Editorial Commentary

Finding chinks in the osimertinib resistance armor

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Introduction

The use of small molecule inhibitors directed at specific oncogene targets, including epidermal growth factor receptor (*EGFR*) mutations, has improved outcomes and defined precision medicine for non-small cell lung cancers (NSCLC). However, despite the impressive responses and improvements in survival seen with these agents, they are rarely curative, and even in patients who initially achieve complete responses to therapy, resistance inevitably develops, and disease progression occurs.

For *EGFR* mutant NSCLC, targeting the T790M resistance mutation defined the role of osimertinib (1), an agent with substantial efficacy but limited toxicities, and resulted in increasing efforts to define resistance mechanisms in the hope that these too could be targeted with further gains in survival.

While initially clonal heterogeneity may be limited early, selection pressures induced by treatments enables the growth of preexisting or new tumor cell clones that are resistant to therapy (2). The varied mechanisms by which resistance can occur, coupled with the co-occurrence of multiple resistance mechanisms within one patient, constitute a major challenge in developing an efficient treatment strategy to counteract tumor progression. The clonal evolution of oncogene-addicted NSCLC can give rise to different molecular aberrations both spatially (between primary and metastasis) and temporally (after treatment failure), further contributing to the complexity of the molecular resistance.

Resistance to *EGFR* TKIs

Resistance to *EGFR* TKIs broadly falls into '*EGFR*-dependent' and '*EGFR*-independent' mechanisms. The first group results in *EGFR* alterations, such as the T790M and C797S mutations; the latter addresses other methods that divert signaling dependence, such as activation of other downstream pathways such as *RAS*, gene fusions, *BRAF*, or even histologic transformation. Resistance to first and second generation *EGFR* TKIs are most often *EGFR*-dependent, with the commonest mechanism being the *EGFR* T790M occurring in 50–60% (3,4). Other *EGFR*-dependent mutations such as *EFGR* D761Y have been described but occur much less frequently (5). *EGFR*-independent mechanisms most frequently involve *MET* (5% to 20%) and *HER2* amplification (8%) (3,4). Histologic transformation to small cell is another cause of resistance (5–14%) (3,4) and *de novo RB1/TP53* mutations enrich for tumors likely to develop such transformation (6).

The spectrum of resistance to osimertinib is distinct to that seen with first or second generation *EGFR* TKIs and varies according to whether the drug is used in the first-line

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or later-line context. When used to treat acquired T790M mutation, between 20% and 30% develop resistance via an *EGFR*-dependent tertiary *EGFR* change, most commonly in the specific binding site of osimertinib, *EGFR* C797 (7,8). Mutations at other sites of the *EGFR* such as G724, L718, G719 and L792, and *EGFR* amplification have also been described (7-10). Resistance due loss of T790M develops in around half the cases, but remarkably in most of these cases is also associated with the emergence of other bypass mutations such as *KRAS* mutations, *MET* amplification, gene fusions or small-cell transformation, thus *EGFR*-independent (7,8). Therefore, in contrast with earlier generation TKIs, around 60% of Osimertinib treated T790M positive cases develop resistance in an *EGFR*-independent fashion, namely amplification of *MET* (6% to 26%) and *HER2* (up to 8%). Other less frequent, but still *EGFR*-independent and potentially targetable mutations have been described: *HER2* insertions, *KRAS* and *BRAF* V600E mutations, *NTRK*, *RET*, *ALK* and *FGFR* fusions, and *MET* exon 14 alterations (7,9,10). Similarly to resistance to earlier-generation TKIs, histologic transformation (to small-cell or squamous) is recognized to occur in between 4% and 15% of osimertinib-resistant cases in the later-line setting (7,10).

Resistance patterns to first-line osimertinib, as observed in the FLAURA trial occurred via *EGFR*-independent mechanisms in 32%, but surprisingly only 8% developed *EGFR* C797S mutations (11). More frequent *EGFR*-independent mechanisms included *MET* amplification (15%), cell cycle gene alterations (10%), *PIK3CA* mutations (7%), *BRAF* or *KRAS* mutations (3% each) and *HER2* amplifications (2%) (11). In later-line osimertinib trials, it is possible that addiction to the EGFR pathway through T790M development is predicated, which may explain why more *EGFR*-dependent resistance pathways are activated. Since the resistance data from FLAURA comes only from plasma genotyping, histologic transformation could not be identified; moreover, other resistance mechanisms could be higher, due to underestimation of gene amplification in plasma. This may explain why a large proportion (40–50%) have unknown resistance mechanisms (10); tissue biopsies may still be important to clarify osimertinib resistance mechanisms in the first-line context.

HER2D16: a potential novel resistance pathway

In this context, Hsu *et al.* (12) describe a patient who developed resistance to osimertinib after multiple other therapies. The resistance mechanism detected was complex; defined by T790M loss plus two *EGFR*-independent causes: a *HER2* amplification and a novel *HER2* ex 16 skipping (HER2D16). The latter has been previously reported in breast cancer only and constitutes one of the three splice variants of *HER2*, but results in addiction to HER2 signaling (13). Its clinical significance warrants further clarification, but is thought to explain some of the variability in the response to HER2 blockade (13). In breast cancer, the oncogenic properties of HER2D16 are mediated through direct coupling with Src kinase (13,14).

