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Review

Primary Resistance to EGFR Tyrosine Kinase Inhibitors (TKIs): Contexts and Comparisons in EGFR-Mutated Lung Cancer

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Abstract: The discovery of the efficacy of tyrosine kinase inhibitors (TKIs) in epidermal growth factor receptor (*EGFR*)-mutated non-small-cell lung cancer (NSCLC) patients has revolutionized lung cancer therapy. Although almost all responders acquire drug resistance within a few years, many studies have revealed several acquired-resistant mechanisms and developed therapeutic strategies countervailing them, most notably against the *EGFR* T790M gatekeeper mutation. However, little progress has been made in terms of elucidating the mechanisms of primary resistance. Primary resistance may be defined into two types of resistance, clinically representing patients that do not respond (non-responders) to EGFR-TKIs. The first group consists of approximately 10% of patients that are insensitive to EGFR-TKIs from the outset (intrinsic primary resistance), and 20–30% of the second group consists of patients that seem to clinically benefit at first, but experience early relapse within six months (late primary resistance). In this review, we first provide an overview of drug-induced lung cancer dynamics. We then delve into the mechanisms of primary resistance, with a primary focus on two specific subtypes of resistance. We suggest that "intrinsic primary resistance" is characterized by pre-existing somatic and genomic changes and cell of origins, while "late primary resistance" is correlated with the drug-tolerant persister state. Developing therapeutic strategies to overcome primary resistance is crucial to prolonging the duration of EGFR-TKI therapy. Ultimately, this will allow for an enhanced understanding of lung cancer's evolutional process, leading to the reversal of acquired resistance and the complete eradication of lung cancer.

Keywords: EGFR-TKI; primary resistance; cancer dynamics; drug-tolerant persister

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1. Introduction

Lung cancer is the most common cause of death due to cancer worldwide [1]. The discovery of epidermal growth factor receptor (*EGFR*) mutations in non-small-cell lung cancer (NSCLC) as a driver oncogene and the effectiveness of EGFR-tyrosine kinase inhibitors (TKIs) for patients have transformed lung cancer therapy, benefiting 60–70% of these patients [2–5].

The most prevalent *EGFR* mutations, accounting for approximately 90%, are deletions in exon 19 and L858R point mutations in exon 21, known as the classical activating mutations [6]. Other, less common *EGFR* mutations include G719X, S768I, and L861Q, insertions in exon 20, with compound mutations being the most frequent among them [7].

In the context of EGFR-TKI treatment, a significant proportion of responders develop drug resistance within a few years, with median progression-free survival periods extending between 9.2 to 18.9 months for the first through third generations of EGFR-TKIs [1,4,5,8–12]. Numerous investigations have elucidated a variety of acquired-resistance mechanisms, prompting the development of therapeutic countermeasures. Notably, the EGFR T790M mutation has emerged as a predominant resistance mechanism against the first- and second-generation EGFR-TKIs. Consequently, third-generation EGFR-TKIs have been engineered with a heightened specificity for the T790M mutation. Moreover, third-generation EGFR-TKIs have been observed to potentiate specific on-target resistance

mutations, notably the EGFR C797S mutation. Resistance mechanisms, such as the activation of bypass signaling pathways, encompassing *MET* and *HER2* amplification, as well as histologic transformations (e.g., transitions to small cell or squamous cell carcinomas), present significant challenges across all EGFR-TKI generations [13–15]. Given these complexities, research endeavors are presently steering towards the development of fourth-generation EGFR-TKIs [16]. Understanding acquired resistance and developing therapeutic approaches to combat this, similarly to the relationship between antimicrobial agents and microbial drug resistance, could be considered as a "cat-and-mouse game".

Conversely, however, 10% of patients exhibit insensitivity to EGFR-TKIs from the outset, and relatedly, 20–30% of patients seem to benefit clinically at first but experience early relapse within six months (Table 1) [17]. They are clinically termed non-responders, and also may be considered as having "primary resistance" to therapy (Figure 1).

Table 1. The prevalence of primary resistance in pivotal clinical studies.

