Identifying Resistance Mechanisms to Osimertinib via Blood Biopsy

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Clinical Practice Points

- The use of osimertinib as front-line treatment for epidermal growth factor receptor-mutated lung cancer has led to identification of various resistance mechanisms, some which may be targetable with standard or investigational treatments.
- Upon progression, patients deserve re-examination of their tumor. Liquid biopsy provides a less invasive technique than tissue biopsy to potentially identify these resistance mechanisms.
- With continued technological advancement, liquid biopsy could replace tissue biopsy in certain situations while providing a more accurate measure of tumor heterogeneity.

Clinical Lung Cancer, Vol. 20, No. 6, e597-600 © 2019 Elsevier Inc. All rights reserved.
Keywords: Circulating tumor DNA, Clonal evolution, EGFR mutation, Liquid biopsy, Resistance mutations

Introduction

Epidermal growth factor receptor (EGFR)-mutated lung cancer represents about one-third of patients with non−small-cell lung cancer (NSCLC) worldwide.[1] Prior to 2018, lung cancers harboring EGFR mutations sensitive to tyrosine kinase inhibitors (TKIs) were treated with erlotinib, gefitinib, or afatinib in the front-line setting.[2-4] Dacomitinib was also United States Food and Drug Administration-approved in the front-line setting for TKI-sensitive EGFR-mutated lung cancer.[5] Nearly all patients develop progressive disease, many owing to secondary mutations including EGFR exon 20 T790M or MET amplification. In multiple small series, T790M mutations account for up to 50% of resistance mechanisms to first-generation TKIs.[6,7] Resistance via T790M can effectively be overcome with osimertinib.[8] With the completion of the FLAURA trial, osimertinib is now approved in the front-line setting for patients who have EGFR exon 19 deletions or exon 21 L858R mutations.[9] As with first- and second-generation TKIs, resistance mechanisms to osimertinib have emerged. Here we present 2 examples of osimertinib resistance and discuss the value of genomic tumor profiling upon progression.

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Submitted: Jul 25, 2019; Accepted: Aug 18, 2019; Epub: Aug 26, 2019

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Case One

A 62-year-old male presented with chronic non-productive cough and chest congestion. A computed tomography scan showed a left hilar mass with ipsilateral mediastinal adenopathy and left-sided pleural effusion. Further workup revealed widely metastatic osseous disease. Transbronchial biopsy of the left hilar mass demonstrated a moderately differentiated adenocarcinoma with an EGFR exon 19 L474_T751 deletion (exon 19del) by next-generation sequencing (NGS). He received afatinib 30 mg daily for 5 months prior to developing progressive pleural disease. Circulating tumor DNA (ctDNA) NGS, at the time, revealed the original exon 19del (allele fraction [AF] 2.8%) and an exon 20 T790M mutation (AF 0.3%). Therapy was switched to osimertinib 80 mg daily. Owing to recurrent pleural effusions, he underwent pleurodesis and pleural catheter placement after 5 months on osimertinib. NGS on a pleural biopsy specimen revealed a BRAF V600E mutation in addition to the prior EGFR exon 19del and T790M mutations. Assuming BRAF was a driver mutation, he received 1 month of dabrafenib and trametinib, but developed rapid clinical progression at the site of his pleural catheter. After progressing on 4 months of chemoimmunotherapy, he re-started osimertinib with the addition of bevacizumab to aid in pleural effusion control. A ctDNA NGS sample collected 1 month after re-initiation of osimertinib revealed an EGFR C797S mutation (AF 5.2%), in addition to the previously identified mutations − exon 19del (AF

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https://doi.org/10.1016/j.cllc.2019.08.002

38.5%), T790M (AF 4.8%), and BRAF V600E (AF 0.06%). Therapy was transitioned to docetaxel and ramucirumab, and he eventually chose to pursue hospice care.

