



Targeting the reversible drug-tolerant state: aurora kinase A, is that the final answer?

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Clinical application of the first-generation (1G) epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib launched the era of molecular targeted therapies for lung adenocarcinoma. At the same time, acquired resistance to these agents has been problematic given that 1G EGFR-TKIs are associated with a median progression-free survival of 9–12 months (1).

The mechanistic origins of acquired resistance to EGFR-TKIs are considered to be multifocal as summarized in our previous review (2). In that review, we classified the origins into three categories: (I) pre-existing minor subpopulation with a genetic resistance mechanism; (II) reversible (non-genetic) drug-tolerant state; (III) roles of the tumor microenvironment such as cancer-associated fibroblasts/dying cancer cells and poor vascularization/hypoxic condition.

In the era of 1G or 2G EGFR-TKIs, *i.e.*, before the approval of front-line osimertinib in 2018, the most problematic acquired resistance mechanism was the *EGFR* T790M secondary mutation (3). One origin of this mutation is that many *EGFR*-mutated lung cancers possess a pre-existing minor subpopulation carrying the T790M mutation. For example, using an ultrasensitive detection method, up to 79.9% of *EGFR*-mutated lung cancers were found to carry a pre-treatment T790M mutation (4). The allele frequency of the *EGFR* T790M mutation ranged

between 0.001 and 0.1% in most cases (95%) (4). Why can cancer cells “prepare” this resistance mutation before the initiation of 1G or 2G EGFR-TKI therapy? Although the evidence is not solid, Fujii and colleagues suggested that hypermethylation of the CpG dinucleotide in *EGFR* codon 790 leads to the C-to-T transition mutation, causing the T790M mutation (5).

However, the 3G EGFR-TKI osimertinib has changed this dynamic because it is also active against lung cancers featuring the *EGFR* T790M mutation (6). This means that *EGFR*-mutated lung cancer cells cannot evade therapy based on the presence of a pre-existing minor subpopulation with T790M mutation. Therefore, it can be hypothesized that drug resistance originating from (II) a reversible (non-genetic) drug-tolerant state or related to (III) the tumor microenvironment will be more important in the era of front-line osimertinib.

The first attempt to combat the reversible drug-tolerant state was reported in 2010 by Sharma and colleagues (7). In this study, which mainly used the PC9 cell line, the authors observed that a small fraction of viable, largely quiescent cells [named drug-tolerant persisters (DTPs)] remained detectable after 9 days in the presence of an EGFR-TKI (gefitinib or erlotinib). DTPs had similar characteristics as cancer stem cells (higher CD133 and CD24 levels than the parental PC9 cells). However, the DTPs eventually

resumed normal proliferation in the presence of the drug (upregulated cancer stem cell markers were decreased), and the authors referred to these cells as drug-tolerant expanded persisters (DTEPs). Through analyses of DTPs and DTEPs, the authors identified that drug-tolerant cells required the histone demethylase KDM5A/RBP2/Jarid1A, and they were selectively ablated by HDAC inhibitors. In addition, DTPs were also sensitive to insulin-like growth factor 1 receptor (IGF-1R) inhibition, and combination therapy with an IGF-1R inhibitor (AEW541) and an EGFR-TKI eliminated these drug-tolerant cells. Following this discovery, many research groups identified a long list of candidate molecules related to the drug-tolerant state upon EGFR-TKI therapy in lung cancer cells with *EGFR* mutation; e.g., NF- κ B, β -catenin, STAT3, Ets-1, endoplasmic reticulum (ER) stress signaling, glucose metabolism, MCL-1, and AXL (8-20).

The next breakthrough in this field was made by Hata and colleagues, who demonstrated that cells with acquired resistance associated with genetic changes such as the T790M mutation evolve from drug-tolerant cells (lacking the T790M mutation), highlighting the importance of targeting drug-tolerant cells (21). By what mechanism can drug-tolerant cells acquire a genetic change (T790M mutation) that causes irreversible drug resistance? One possible mechanism was reported by El Kadi and colleagues (22). EGFR-TKI therapy led to activation of the NF- κ B pathway, thereby inducing the expression of activation-induced cytidine deaminase, which generated the T790M mutation by inducing the deamination of 5-methylcytosine to thymine at position c.2369.