Study	EGFR-TKI	TKI-Generation	Overall Response Rate, %	Primary Resistance, %	Intrinsic Primary Resistance (PD), %	Late Primary Resistance (SD), %
IPASS	Gefitinib	1	71.2	28.8	7.6	20.5
NEJ003	Gefitinib	1	73.7	26.3	11	15.8
WJTOG-3405	Gefitinib	1	62.1	37.9		
EURTAC	Erlotinib	1	58	42		
OPTIMAL	Erlotinib	1	83	17		
LUX-Lung-7	Afatinib	2	72.5	27.5	6	21
ARCHER-1050	Dacomitinib	2	75	25	5	13
FLAURA	Osimertinib	3	80	20		

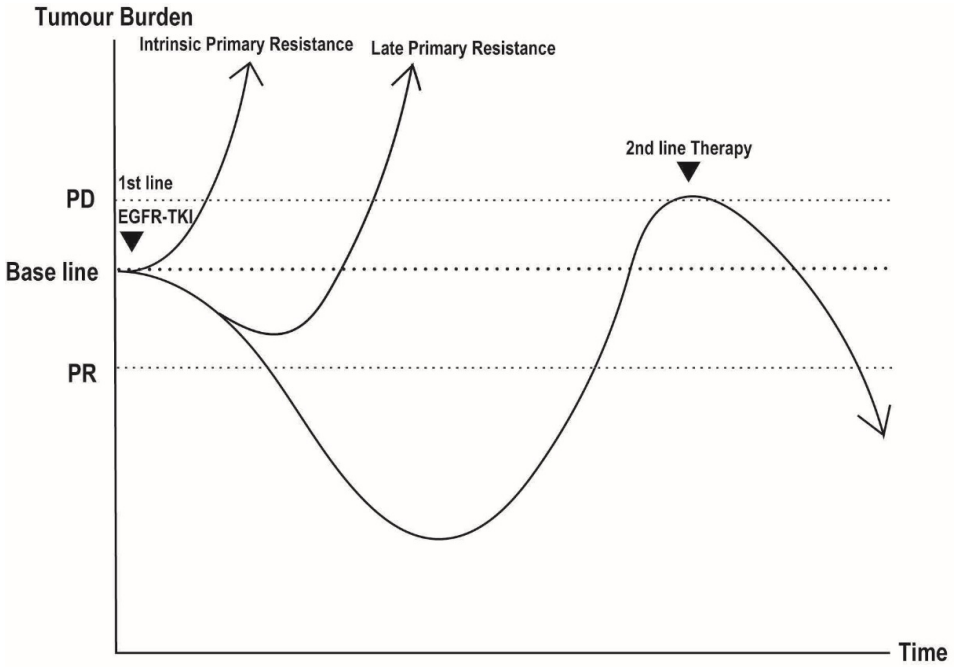

Figure 1. The clinical time courses of patients with *EGFR*-mutated non-small-cell lung cancer undergoing EGFR-TKI treatment. The post-treatment dynamics of *EGFR*-mutated non-small cell lung cancer patients receiving EGFR-TKI therapy can be broadly categorized into three groups. The first group is referred to as "intrinsic primary resistance", where patients show resistance to EGFR-TKI even before treatment initiation. The second group is known as "late primary resistance", in which patients initially respond to EGFR-TKI therapy for a short period but quickly develop resistance. The third group represents the typical treatment course, with patients undergoing approximately a one-year period of EGFR-TKI exposure before acquiring resistance.

Based on the definition of primary resistance, we derived it by subtracting ORR from 100 (%). We could further separate the group into patients with PD (intrinsic primary resistance) and SD (late primary resistance).

Minimal advancements have been achieved in terms of understanding the mechanisms behind primary resistance, as opposed to acquired resistance. One reason may be that it has been difficult to routinely perform comprehensive genomic and epigenetic profiling in the clinical setting until recently. In particular, patients with primary resistance may have limited pre-treatment tissue for deep sequencing and, thus, be less willing to undergo serial tumor tissue biopsies. Therefore, this remains a significant area of unmet research need.

Overcoming primary resistance might herald a new era in lung cancer therapy. By mitigating primary resistance, the efficacy of initial EGFR-TKI treatments could be extended, offering deeper insight into the progression of lung cancer and ultimately enhancing patient prognosis.

This review delves into the present understanding of mechanisms behind primary resistance, drawing from both foundational and translational research. Additionally, it offers insights into future directions for comprehending primary resistance in the context of EGFR-TKI treatment.

2. Overview of Drug-Induced Cancer Dynamics

A broad and all-encompassing overview of lung cancer dynamics is vital to allow for an understanding of primary resistance mechanisms.

When the drug enters the body, it has a more significant effect on cancer cells than on normal cells, but it can also affect normal cells as a side effect. Lung cancer cells may engage with cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAM), and normal cells, inducing a range of alterations in the tumor microenvironment (TME) (Figure 2).