Case Two

A 52-year-old female non-smoker presented with left-sided pleuritic chest pain. Chest x-ray showed a left-sided pleural effusion. Pleural biopsy revealed metastatic adenocarcinoma. NGS demonstrated an EGFR exon 19 E746_A750 deletion. She started erlotinib 150 mg daily, requiring dose reduction at 3 months for grade 3 rash. Re-staging scans after 9 months showed progression in the lungs, liver, and regional/distant lymph nodes. A left cervical lymph node biopsy was consistent with primary lung adenocarcinoma. NGS of the lymph node biopsy and ctDNA both revealed an exon 19del (AF 15.3%) and a new T790M mutation (AF 5%). Given the mutational profile, she enrolled on a phase I trial of osimertinib and an oral JAK inhibitor (INCB 039110). She initially had disease response, but rapidly progressed after 4 months. Repeat lymph node biopsy and NGS re-demonstrated the exon 19del but loss of T790M. She received 1 additional month of single-agent osimertinib without response. Circulating tumor DNA was obtained and showed exon 19del (AF 15.3%), confirmed loss of T790M, and revealed gain of BRAF V600E (AF 0.9%) and KRAS amplification. The patient transitioned to carboplatin and pemetrexed for 2 cycles, but stopped owing to disease progression. She began fourth-line therapy with single-agent pembrolizumab but passed away shortly thereafter from hypoxic respiratory failure owing to overwhelming tumor burden.

Discussion

As osimertinib use increases in the front-line setting, it is increasingly important to gain further understanding into the various acquired resistance mechanisms to this drug. Whereas the exon 20 T790M mutation serves as a gatekeeper mutation and accounts for up to 50% of resistance to first-generation TKIs, no dominant resistance mechanism to osimertinib has emerged. Some of the more common alterations noted in this setting include EGFR C797S, PIK3CA, MET amplification, BRAF, and histologic transformation to small-cell lung cancer (SCLC).[10]

The EGFR C797S mutation is a common resistance mechanism to osimertinib, occurring in up to one-third of patients.[11] Two recent analyses retrospectively focused on using serial ctDNA from 2 AURA trials to analyze osimertinib resistance. From the AURA17 trial, 15 of 82 patients with detectable ctDNA EGFR sensitizing mutations at time of progression had an acquired EGFR C797S mutation.[12] Plasma samples from the AURA trial showed that 68% of cases lost T790M at the time of resistance and 22% of cases acquired EGFR C797S.[10]

Table 1 Osimertinib Combination Trials

NCT Identifier	Drug in Combination With Osimertinib	Mechanism	Phase	Study Size	Population
NCT02496663	Necitumumab	Recombinant human IgG1 antibody targeting EGFR1	I	100	Osimertinib and necitumumab in EGFR-mutated stage IV or recurrent NSCLC with previous progression on EGFR TKI
NCT03940703	Tepotinib	MET-targeting TKI	II	90	Tepotinib and osimertinib in EGFR TKI relapsed MET-amplified NSCLC
NCT03784599	T-DM1	HER2-targeting antibody-drug conjugate	II	58	T-DM1 and osimertinib to target HER2 bypass track resistance in EGFR-mutant NSCLC
NCT03778229	Savolitinib	Small molecular inhibitor of c-Met	II	172	Osimertinib and savolitinib in EGFRm+/MET+ NSCLC following prior osimertinib
NCT03532698	Aspirin		II	200	Combination of osimertinib and aspirin to treat osimertinib-resistant NSCLC
NCT02503722	Sapanisertib	Small molecular inhibitor of mTOR	I	36	Sapanisertib and osimertinib in patients with stage IV EGFR-mutated NSCLC after progression on EGFR TKI
NCT03944772	Savolitinib	Small molecular inhibitor of c-Met	II	150	Platform study in patients with EGFR-mutated NSCLC with progression following first-line osimertinib therapy.
	Gefitinib	Selective EGFR TKI			
	Necitumumab	Recombinant human IgG1 antibody targeting EGFR1			

The table summarizes current clinical trials combining osimertinib with additional targeted therapies in EGFR-mutated tumors after progression on an EGFR TKI. The trials were captured from clinicaltrials.gov on July 17, 2019.
Abbreviations: EGFR = epidermal growth factor receptor; HER2 = human epidermal growth factor receptor 2; IgG1 = immunoglobulin G1; mTOR = mammalian target of rapamycin; NSCLC = non–small-cell lung cancer; TKI = tyrosine kinase inhibitor.