Following these studies, in 2019, Shah and colleagues reported that non-genetic resistance through the activation of aurora kinase A (AURKA) by its coactivator TPX2 emerges in response to osimertinib monotherapy by preventing drug-induced apoptosis (23). Via compound screening (a 94-compound cancer-focused library), they achieved synergistic inhibition of AURKA inhibitors with

osimertinib or another 3G EGFR-TKI, namely rociletinib, using four *EGFR*-mutated lung cancer cell lines (PC9, HCC827, HCC4006, and NCI-H1975) after chronic EGFR-TKI exposure with stepwise dose escalation over a 9-day period followed by maintenance treatment at a dose of 1 μ M for 6 weeks. In addition to experimental models, the authors observed that TPX2 expression was significantly increased [Immunohistochemistry (IHC) score >2] in six of nine paired tumor tissue samples obtained after EGFR-TKI treatment failure compared with the findings in pre-treatment samples. Interestingly, the importance of AURK inhibition in enhancing apoptosis upon EGFR-TKI therapy in *EGFR*-mutated lung cancer cells was also described at the recent 2019 AACR annual meeting by another research group (24). Are these reports just adding one molecule at the end of the aforementioned long list of candidate molecules related to the drug-tolerant state in lung cancer cells with *EGFR* mutation, or is AURK the final target for combination therapy featuring osimertinib to suppress drug-tolerant cells? At the end of this commentary, I would like to raise three points that would support the importance of AURKA over other candidate molecules.

First, it is easy to understand why some cancer cells have high AURKA activation at the time of EGFR-TKI therapy. It is well known that in G2/M phase, cells have high levels of phosphorylated AURKA, and in fact, Shah and colleagues also reported that parental PC9 cells synchronized into G2/M phase using serum starvation or thymidine block were less sensitive to EGFR-TKIs than cells in G1/S phase or asynchronous cells (23). The second advantage of AURKA inhibition in combination with osimertinib is that many AURK inhibitors are currently in clinical development (25). Last, as illustrated in *Figure 1*, AURKA is related to many molecules that have been reported as causes of drug-tolerant states. Therefore, it is possible that AURKA inhibition can be a “master key” to repress important signaling pathways for drug-tolerant cells at an early phase of EGFR-TKI treatment.

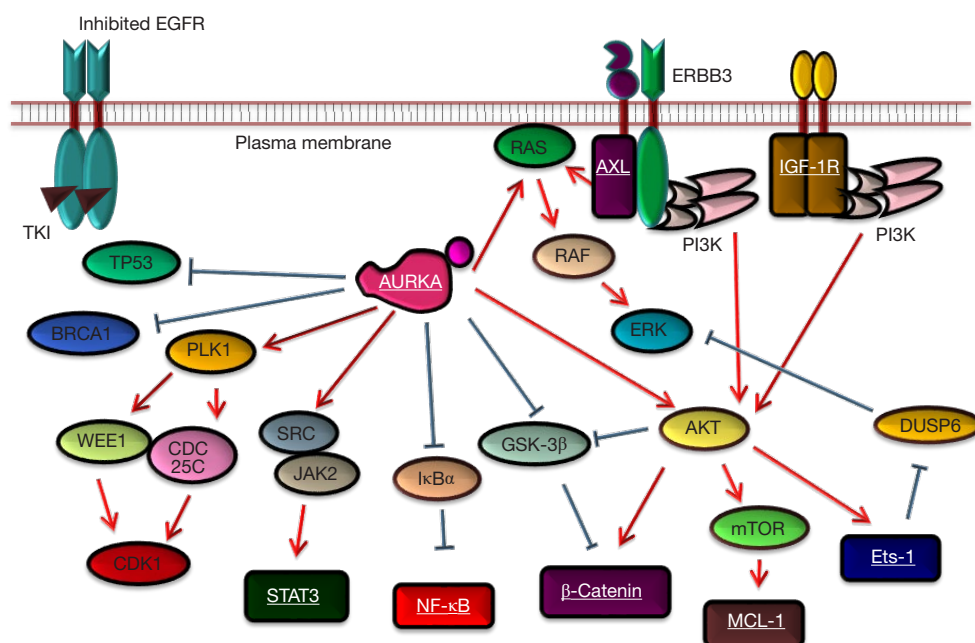


Figure 1 Scheme of the AURKA pathway and molecules that are reported to cause the drug-tolerant state [AXL (20), IGF-1R (7,21), NF- κ B (8,9,13,14,17), β -catenin (10), STAT3 (11,12), Ets-1 (15), and MCL-1 (18), which are highlighted in white bold letters] in lung cancer cells carrying *EGFR* mutations upon EGFR-TKI treatment. It is interesting that the compound screening by Shah and colleagues also identified that compounds with the 3rd to 7th highest synergy scores were inhibitors of PI3K, AKT, mTOR, or Src (although they are not highlighted, they may have some roles in the drug-tolerant state; as a side note, the compounds with the highest two synergy scores were AURK inhibitors) (23). Some other candidate factors that might be related to drug tolerance; e.g., altered chromatin state (7), ER stress signaling (19), or glucose metabolism (16), are not illustrated in this figure; however, it is noteworthy that AURKA inhibition will lead to the inhibition of multiple potential causes of drug tolerance. The figure was generated using a combination of results from multiple published works related to the AURKA pathway (26–29) or drug tolerance states (15,18,20,23); therefore, it is possible that it may overestimate the roles of AURKA. AURKA, aurora kinase A; EGFR, epidermal growth factor receptor; TKIs: tyrosine kinase inhibitors; ER, endoplasmic reticulum.

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Footnote

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