co-drive resistance to abivertinib. In the third patient, *EGFR* amplification (CN = 2·65) was present prior to abivertinib treatment. This patient developed PD (enlarged brain metastasis) upon first radiological evaluation; *EGFR* copy number gain (CN = 3·24) was observed. She continued abivertinib after brain radiotherapy for a further 3·6 months and NGS analysis of plasma at clinical PD showed further copy number gain in *EGFR* (CN = 5·96) and a newly emerged *AXL* amplification (CN = 2·67). Paired FISH analysis of her pre-abivertinib and post-abivertinib samples also demonstrated *EGFR* amplification (Supplementary Fig. S1).

3.5. EGFR-independent resistance mechanisms

We also observed multiple bypass and downstream alterations upon abivertinib progression. *MET* amplification was detected in three (10%) patients (one co-occurred with *EGFR* amplification, one co-occurred with *EGFR* amplification and *EGFR* C797S mutation). Other copy number variations mediating resistance in this category included *HER2* amplification and *AXL* amplification, each in one (3%) patient. Mutations in downstream pathway were also detected, including *PIK3CA* hotspot E545K and E542K mutation in two (7%) patients.

In addition to activation of bypass and downstream pathways, inactivation of tumour suppressor genes was also detected, primarily involving loss of heterozygosity (LOH) or copy number loss of tumour suppressor genes such as *CDKN2A*, *Rb1*, *TP53* and *APC* (Fig. 3, Supplementary Table S2).

3.6. Histologic transformation

Among 15 patients with identified histology by routine pathological evaluation of samples obtained upon abivertinib PD, 14 (93%) patients retained adenocarcinoma and small-cell lung cancer transformation