Additional mutations have been identified in osimertinib-resistant patients. In the cases presented here, BRAF mutations emerged, which have previously been described as a potential resistance mechanism.[13] Other studies have demonstrated genomic alterations including mutations, amplifications, and fusions in different genes at the time of progression on osimertinib.[14,15] However, it is unclear if these alterations are truly drivers for resistance, as demonstrated by the lack of benefit with dabrafenib and trametinib in the first case presented above. EGFR-driven NSCLC has also been noted to transform to SCLC.[10] Inactivation of both *RB1* and *TP53* demonstrated up to a 40× greater risk of transformation to SCLC in a predefined cohort of patients.[16]

With various molecular aberrations being detected, there remains a need for better understanding of the true resistance pathways to osimertinib. The use of plasma-based testing is a significant technological advancement that has made this easier for the patient. Plasma-based testing encompasses multiple techniques to analyze various components of tumor-derived materials. These materials include ctDNA, circulating tumor cells, exosomes, ctRNA, and microRNA (miRNA).[17] The clinically available analytic techniques focus primarily on detecting ctDNA. Allele-specific polymerase chain reaction (PCR) or emulsion PCR can be applied when attempting to identify a limited number of specific mutations (ie, *EGFR*). Larger gene panels or untargeted approaches, such as whole genome sequencing, provide more information, but at a lower sensitivity when looking for a particular mutation.[18] In the largest prospective study to date, ctDNA was shown to provide higher rates of guideline-recommended genotyping with similar detection rates in a shorter period of time when compared with standard of care genotyping.[19] Being able to detect small fractions of ctDNA with a simple blood sample makes plasma-based testing an attractive option while determining a patient's treatment plan.

In the 2 preceding cases, potential resistance mutations were identified from ctDNA, allowing for alterations in therapy without a biopsy. A retrospective analysis of patients on the AURA trial demonstrated a $>$ 80% sensitivity and $>$ 96% specificity for EGFR-sensitizing mutations when comparing plasma (via BEAMing, a type of emulsion PCR) and tumor genotyping.[20] This data suggests ctDNA can be used as a non-invasive screening tool to serially study the clonal evolution of the tumor in real time and guide further treatment options. A recent retrospective study analyzed patients who underwent comprehensive genomic testing to determine the effect on treatment decisions. This study included treatment-naive patients and patients who had progressed on EGFR TKIs or chemotherapy and/or immunotherapy. Depending upon the patient cohort, results of the ctDNA NGS affected 23% to 32% of the subsequent treatment decisions.[21]

This information becomes clinically relevant when there are resistance mechanisms that have approved or experimental targeted therapies. A phase Ib/II trial of patients with EGFR-mutated, MET-dysregulated lung cancer showed a safe and promising regimen for MET-amplified disease when combining capmatinib and gefitinib.[22] Ongoing trials combining osimertinib with other targeted therapies will help determine whether certain resistance mechanisms can be overcome (Table 1). Other approaches include attempts at delaying the development of acquired resistance to osimertinib in the front-line setting by targeting intrinsic resistance pathways such as the overexpression of Cripto-1 with dasatinib, a Src inhibitor.[23]

Conclusion

As osimertinib enters the front-line setting of EGFR-mutated lung cancer, we are provided an opportunity to learn more about the resistance mechanisms that ultimately lead to drug inefficacy. Uncertainty remains in some cases as to whether the newly detected genetic abnormalities are driver mutations suggestive of tumor heterogeneity or passenger mutations detected artifactually owing to technical limitations in methodology of calling variant allele frequencies.[24] Identifying such artifacts is necessary to develop filtration techniques leading to improved signal-to-noise ratio and thus improved testing precision. Circulating tumor DNA provides an easy and cost effective method of studying these mutations. However, in cases where ctDNA does not reveal a resistance mechanism, a tissue biopsy remains warranted. ctDNA may provide a better understanding of tumor heterogeneity and provide a more comprehensive interpretation of the genotype of different tumor clones compared with a directed, single tumor biopsy. These findings urge for continued study of ctDNA as a method of obtaining insight into resistance patterns, and ultimately, lead to new drugs or combination therapy to overcome these therapeutic obstacles.

Disclosure

D.S. Subramaniam is an employee of and holds stock options with AstraZeneca and is a speaker at Genentech. The remaining authors have stated that they have no conflicts of interest.

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