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Short Communication

The spatiotemporal evolution of *EGFR* C797S mutation in *EGFR*-mutant non-small cell lung cancer: opportunities for third-generation EGFR inhibitors re-challenge

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Third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have demonstrated impressive activity in *EGFR* T790M-positive non-small cell lung cancer (NSCLC) progressed from prior EGFR-TKIs [1,2]. However, resistance inevitably occurred after approximately 10 months of treatment. *EGFR* C797S mutation has been reported as one of the major resistance mechanisms to osimertinib, occurring in around 14%–24% of patients progressed from osimertinib [3,4]. Till now, multiple studies have focused on the onset of *EGFR* C797S in this subset of patients. Little is known about the spatiotemporal evolution of *EGFR* C797S mutation during subsequent treatment course. Here we present four cases showing the spatiotemporal evolution of *EGFR* C797S during subsequent treatment after progression from third-generation EGFR-TKI AC0010 (Abivertinib), which is a pyrrolopyrimidine-based, irreversible, EGFR-TKI, structurally distinct from pyrimidine-based osimertinib and rociletinib [5]. Abivertinib has also been reported to demonstrate a distinct resistance profile as compared to osimertinib [6,7]. As previous study has suggested a rational for sequencing different third-generation EGFR-TKIs with distinct resistance profiles [8], we here reported for the first time on re-challenge osimertinib after progression from abivertinib based on the "on and off" switch of *EGFR* C797S mutation.

Longitudinal genomic profiling by NGS was performed on a total of 19 plasma, tissue and cerebrospinal fluid biopsies from 4 patients with advanced *EGFR* T790M-positive lung adenocarcinoma who progressed upon a third-generation EGFR-TKI, abivertinib and further response to osimertinib. Detailed methods are in Supplementary. Two evolution patterns of *EGFR* C797S mutation post-abivertinib treatment were identified, including an "on-off-on" pattern and "off-on" pattern tracked in 1 and 3 patients, respectively (Fig. 1, Table S1 online).

A woman (P01) with advanced *EGFR*-mutant NSCLC developed *EGFR* T790M mutation-mediated resistance after 14 months of gefitinib treatment (Table S1 online). NGS of the pre-treatment plasma demonstrated the presence of *EGFR* 19 deletion, *EGFR* T790M mutation and *TP53* N29fs mutation, with no other alterations in *EGFR* and bypass drivers detected (Fig. 2). She received abivertinib 300 mg BID in a phase I dose finding study and achieved a best response (BOR) of partial response (PR) lasting for 8.4 months (Fig. 2b). Upon disease progression (PD), plasma NGS demonstrated a newly emerged *EGFR* C797S mutation (T → A) at an allelic fraction (AF) of 1.34%, in *cis* with *EGFR* T790M mutation (AF: 1.60%). *EGFR* 19 deletion (AF: 4.52%) and *TP53* mutation were also detected. Alterations in bypass drivers were not detected (Fig. 2c, d). Pemetrexed plus cisplatin was administered and the best response was stable disease (SD) with an 18% tumor shrinkage. The patient then experienced PD after 5 months of chemotherapy with new brain and leptomeningeal metastases. She underwent lumber puncture and NGS of CSF showed *EGFR* 19 deletion, while synchronous plasma NGS demonstrated *EGFR* 19 deletion and *EGFR* T790M mutation, with *EGFR* C797S mutation lost (Fig. 2c, d).

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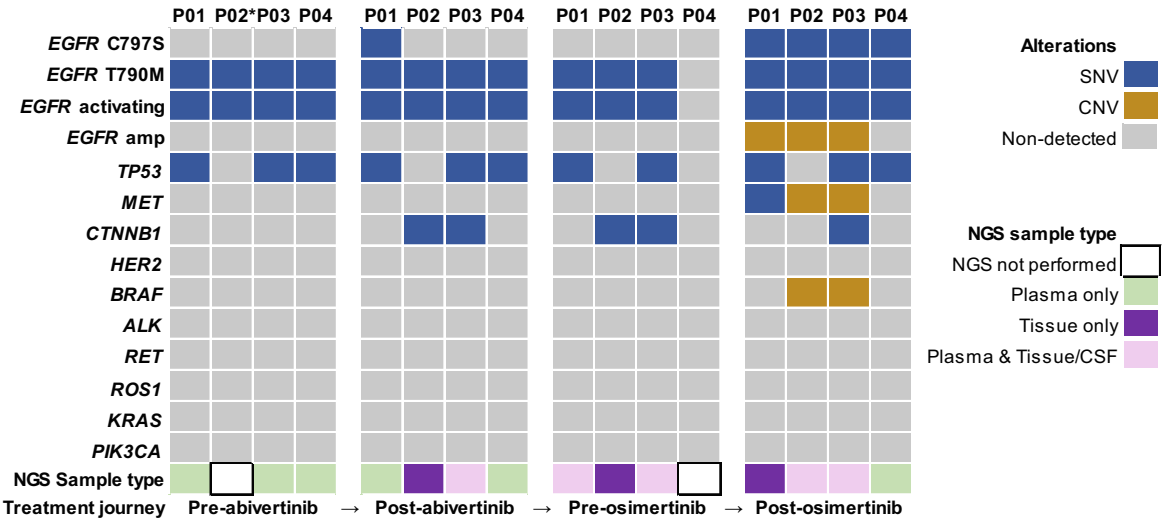