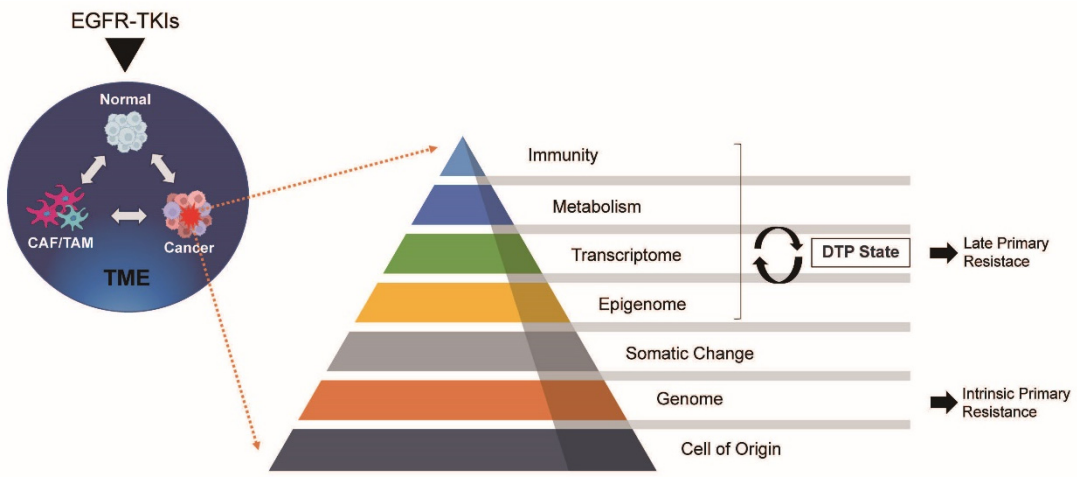

Figure 2. Overview of Drug-Induced Cancer Dynamics. The medication can influence tumor cells throughout a seven-tiered pyramidal structure, potentially leading to diverse adaptations in cancer cells in response to the altered conditions induced by the drug.

The drug can infiltrate and affect tumor cells from the top to the bottom of a seven-layer pyramidal hierarchy. Cancer cells adapt and transform in response to the altered conditions brought about by medication exposure.

We propose that, at first, immunity and metabolism will react and bring about tumor changes. Then, after a short period, the drug affects the mRNA known as the transcriptome. Then, it will affect the epigenome, the genetic information unrelated to DNA sequence changes. As more time passes, it can affect the DNA sequence, leading to somatic and genomic changes. The lowest level is "cell of origin", as exemplified by the metamorphosis

of NSCLC into either small cell or squamous cell lung cancer [15,18]. These changes may occur sequentially or simultaneously by mutually affecting each other.

A small subset of cancer cells might evade cell death induced by anticancer drugs by transitioning into a reversible state of slow proliferation, referred to as the drug-tolerant persister (DTP) state [19–21]. This adaptable state allows cancer cells to withstand drug treatment and may later lead to the development of primary or acquired resistance.

Numerous genetic modifications, like *EGFR* T790M or *MET* amplification, might emerge during the DTP state. Thus, the DTP state might act as a reservoir for the evolution of drug-resistant cells [22].

The lower layers of hierarchy represented by genetic alterations or lineage plasticity can show more solid phenotypes. They might be involved in developing "intrinsic primary resistance". Conversely, at the upper levels of the hierarchy, there are no solid phenotypes of resistance, which might be associated with the emergence of late primary resistance. One reason is that DTP cells can mainly be related to these upper layers [23]. DTP cells are reversible, in a slow proliferation state, against drug exposure and can survive for some time without undergoing any genetic alterations. This is very similar to the biology of some lung cancer patients, who initially appear to be improved but develop drug resistance within six months, which is known as late primary resistance.

The key components of lung cancer dynamics may be roughly divided into three categories: (1) drug-related factors, (2) tumor-related factors, and (3) tumor microenvironment (TME). We discuss these three items in the subsequent sections.

3. Drug-Related Factors

Drug efficacy depends on pharmacokinetic and pharmacodynamic analysis, and is related to the drug concentration in a body compartment rather than the drug dose. Whether treatment is given once or as a combination therapy may also influence the drug's efficacy on tumors.