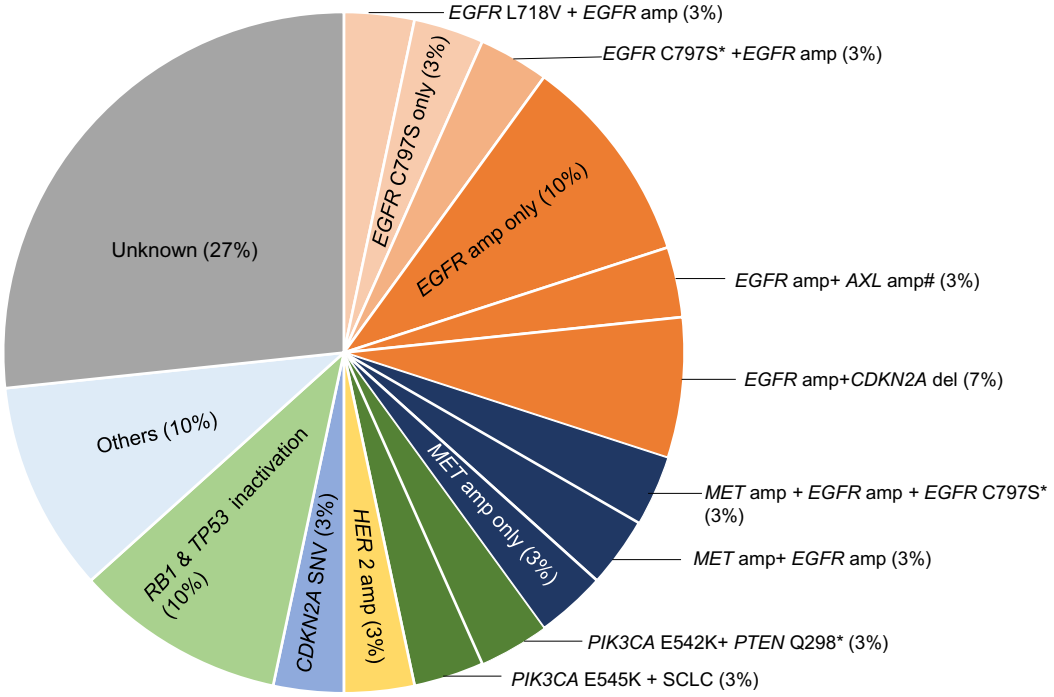

Fig. 3. Landscape of potential resistance mechanisms to abivertinib. Amp, amplification; del, copy number deletion; SCLC, small cell lung cancer; SNV, single nucleotide variant *EGFR* C797S* in one patient was detected at a low allelic fraction (0·52%), co-occurred with *MET* amplification and *EGFR* amplification. *EGFR* C797S in another patient was detected only in plasma NGS, while NGS of both the plasma and tissue detected *EGFR* amplification upon abivertinib progression (not presented in pre-treatment tissue NGS). *AXL* amp#, *EGFR* copy number gain was observed upon RECIST disease progression, while *AXL* amp was detected upon clinical disease progression with further copy number gain of *EGFR*. Others include concurrent *APC* S811* and *MLH1* amp ($n = 1$), *CD79A* A32G mutation ($n = 1$) and *HGF* G396D mutation ($n = 1$). Unknown refers to patients in whom putative resistance mechanisms were not identified.