Fig. 1. Summary of mutation spectrum evolution. *EGFR* and other known drivers of resistance were listed in the heatmap. SNV, single nucleotide variant; CNV, copy number variation; NGS, next-generation sequencing; pre-abivertinib; before the start of abivertinib; post-abivertinib, upon abivertinib progression; pre-osimertinib, before the start of osimertinib; post-osimertinib, upon osimertinib progression; * *EGFR* mutations were detected by amplified refractory mutation system in pre-abivertinib sample for P02 and next-generation sequencing was not performed for this sample.

Due to *EGFR* C797S lost and the presence of *EGFR* T790M mutation, the patient was enrolled into the ASTRIS trial and started osimertinib 80 mg qd. She experienced a SD response (0%) for 10 months (Fig. 2a). Upon PD to osimertinib, NGS of enlarging liver biopsy identified the re-emergence of another *EGFR* C797S mutation (G → C) at an AF of 26.36%, in *cis* with *EGFR* T790M mutation (AF: 34.58%) and the presence of *EGFR* 19 deletion (AF: 58.82%) without detection of the previous T → A mutation encoding *EGFR* C797S (Fig. 2c, d). In addition, *EGFR* amplification (copy number, CN = 5.2) and *MET* V546M mutation (AF: 48.47%) were also detected. Alterations in other bypass drivers were not detected.

The patient then received paclitaxel plus bevacizumab treatment for 4 cycles and experienced PD in brain metastases after 3 months of treatment. NGS of CSF upon PD demonstrated no *EGFR* C797S mutation, but *EGFR* 19 deletion, *EGFR* amplification, *MET* amplification and *MET* V546M mutation (Fig. 2c).

A woman (P02) in her 50 s with *EGFR* T790M-positive advanced NSCLC was enrolled in the phase I dose finding study of abivertinib after progression from erlotinib and subsequent chemotherapy. The patient received abivertinib 150 mg BID and the best response was PR. She experienced PD after 23.3 months of abivertinib treatment with enlargement of lung lesions (Table S1, Fig. S1 online). NGS of lung biopsy demonstrated the presence of *EGFR* 21L858R mutation, *EGFR* T790M mutation and *CTNNB1* S37C mutation. Alterations in bypass drivers were not detected (Figs. 1, S1 online). Osimertinib was tailored and achieved SD response (−13%) that lasted for 14 months. NGS of enlarging lung lesion upon progression to osimertinib demonstrated an acquired *EGFR* C797S mutation (G → C), in *cis* with *EGFR* T790M mutation (Figs. 1, S1 online). In addition, *EGFR* amplification, *MET* amplification and *BRAF* amplification were also identified. NGS of paired plasma confirmed the presence of *EGFR* C797S mutation (G → C), in *cis* with *EGFR* T790M mutation, but none of abovementioned amplification events was detected. The patient was then treated with crizotinib and osimertinib and achieved a SD response after 1 month of combination treatment.

