EGFR-independent mechanisms of acquired resistance to AZD9291 in EGFR T790M-positive NSCLC patients

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Received 27 May 2015; revised 11 July 2015; accepted 20 July 2015

Background: AZD9291 is an oral, irreversible, mutant-selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (EGFR-TKI), which specifically targets both sensitizing and resistant T790M mutations. This compound has shown outstanding activity, in a phase I/II (AURA) trial. However, despite impressive tumor responses in T790M-positive patients, acquired resistance to this drug limits the benefit of this compound. Mutations at the EGFR C797 codon, located within the kinase-binding site, were very recently reported to be a potential mechanism of resistance to AZD9291 in T790M-positive patients.

Patients and methods: To identify potential mechanisms of resistance to AZD9291, we report here on two patients with resistant biopsy specimens that had been treated with AZD9291.

Results: We identified in two distinct cases, HER2 and MET amplification by FISH and CGH as a potential mechanism of acquired resistance to third-generation EGFR-TKI. Interestingly, this event occurred with complete loss of the T790M mutation. In one case, we observed a different molecular status at two biopsy sites (the T790M mutation at the primary site and wild-type T790M at the metastatic site with different pathways of acquired resistance to AZD9291).

Conclusion: Our observations suggest that T790M-positive and wild-type T790M clones may coexist at baseline. AZD9291 efficiently suppresses the growth of T790M-positive cells, but a population of wild-type T790M cells at baseline will mediate the development of resistance, here via a by-pass pathway activating either HER2 or MET.

Key words: NSCLC, EGFR, AZD9291, T790M, MET, HER2

introduction

Activating mutations in the epidermal growth factor receptor (EGFR) are key drivers of non-small-cell lung cancer (NSCLC) in 10%–15% of non-Asian patients [1, 2]. Patients with one of the most common EGFR mutations [i.e. the p.Leu858Arg (L858) mutation in exon 21 and delE746-A750 deletions in exon 19] achieve typically good responses to therapy with first- or second-generation EGFR tyrosine kinase inhibitors, such as erlotinib, gefitinib or afatinib [3–5]. Despite a striking initial response to treatment, practically all patients experience disease progression generally after 9–14 months of treatment. This acquired resistance is driven in ~60% of cases by a second-site EGFR point mutation, p.Thr790Met (T790M), whereas MET amplification, HER2 amplification, EGFR amplification, PIK3CA mutations and transformation into small-cell components occur less frequently [6, 7]. AZD9291 is an oral, irreversible, mutant-selective EGFR tyrosine kinase inhibitors (EGFR TKI), that selectively targets both drug-sensitive (like L858R or exon 19 deletion) and resistant T790M mutations. AZD9291 has shown promising activity (with an overall response rate of 61% in EGFR T790M-positive patients), in a phase I (AURA trial) even at the first dose [8, 9]. However, despite impressive tumor responses in T790M-positive patients, acquired resistance to this drug eventually occurs after a median duration of response of ~10 months. EGFR p.Cys797Ser (C797S) mutations, located within the kinase-binding site, were recently reported to be a potential mechanism of resistance to irreversible EGFR inhibitors such as AZD9291 in T790M-positive patients [10, 11]. No other molecular mechanisms underlying resistance to AZD9291 have been identified to date. A better understanding of the mechanisms of resistance to these third-generation EGFR inhibitors is critical for envisioning new strategies for these patients. To identify potential mechanisms of resistance to AZD9291, we studied resistant biopsy specimens of patients.
treated with AZD9291 in the AURA study. Here we report a genomic analysis of acquired resistance in two patients.

**methods**

Tumor samples were analyzed for the presence of genetic alterations at baseline in the MOSCATO (Molecular screening for cancer treatment optimization; an institutional biospecimen protocol) trial and at recurrence in the MATCH-R trial [using next-generation sequencing (NGS, Ion Torrent PGM); whole-exome sequencing (Illumina technology), RNA seq (Illumina technology) and CGH (Agilent technology)] [12].