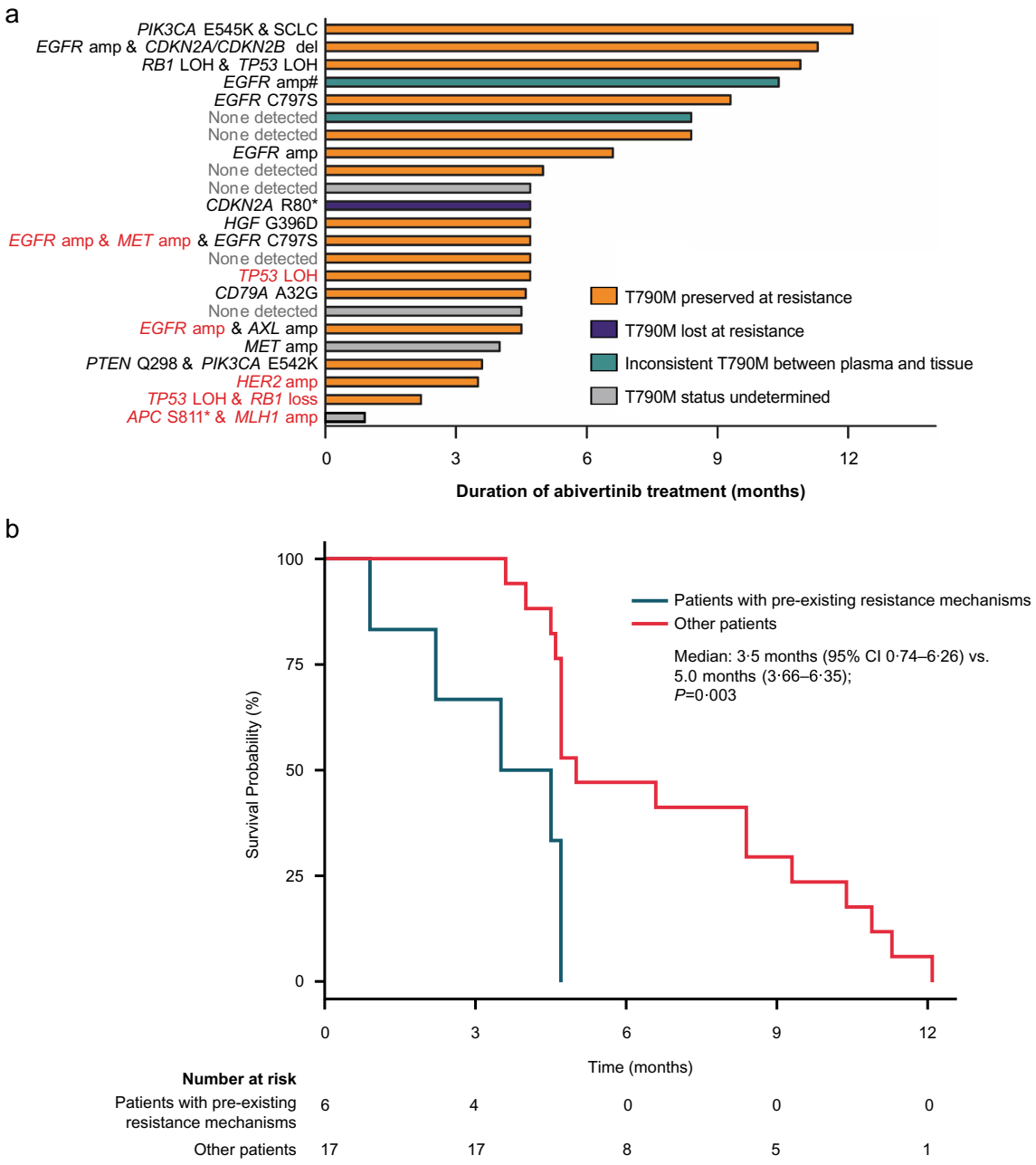

Fig. 4. (a) Pre-existing and acquired resistance mechanisms for abivertinib and clinical outcomes. NGS analysis of patients with matched pre- and post-abivertinib plasma samples ($n = 23$; paired tissue samples were also available in two patients upon abivertinib progression). amp, amplification; del, deletion; LOH, loss of heterozygosity; mut, mutation; non-detected, no putative resistance mechanism was identified; SCLC, small cell lung cancer. 'Inconsistent T790M status for plasma and tissue' refers to plasma-negative but tissue-positive ($n = 1$) or *vice versa* ($n = 1$). 'T790M status undetermined' refers to the following 2 circumstances: no *EGFR*-activating mutation detectable upon progression ($n = 2$) or *EGFR* T790M mutation undetectable in pre-treatment ctDNA ($n = 1$, this patient was categorized as *EGFR* T790M loss when only considering the post-treatment sample). *EGFR* amp# refers to *EGFR* amplification detected in pre- and post- abivertinib tissue biopsy rather than in plasma in this patient. Pre-existing resistance mechanisms in ctDNA are shown in red. (b) Patients with pre-existing resistance mechanisms in ctDNA remained on abivertinib for an inferior duration *versus* other patients. Pre-existing resistance mechanisms refers to individuals with putative resistance mechanisms identified prior to abivertinib initiation in circulating tumour DNA. Statistical analysis was performed by using Kaplan-Meier analysis. Log-rank test was used to compare subgroups.

was observed in one (7%) patient who maintained *EGFR* 21L858R mutation and T790M mutation upon disease progression (Fig. 2).

3.7. Resistance mechanisms for abivertinib among patients who received RP2D dose

Putative resistance mechanisms for abivertinib were identified in five of the six patients who received abivertinib at the RP2D dose (300 mg BID). These included *EGFR* amplification (detected in $n = 3$ and considered as a putative resistance mechanism in two individuals with matched pre- and post-treatment samples) and *EGFR* C797S mutation ($n = 2$, including one patient with concurrent *EGFR* amplification). Other putative resistance mechanisms identified in these individuals were *MET* amplification ($n = 1$, with concurrent *EGFR* amplification without a matched pre-treatment sample) and concurrent small-cell lung cancer transformation and *PIK3CA* E545K mutation ($n = 1$).

3.8. Pre-existing and acquired resistance mechanisms for abivertinib

We next sought to distinguish resistance mechanisms which were present prior to abivertinib treatment (pre-existing) from those acquired post-abivertinib treatment by comparing matched pre- and post-abivertinib samples. Twenty-five patients had pre- and post-abivertinib samples, including 23 with plasma NGS and three with tissue NGS. One patient had paired plasma and tissue NGS at both timepoints. Resistance mechanisms were first analysed in the 23 patients with paired plasma samples. Abivertinib resistance was attributed to pre-existing and acquired resistance mechanisms in six and 12 patients, respectively, while two patients harboured both pre-existing and acquired resistance mechanisms (Fig. 4a). Pre-existing resistance mechanisms detected in circulating tumour DNA (ctDNA) before abivertinib treatment included *EGFR* amplification, *HER2* amplification, *MET* amplification, *APC* S811* mutation, *MLH1* amplification, *RB1* loss and *TP53* LOH. Patients with pre-existing resistance mechanisms in ctDNA demonstrated an inferior duration of abivertinib treatment compared with those without pre-existing resistance mechanisms (median: 3·5 months [95% CI 0·74–6·26] *vs.* 5·0 months [3·66–6·35], log rank $p = 0{\cdot}003$; Fig. 4b). Subsequent analysis of the three patients with matched pre- and post-abivertinib tissue NGS revealed abivertinib resistance was attributable to acquired *EGFR* amplification and pre-existing *EGFR* amplification in two and one patient (in this patient paired pre-and post-treatment plasma, *EGFR* amplification was not detected), respectively.