The second case (P03) was a man in his 40 s presented with stage IV *EGFR* T790M-positive lung adenocarcinoma after progression from 19 months of gefitinib treatment (Table S1, Fig. S2 online). He was enrolled in the phase I dose finding study of abivertinib and received abivertinib 300 mg BID. He achieved a best response of PR that lasted for 8.4 months. After further progression, NGS of the enlarging lung lesion biopsy showed *EGFR* 19 deletion with *EGFR* T790M mutation lost and *CTNNB1* S37F mutation (Fig. 1). Whereas, concurrent plasma sequencing demonstrated the presence of *EGFR* 19 deletion and *EGFR* T790M mutation. Neither NGS of the lung or plasma detected alterations in bypass drivers. The patient was then treated with paclitaxel (175 mg/m^2), carboplatin (AUC = 5) and bevacizumab (7.5 mg/kg) for 4 cycles followed by bevacizumab maintenance. The best response was SD with decrease in the number of lung metastases. After 7 months of chemotherapy, he experienced PD and NGS demonstrated the presence of *EGFR* 19 deletion, *EGFR* T790M mutation and *CTNNB1* S37F mutation both in lung and plasma biopsies. The patient was enrolled into the ASTRIS trial and started osimertinib 80 mg qd. He experienced a SD response (−14%) for 12.8 months. Upon PD to osimertinib, NGS of lung biopsy identified *EGFR* C797S mutation (T → A and an additional G → C), both in *cis* with *EGFR* T790M mutation. In addition, acquired *EGFR* amplification, *MET* amplification and *BRAF* amplification were also detected, along with *CTNNB1* S37F mutation (Figs. 1, S2 online). Concurrent plasma sequencing showed *EGFR* C797S mutation (G → C), in *cis* with *EGFR* T790M mutation and acquired *MET* amplification. The patient then started pemetrexed monotherapy for 1 cycle without back to hospital for radiologic evaluation and died 1-month post-treatment.

The third case (P04) was a man developed *EGFR* T790M-positive lung adenocarcinoma after progression from 14 months of gefitinib treatment. He was enrolled in the phase I dose finding study of abivertinib and started abivertinib 100 mg BID. The best response was PR (Table S1 online). After 4.6 months of abivertinib treatment, he experienced PD to abivertinib with enlargement of liver metastases. Plasma NGS identified *EGFR* 19 deletion and *EGFR* T790M mutation (Fig. 1). Other alterations in *EGFR* and bypass drivers were not detected. The patient then received local ablation of liver metastases and underwent 3 cycles of paclitaxel (175 mg/m^2), carboplatin (AUC = 5) and bevacizumab (7.5 mg/kg). The best response was PR and he experienced PD with cognitive disorder after 2.3 months of chemotherapy. Whole brain radiotherapy was performed (30GY/10f). The patient started osimertinib 80 mg qd