**results**

The first patient (subject 1) was a 54 year-old man, a former smoker (20 PY, had stopped smoking 25 years earlier) presented with an epileptic seizure (Figure 1). A unique 3-cm-diameter brain metastasis was detected by MRI. Additional exams corroborated the diagnosis of a right upper lobe adenocarcinoma with mediastinal lymph node involvement and a brain metastasis (T2N2M1b, according to the current 7th TNM classification) in July 2009. The patient received stereotactic brain irradiation then a four-cycle platinum-based chemotherapy doublet followed by thoracic irradiation (42 Gy). Nine months after the end of treatment (October 2010), the patient presented with tumor progression; a new 4-mm-diameter right cerebellar brain lesion and a lesion in the right femur for which he received femoral irradiation alone (20 Gy). The brain metastasis was considered too small for stereotactic irradiation. At that time, a mutation analysis was carried out on the initial tumor biopsy specimen which unveiled an activating mutation of EGFR Del19 (p.Glu746_Ala750delins). Second-line treatment with gefitinib was started in December 2010 with excellent tolerance and a clinical benefit. In November 2012, right shoulder pain led to detection of a new bone metastasis of the tip of the right scapula that was treated with local irradiation (20 Gy) in December 2012. Stereotactic brain irradiation was delivered to four new brain metastases in January 2013. Treatment with gefitinib was continued but lung and right scapula CT scan-guided tumor biopsy specimens were collected in October 2013 following tumor progression at these two sites (Moscato trial at Gustave Roussy) [12]. The molecular analysis found an **EGFR** del19 (p.Glu746_Ala750delins) mutation as well as an **EGFR** exon 20 T790M mutation in the lung tumor samples. The **EGFR** Del19 (Glu746_Ala750delins) mutation was found in the scapula samples but not the T790M mutation (Figure 1). After discussion of the case during the phase I Molecular Tumor Board meeting, the patient was enrolled in the phase I study of...
AZD9291 (AURA) started in November 2013. He received a fixed daily dose of 80 mg. An excellent tumor response was observed with a partial response of the different lung lesions and the right scapula lesion showing stable disease. RECIST progression was observed at different tumor sites after more than 12 months of treatment. The patient was enrolled in the MATCH-R trial at Gustave Roussy and underwent further lung and right scapula CT scan-guided tumor biopsies. The results of the molecular profile are reported in Figure 1. Significant HER2 amplification was found by CGH in the lung sample together with a persistent EGFR Del19 mutation. Intriguingly, NGS revealed complete loss of the EGFR T790M mutation (with a limit of detection at least of 1%). No EGFR C797S mutation was found. Neither HER2 amplification nor the EGFR T790M mutation was observed on the scapula biopsy specimen but the EGFR Del19 mutation was still observed. The HER2 amplification observed on the lung biopsy specimen by CGH was confirmed by FISH (50 nuclei analyzed, average number of centromeres per cell: 3.16, HER2/chromosome-specific centromeric enumeration probe (CEP) 17 ratio: 6.65, DNA probe kit from Dako) (Figure 2).

Given this molecular profile, the patient started a new therapeutic line with a combination of paclitaxel and trastuzumab in March 2015. The patient presented a stable disease on the lung lesions and the right scapula lesion after 6 and 12 weeks of treatment.

The second patient (subject 2) was a 60-year-old lady, a never smoker, diagnosed with a stage IV lung adenocarcinoma (lung and pleural metastasis) (Figure 3). She received six cycles of cisplatin–pemetrexed as first-line therapy and achieved a partial response. Due to the discovery of an activating EGFR mutation (exon 21, L858R) on the initial biopsy specimen, she received second-line therapy with erlotinib that caused grade 3 skin toxicity. Erlotinib was switched to gefitinib. The treatment was continued for 12 months with a good safety profile and stable disease. However, in January 2012 she developed a brain metastasis that was treated neurosurgically, followed by a whole-brain irradiation. NGS analysis of the brain tumor revealed a T790M mutation (with an exon 21 L858R mutation). Due to extra-brain tumor progression, she received six cycles of carboplatin–pemetrexed–bevacizumab followed by bevacizumab maintenance therapy. In September 2012, she was enrolled in a phase I trial ( cetuximab combined with an mTor inhibitor) due to tumor progression. Disease stabilization was obtained for 17 months. In parallel, molecular screening was carried out in a cMET inhibitor phase I trial but FISH analysis of the tumor was negative for MET amplification. In March 2014, a core needle lung biopsy was carried out on a progressive lung nodule. This
Figure 3. Patient 2 clinical course including treatment history and relevant imaging studies and tumor biopsy specimens. (A) Next-generation sequencing readings from sequential tumor biopsy specimens (pre-erlotinib, pre and post-AZD9291). (B) Representative images from a CT of the chest with different timings. (i) At baseline before AZD9291 with right pulmonary lesions [grey (red online) arrows]. (ii) At 6 months with a partial tumor response of pulmonary lesions. (iii) At 10 months of treatment with AZD9291: lung progression. (C) Pulmonary disease course with AZD9291 according to RECIST criteria 1.1. (D) Patient (subject 2) clinical course between 2008 and 2015.

Figure 4. Patient 2 clinical course including NGS, CGH and FISH results on tumor biopsy specimens. Post-AZD9291 treatment sample analysis: (A) Integrative genomic viewer screenshots of next-generation sequencing readings from sequential tumor biopsy specimens. The analysis shows the absence of both T790M and C797S EGFR mutations. (B) Copy number alteration profiles of primary lung by array CGH. Frequencies of DNA gain and loss according to chromosomal positions indicated along the x-axis. Chromosomal amplification is clearly demonstrated for 7q comprising the MET Locus. (C) Fluorescent in situ hybridization with MET amplification [MET in light grey (green online); centromere 17 in dark grey (red online)].
biopsy confirmed the presence of a T790M resistance mutation. The patient was enrolled in the phase I of AZD9291 (AURA). She started the treatment in April 2014 at a dose of 80 mg/day. A confirmed partial tumor regression (45%) was achieved with a clinical benefit until February 2015. She received AZD9291 for 10 months until progression of pulmonary disease. She was enrolled in the MATCH-R trial at Gustave Roussy and a core needle lung biopsy of a progressive pulmonary nodule was carried out. FISH results showed significant amplification of MET (41 nuclei analyzed, average number of cMET-positive cells: 21, ratio cMET/CEP7: 5.32, DNA probe kit from Zytovision) confirmed by CGH (log ratio 0.85) and by immuno-histochemistry (100% positivity on tumor cells) (Figure 4). NGS analyses showed an EGFR-activating mutation (exon 21, L858R) but no EGFR T790M or C797S resistance mutation, and no other acquired mutations. The patient is currently being treated with a cMET inhibitor in a phase I trial.