4. Discussion

In this study, we found heterogenous resistance mechanisms to abivertinib involving multiple genes such as *EGFR*, *CDKN2A*, *MET*, *PIK3CA, HER2, TP53, Rb1* as well as histologic transformation. This resistance landscape observed for abivertinib is distinct to that previously reported for osimertinib. For example, *EGFR* T790M loss (15%) with abivertinib resistance was less frequent than reported in osimertinib resistance cohorts (42%–68%) [5–7,21,22]. In addition, *EGFR* tertiary mutations, reported in 21%–45% of patients developing resistance to osimertinib [4,7,22], occurred only in 13% of patients developing resistance to abivertinib. This observation was supported by a previous study involving 16 patients progressing upon abivertinib in which no *EGFR* tertiary mutation was identified (0/16) [2]. *MET* amplification, another predominant resistance mechanism for osimertinib, was also only captured in 10% of patients in the present study and in 6% (1/16) of patients progressing upon abivertinib in the previous report [2]. Structural differences between pyrimidine-based osimertinib and pyrrolopyrimidine-based abivertinib might account for the development of different resistance profiles [17,23].

In this study, *EGFR* amplification was the most common resistance mechanism for abivertinib. Analysis of matched pre- and post-treatment samples demonstrated that *EGFR* amplification was acquired or increased in copy number upon abivertinib progression in 23% of patients, indicating the role of *EGFR* amplification in mediating resistance to abivertinib. Both acquired and increased *EGFR* amplification have been reported as potential mechanisms of resistance in 5% (5/107) of who progressed on osimertinib in the AURA 17 study [21].

In some patients, *EGFR* amplification co-occurred with known resistance mechanisms for third-generation EGFR TKIs including *EGFR* tertiary mutations and *MET* amplification, suggesting different clones may co-drive resistance to abivertinib. The role of *EGFR* amplification as a resistance driver co-existing with other resistance mechanisms was previously reported in patients who developed *EGFR* T790M-mediated resistance to first-generation EGFR TKIs [24]. Similar to the patient in our study who developed co-occurring *EGFR* C797S mutation at very low allelic fraction with *EGFR* amplification, *EGFR* amplification was the dominant resistance for osimertinib in a patient who developed commitment *EGFR* C797S mutation at very low allelic fraction upon osimertinib progression [25]. These observations suggested distinct clones, with one carrying amplified *EGFR* and another carrying *EGFR* C797S mutation, may co-drive resistance to third-generation EGFR TKIs. The co-occurrence of *EGFR* amplification with other *EGFR* tertiary mutations such as *EGFR* C797G, *EGFR* C796S and *MET* amplification have also been documented in patients who progressed on osimertinib [26–28]. However, the potential co-driving role of *EGFR* amplification with other well-characterized drivers is not well established. Future studies are needed to assess the role of *EGFR* amplification in patients with other concomitant drivers of resistance to third-generation EGFR TKIs in preclinical and clinical settings. We envisage that combination treatment targeting both *EGFR* amplification and concomitant co-drivers of resistance might be beneficial for some patients with disease progression on third-generation EGFR TKIs, including those with co-drivers such as *EGFR* L718V, which have demonstrated sensitivity to afatinib *in vitro* [29].

Genomic profiling of matched samples pre- and post-abivertinib treatment provides insights into the biology of abivertinib resistance by distinguishing putative resistance mechanisms existing prior to treatment from those acquired post-treatment. In our study, pre-existing resistance mechanisms co-occurred with *EGFR* T790M mutation in ctDNA from 26% of patients and were associated with an inferior duration of abivertinib treatment. Our data suggested that comprehensive genomic profiling by NGS can facilitate the early detection of potential resistance mechanisms. Future studies are needed to understand how NGS-based genomic profiling may guide treatment decisions, including the optimal timepoint for early intervention of combinational targeted therapy strategies.