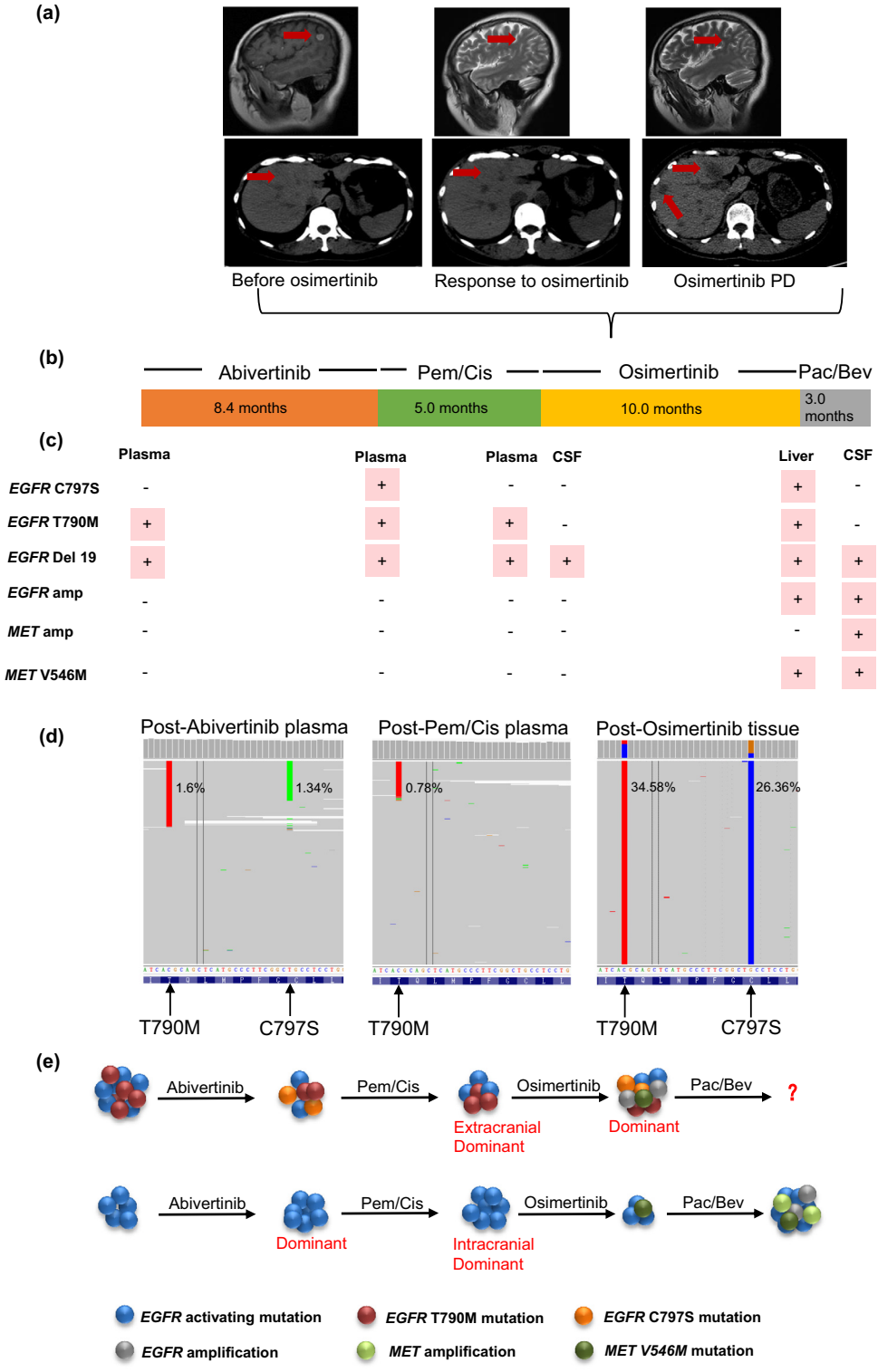

Fig. 2. The "on-off-on" evolution pattern of *EGFR* C797S mutation. (a) Re-sensitization to osimertinib. Images of chest computed tomography and brain magnetic resonance before osimertinib initiation, response to osimertinib and further resistance to osimertinib. PD, disease progression (b): Timeline of treatment. Pem/Cis, Pemetrexed plus cisplatin for 4 cycles followed by pemetrexed maintenance. Pac/Bev, paclitaxel plus bevacizumab. (c) The spatiotemporal evolution of *EGFR* C797S mutation and other major genomic alterations. Longitudinal plasma, tissue and CSF tests were performed at various timepoints including abivertinib resistance, further progression to pemetrexed plus cisplatin (synchronous plasma and CSF tests), resistance to osimertinib (liver test) and further resistance to paclitaxel plus bevacizumab (CSF test). amp, amplification (d): Allelic context of *EGFR* C797S mutation and *EGFR* T790M mutation during subsequent treatments after progression from abivertinib. (e) Conceptual models showing genomic evolution during the course of subsequent treatment.

and achieved a best response of PR lasting for 7 months. During further PD, plasma NGS identified *EGFR* C797S mutation (T → A), in *cis* with *EGFR* T790M mutation. Alterations in bypass drivers were not detected (Fig. 1). The patients then received gefitinib plus osimertinib for 2 weeks without clinical benefit and unfortunately died 4 months after *EGFR* C797S mutation occurred.

These findings suggest that the status of *EGFR* C797S mutation could dynamically change under selective treatment pressure and underscore the necessity for longitudinal genomic profiling after progression from third-generation EGFR-TKIs. Most importantly, we proposed two evolutionary strategies to optimize the sequence of different third-generation EGFR-TKIs for advanced EGFR-mutant NSCLC. One evolutionary strategy is to delay the emergence of *EGFR* C797S mutation by tailoring abivertinib first, which has been reported with less vulnerability to develop *EGFR* C797S mutation in recent studies [6,7]. Another evolutionary strategy is to eliminate *EGFR* C797S-positive clones by chemotherapy. The feasibility of switching off *EGFR* T790M mutation by chemotherapy has been previously reported by Hata et al. and our group [9,10]. In this study, we further showed that *EGFR* C797S mutation might also be switched off by chemotherapy, even if it occurs in *cis* with *EGFR* T790M mutation. In addition, we demonstrate a proof-of-concept to treat *EGFR* C797S mutation, in *cis* with *EGFR* T790M mutation, by precisely osimertinib re-challenge, rather than directly targeting this undruggable mutation.