**Discussion**

These two cases illustrate the potential importance of sequential biopsies of growing tumors at clinical progression as the results may lead to immediate changes in therapy. EGFR T790M mutation is the most common mechanism of acquired resistance under first-generation EGFR tyrosine kinase inhibitors (EGFR-TKI), seen in nearly two thirds of cases. HER2 and MET amplification may be the second most common finding at the time of resistance, seen in ~10%–20% of patients with acquired resistance [6, 7, 13, 14]. This is the first report on the potential escape of tumor cells via the EGFR-independent pathway under third-generation EGFR-TKI. We did not identify HER2 or MET amplification in the pretreatment samples using CGH or FISH. Preclinical findings suggested that HER2 and MET play an important role in mediating sensitivity and resistance to EGFR-TKI and in driving tumor growth and survival [15, 16]. The EGFR T790M mutation and HER2 amplification appear to be mutually exclusive while MET amplification occurs with or without T790M mutations [14, 16]. In our two patients, HER2 or MET amplification appeared to be exclusive of the EGFR T790M mutation with loss of the T790M mutation. In subject 1, due to a divergent radiological response, we carried out whole-exome sequencing on lung and scapula biopsy specimens. HER2 amplification was observed only on tumor lung biopsy samples which previously escaped first-generation EGFR-TKI through the acquired resistance T790M mutation and lost it at progression with AZD9291. The significant partial tumor response achieved by subject 1 with AZD9291 was observed only in the T790M-positive lung metastases whereas the bone scapula metastasis (Del19 and T790M wild-type) was stable. These results are comparable with what has been reported with AZD9291 in the phase I AURA trial according to the T790M mutation status (ORR of 61% in T790M-positive patients versus 21% in patients with wild-type T790M) [9]. In subject 2, we also observed a sustained partial response in T790M-positive lung disease with loss of T790M at acquired resistance to AZD9291. These cases reflect potential intratumor heterogeneity, suggesting spatial and temporal patterns of subclone evolution that need to be taken into consideration in treatment.
strategies. Both observations suggest that T790M-positive and T790M wild-type clones may coexist in some cancers with acquired resistance to initial EGFR-TKIs. In both cases, we observed loss of the T790M mutation at the time of acquired resistance to AZD9291 associated with the emergence of new oncogenic drivers that are independent of the EGFR pathway. We hypothesize that this might be due to the selection of T790M wild-type clones present in the tumor at AZD9291 treatment initiation and which led to resistance via an independent mechanism such as HER- or MET-amplified subclones. Similar results were recently reported by Thress et al. They showed that some cancers may convert from T790M-positive to T790M wild-type cancer cells upon AZD9291 exposure and also identified cases harboring acquired C797S mutations [10]. If these findings are related to tumor heterogeneity, this would reinforce the importance of studying an underappreciated genomic heterogeneity through the proportion of T790M-positive or -negative tumor cells. Table 1 summarizes the potential mechanisms of acquired resistance to third-generation EGFR inhibitors already published in NSCLC. We need to better understand this heterogeneity which is an important issue in cancer genome studies and may lead clinicians to combine therapies in order to stop or prevent the emergence of resistance.

We identified HER2 and MET amplification as a potential mechanism of acquired resistance to third-generation EGFR-TKIs such as AZD9291 in EGFR T790M-positive NSCLC. Further investigations are needed to confirm this report on two clinical cases. Our findings may provide the rationale for targeting HER2 or MET pathways in this clinical setting. In both cases, our observations suggest that T790M-positive and T790M wild-type clones may coexist at baseline in cancers with acquired resistance to initial EGFR-TKI. AZD9291 can efficiently suppress the growth of T790M-positive cells, but a population of T790M wild-type cells at the baseline will mediate the development of resistance.

**Acknowledgements**

We thank Lorna Saint-Ange for English editing.

**Funding**

This work was funded by Institut National du Cancer (INCa) – Direction générale de l’offre de soins (DGOS) – Institut national de la santé et de la recherche médicale (INSERM) 6043 and Philanthropia-Lombard Odier.

**Disclosure**

JCS has received consultancy fees from AstraZeneca. All remaining authors have declared no conflicts of interest.

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