While subsequent treatments options following resistance to third-generation EGFR TKIs may be exhausted, emerging clinical trials are investigating combination treatment approaches targeting EGFR and bypass pathways in patients who developed resistance to osimertinib. For example, savolitinib in combination with osimertinib is currently being investigated in advanced *EGFR*-mutant patients with *MET*-positive tumour after osimertinib failure (NCT03778229). T-DM1 and osimertinib combination treatment is also being investigated in patients with *EGFR*-mutant NSCLC and HER2 bypass pathway activation after progression on an EGFR TKI, including osimertinib (NCT03784599). However, at present there are no clinical trials for patients who develop resistance to abivertinib, potentially due to the fact that the resistance mechanisms to abivertinib remain largely unknown. Therefore, our study has potential translational implications for the care of patients who develop resistance to abivertinib. Future preclinical and clinical studies are needed to provide insights into the biology of *EGFR* amplification-mediated abivertinib resistance and investigate potential combinational strategies to overcome resistance to abivertinib. Regarding the heterogeneity of resistance mechanisms to abivertinib, innovative clinical trials which integrate NGS-based testing of multiple biomarkers and matched combinational treatments are also urgently needed for patients who develop resistance to abivertinib.

Our study was associated with several limitations. Firstly, not all patients underwent NGS-based genomic profiling of pre-treatment samples prior to abivertinib treatment, thus the resistance mechanisms to abivertinib observed in this study warrant validation in a larger cohort with matched pre- and post-treatment samples. Secondly, patients enrolled for this resistance analysis were from a phase I dose escalation and expansion study and received different doses of abivertinib. While nearly all (97%) patients received effective dose of abivertinib, the resistance mechanisms observed in this study require validation in patients receiving abivertinib at the RP2D dose who develop PD. Thirdly, as the majority of patients underwent plasma NGS upon abivertinib PD, events of CN variations such as *EGFR* amplification, *MET* amplification might be underestimated. Future studies need to precisely characterise the frequency of resistance associated with CN variations by tissue NGS upon abivertinib progression.

In conclusion, this study reveals a heterogenous landscape of resistance to abivertinib, which appears to differ from that previously reported for osimertinib in terms of *EGFR* T790M loss, *EGFR* tertiary mutation frequencies, and predominant resistance mechanisms. *EGFR* amplification was the most common mechanism of resistance to abivertinib in our study. Our study underscores the need to evaluate resistance mechanisms to different third-generation EGFR TKIs individually. Larger studies are needed to validate these observations and provide further insight into how comprehensive genomic profiling can guide treatment decisions to overcome resistance to third-generation EGFR TKIs.

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Declaration of interests

QZ declares speaker fees from AstraZeneca and Roche. Y-LW declares speaker fees from AstraZeneca, Eli Lilly, Pfizer, Roche, and Sanofi. XX is the CEO and stock owner of ACEA Therapeutics Inc. WT, FRL are employees of ACEA Therapeutics Inc. S-KC, J-YY, HH-Z, ZZ are employees of Burning Rock Biotech. Other authors declare no competing interests.

Author contributions

Y-LW and QZ designed the study. QZ and Y-CZ perform the literature search. QZ, Y-CZ, Z-HC and BG contributed to clinical samples collection. S-KC, J-YY, HH-Z and ZZ performed the genomic analysis. Y-CZ, Z-HC, J-YY and H-HY were involved in data interpretation and statistical analysis. J-YY and W-FL were involved in figure editing. ZX and Y-CZ performed the FISH analyses. Y-CZ, QZ and Y-LW developed the initial draft of the manuscript. Y-LW, QZ, X-CZ, WT, RFL, XX contributed to critically review the initial draft of the manuscript. Y-CZ, QZ, Y-LW, X-CZ, C-RX, X-YB, JS, J-JY involved in producing the subsequent drafts of the manuscript and finalization of the report.

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