To the best of our knowledge, this is the first study that demonstrated the feasibility of successful osimertinib re-challenge based on longitudinal comprehensive genomic profiling after progression from another third-generation EGFR-TKI. Different from previous studies which suggested that the clinically significant benefit from osimertinib after rociletinib and HM61713 failure may be due to incomplete target inhibition [11,12], three of our patients demonstrated impressive response to both inhibitors. Our data not only suggest a rational for re-challenging third-generation EGFR-TKIs with distinct resistance profile, but also underscore the need for re-evaluating the function of chemotherapy in facilitating the re-sensitization to third-generation EGFR-TKIs after the initial failure.

The current study is associated with several limitations. First, we did not observe well-characterized resistance mechanisms in the last three patients upon abivertinib resistance. Thus, the role of *EGFR* C797S mutation in determining third-generation EGFR-TKIs re-challenge warrants further investigation in context with other bypass mechanisms. Second, we did not show here if abivertinib re-challenge after osimertinib failure may also work or not. Future studies are warranted to precisely define the subset of patients that may benefit from third-generation EGFR-TKIs re-challenge.

Collectively, we showed here the spatiotemporal evolution of *EGFR* C797S mutation after progression from third generation EGFR-TKIs. Dynamically genetic profiling unveiled different evolution patterns of *EGFR* C797S mutation including "on-off-on" as well as "off-on" patterns, suggesting the necessity of dynamically tracking the status of *EGFR* C797S mutation and other potential resistance mechanisms after progression from third-generation EGFR-TKIs to guide successful third-generation EGFR-TKIs re-challenge in future.

Conflict of interest

Yi-Long Wu received honoraria from AstraZeneca, Roche, Eli Lilly, Pfizer and Sanofi. All the other authors declare no conflict of interest.

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Author contributions

Yi-Long Wu designed the study. Yi-Chen Zhang, Qing Zhou, Zhi-Hong Chen and Ming-Feng Zhang collected the clinical data. Shao-Kun Chuai and Jun-Yi Ye performed the genomic analysis. Yi-Chen Zhang and Yi-Long Wu wrote the initial manuscript. All the authors critically revised the initial manuscript and approved the final manuscript for submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2019.03.031.

References

[1] Mok TS, Wu YL, Ahn MJ, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 2017;376:629–40.
[2] Wu YL, Zhou Q, Liu X, et al. Phase I/II study of AC0010, mutant-selective EGFR inhibitor, in non-small cell lung cancer (NSCLC) patients with EGFR T790M mutation. J Thorac Oncol 2017;12(Suppl 1):S437.
[3] Papadimitrakopoulou VA, Wu YL, Han JY, et al. Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. Ann Oncol 2018;29(Suppl 8). LBA51.
[4] Yang Z, Yang N, Ou Q, et al. Investigating novel resistance mechanisms to third-generation EGFR tyrosine kinase inhibitor osimertinib in non-small cell lung cancer patients. Clin Cancer Res 2018;24:3097–107.
[5] Xu X, Mao L, Xu W, et al. AC0010, an irreversible EGFR inhibitor selectively targeting mutated EGFR and overcoming T790M-induced resistance in animal models and lung cancer patients. Mol Cancer Ther 2016;15:2586–97.
[6] Ma Y, Zheng X, Zhao H, et al. First-in-human phase I study of AC0010, a mutant-selective EGFR inhibitor in non-small cell lung cancer: safety, efficacy, and potential mechanism of resistance. J Thorac Oncol 2018;13:968–77.
[7] Zhou Q, Zhang YC, Chen ZH, et al. Circulating tumor DNA portrays the resistance landscape to a novel third generation EGFR inhibitor, AC0010. J Thorac Oncol 2018;13(Suppl 10):S408–9.
[8] Chabon JJ, Simmons AD, Lovejoy AF, et al. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun 2016;7:11815.
[9] Hata A, Katakami N, Yoshioka H, et al. Spatiotemporal T790M heterogeneity in individual patients with EGFR-mutant non-small-cell lung cancer after acquired resistance to EGFR-TKI. J Thorac Oncol 2015;10:1553–9.
[10] Zhang YC, Pi C, Ke EE, et al. Tracking spatiotemporal T790M heterogeneity in patients with EGFR-mutant advanced NSCLC after acquired resistance to EGFR TKIs. Ann Oncol 2016;27(Suppl 9). Abstract 439O.
[11] Sequist LV, Piotrowska Z, Niederst MJ, et al. Osimertinib responses after disease progression in patients who had been receiving rociletinib. Jama Oncol 2016;2:541–3.
[12] Chen CW, Kuo CW, Chen YL, et al. Successful AZD9291 therapy in advanced non-small cell lung cancer after failure of HM61713. J Thorac Oncol 2017;12:e72–4.

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