Interestingly, in the article by Hsu *et al.* (12) the HER2D16 mutation was detected in the plasma prior to commencing Osimertinib, but the allelic fraction increased with disease progression, leading to the hypothesis that this novel mutation mediated resistance. To further this, the authors use an *EGFR* -T790M/L858R positive cell line (H1975), to stably express HER2D16 and demonstrate elegantly that this novel protein cooperates with the EGFR, in both wild-type (WT) and mutant cells to allow constitutive activation despite osimertinib inhibition. Remarkably, Src levels did not alter after treating cells with dasatinib (a known Src inhibitor), and therefore failed to suppress cellular proliferation, with or without the presence of osimertinib. However, in an attempt to better suppress HER2 signaling *in vitro*, the authors combined osimertinib with afatinib (a known pan-*HER2* TKI). The combination of afatinib and osimertinib was indeed synergistic *in vitro*, but this was in a construct where both mutations are present in the one cell. Whether this is what actually occurs *in vivo* is difficult to know.

Discussion

HER2 alterations include amplifications and mutations, but are most commonly in-frame exon 20 insertions and occur *de novo* in about 1% to 5% of lung adenocarcinomas (15). They have been previously reported as osimertinib resistance mechanisms; in FLAURA, *HER2* amplifications were detected in 2%, and *HER2* mutations in 1% (11); in the later-line context, up to 5% of *HER2* amplifications have been described, but no *HER2* mutations (8,10,15). A recent publication described the *in vitro* use of trastuzumab-emantisine (TDM-1) combined with osimertinib to overcome *HER2* amplification-mediated resistance in *EGFR*-T790M-positive NSCLC cell lines, another example that combination strategies could be used to overcome resistance (16). A phase I–II trial is testing this combination

in patients with *EGFR*-mutant NSCLC, progressing after standard *EGFR* treatment who developed a HER2 bypass track mechanism of resistance (NCT03784599).

The real question from these data is whether this novel resistance mechanism and the *in vitro* targeting will translate in the *in vivo* context. Afatinib binds to Cys797, and preclinical evidence suggested effective inhibition in several *EGFR* activating mutations including T790M (17), but its clinical performance in patients with erlotinib-resistant cancers harboring T790M was minimal (18). Afatinib is equally potent against WT *EGFR* and *EGFR* T790M, so the toxicity resulting from inhibiting WT *EGFR* precludes the use of doses that would be needed to effectively suppress T790M. This same caveat was reported by the authors (12), since the drug concentration of afatinib which is effective with osimertinib may not be deliverable due to toxicity. Moreover, there is very limited experience with the combination of afatinib-osimertinib in literature, with debatable benefit (19). Similarly, while there was clear preclinical evidence suggesting activity of afatinib on *HER2* mutant NSCLC, the largest prospective trial that attempted to test its efficacy was stopped due to futility (20).

In the same context, preclinical data in a T790M-positive cell line suggested that the configuration of the T790M and C797S affected the response to therapy (21): if the two EGFR mutations were in *cis* (same DNA strand), the cells were refractory to combination first and third-generation TKIs; on the contrary, when the two mutations were in *trans* (on different DNA strands), a combination of EGFR inhibitors showed clear evidence of *in vitro* response (21). While this combination has shown some efficacy, unfortunately it is limited, making routine C797S/T790M testing less clinically meaningful (22).

The patient detailed by Hsu *et al*. (12) was heavily pre-treated, with systemic treatment including chemotherapy, TKIs and even with radiofrequency ablation. The exposure to several treatments is likely to have imposed different selection pressures, leading to multiple different clones. The mixed response to osimertinib, is suggestive of tumoral heterogeneity and is known to be poorly prognostic (23). Since these mutations were found in separate blood samples and the response was heterogeneous, raises the question whether these mutations coexisted in the one site or is the result of spatial heterogeneity. This then draws into challenge whether targeting this resistance pathway would be likely to be broadly effective.

The importance of identifying resistance mechanisms is based on the principle that further specific targeting may evoke durable benefit and minimal toxicity. Considering that resistance to osimertinib usually involves combined mechanisms, such as the activation of alternative cellular pathways and/or aberrant downstream signaling, osimertinib-based combination therapies are currently being investigated (10). Moreover, several case reports and small clinical series including novel combinations with other TKIs against *EGFR*, *RET*, *ALK*, *ROS*, *MET* and *BRAF* inhibitors have been reported (10,22,24-26). However, again, all this initial evidence requires further confirmation. An interesting approach is the recently launched ORCHARD Phase II trial (NCT03944772) which will explore treatment options after disease progression on first-line osimertinib according to the onset of acquired resistance mechanisms. In this innovative platform trial, patients will be allocated to a biomarker-matched study treatment: osimertinib plus gefitinib - osimertinib plus savolitinib (a novel *MET* inhibitor)—osimertinib plus necitumumab or platinum-based doublet plus durvalumab—within each group based on tumour molecular profile.

Conclusions

Since no clear mechanism of resistance is identified in between 30–40% of patients treated with later-line osimertinib and up to 50% of patients treated with first-line osimertinib (10), studies like Hsu *et al*. (12) are key to identify targetable alterations. The combination TKI approach is familiar and easy to implement although toxicity would need to be considered and needs confirmation of benefit. But more importantly, this study shows us that resistance is a complex process. It may incorporate both *de novo* clonal heterogeneity and clonal selection, but also may be the result of mutagenesis with single cells developing multiple resistance mechanisms.

The data from the cell line construct suggest that the HER2D16 mutation is targetable and the authors argue that it should be included as standard testing for reversible mechanisms of osimertinib resistance. However, it is important to acknowledge that while it is useful to identify potential mediators of resistance, the main impetus to implementing such testing is if this mutation is targetable *in vivo* with an effective yet non-toxic regimen. Until then, while these results are fascinating and important in better understanding the biology of this disease, they remain primarily of academic interest.

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