3.1. Pharmacokinetic Mechanisms

The metabolism of each generation of EGFR-TKIs is similar, but distinct. Based on its metabolism by CYP3A enzymes, most EGFR-TKIs, other than afatinib (second-generation), may be affected by CYP enzyme inducers, which might result in false resistance. For instance, dexamethasone, a CYP inducer, led to a 0.6-fold reduction in erlotinib (first-generation) [24]. Other potent CYP inducers like phenytoin and rifampin can lower the level of gefitinib plasma concentrations [25,26]. On the other hand, hepatic enzymes barely metabolize afatinib; hence, CYP-interacting substances are unlikely to have an effect on afatinib PK [27,28]. However, afatinib both acts on and inhibits the P-glycoprotein (P-gp) drug transporter, and the presence of a P-gp enhancer, rifampin, reduces the efficacy of afatinib [29].

First- and second-generation EGFR-TKIs possess limited capability to penetrate the central nervous system (CNS). In contrast, third-generation EGFR-TKIs are specifically engineered for enhanced CNS penetration [30].

3.2. Pharmacodynamic Mechanisms

This mechanism is mainly associated with intrinsic primary resistance. For instance, first- and second-generation EGFR-TKIs are ineffective against the *EGFR* T790M mutation due to the consequent altered conformational structure of *EGFR*. Thus, patients who had such a mutation originally are non-responders, known as those with intrinsic primary resistance.

Moreover, numerous studies indicate that responses to all-generation EGFR-TKIs vary among EGFR exon 20 mutations due to their heterogeneity [31,32]. A recent study revealed that *EGFR* mutations, encompassing atypical variations, can be organized into four unique subgroups based on their structures and functions to improve drug sensitivity prediction to targeted therapies: (1) classical-like mutations located away from the ATP-binding pocket;

(2) T790M-like mutations within the hydrophobic core; (3) insertions in the loop at the C-terminal end of the αC-helix in exon 20 (Ex20ins-L); and (4) mutations on the inner surface of the ATP-binding pocket or at the C-terminal end of the αC-helix, anticipated to cause compression of P-loop and αC-helix (PACC) [33]. Based on this structural classification, exon 20 mutations are sorted into one of three structural classes ((2), (3), and (4)). Especially among the (3) Ex20ins-L group, patients in the exon 20 far-loop insertions (Ex20ins-FL) subgroup are not sensitive to any targeted drugs, leading to intrinsic primary resistance.

4. Tumor-Related Factors

4.1. Metabolism

4.1.1. DTP Cells and Late Primary Resistance

Cancer cells modify their metabolic processes to adjust to the changes brought about by drug exposure. For example, the Warburg Effect, proposed by Otto Warburg more than 50 years ago, is a phenomenon by which cancer cells harness anaerobic glycolysis to fuel their proliferation rather than mitochondrial oxidative phosphorylation [34]. In contrast, recent research indicates that cancer cells use oxygen via mitochondrial oxidative phosphorylation as much as or more than normal cells [35]. DTP cells, which are exposed by drugs, perform a pivotal role in this situation, leading to late primary resistance for EGFR-TKIs.

Since DTP cells demonstrate a heightened reliance on mitochondrial oxidative phosphorylation, they must regulate the overproduction of oxygen-rich molecules, such as superoxide and lipid peroxide, which arise from the mitochondrial oxidative phosphorylation process. Therefore, DTP cells use glutathione peroxidase 4 (GPX4) to neutralize lipid peroxides. It is confirmed in NSCLC cells that inhibiting GPX4 can lead to an accumulation of lipid peroxides, resulting in ferroptosis, a type of oxidative cell death [36,37].

Likewise, DTP cells have elevated aldehyde dehydrogenase (ALDH) activity to shield themselves from the toxic impacts of lipid peroxidation [21]. For instance, a combined treatment using an ALDH inhibitor (disulfiram) and cisplatin-based chemotherapy enhanced the survival of advanced NSCLC patients, indirectly proving that ALDH inhibition can counteract late primary resistance [38].

4.1.2. Lipid Metabolism and Autophagy

Lipid metabolism might also play a role in late primary resistance. Fatty acid β-oxidation is essential for energy generation in mitochondrial oxidative phosphorylation. CD36 acts as the primary transporter for fatty acid absorption. The high methylation of CD36 in lung cancer fills a vital role in cancer progression through fatty acid β-oxidation. As a matter of fact, de-methylation of CD36 can lead to removal of the resistance. Combined treatment with the DNA methylation inhibitor (decitabine) and the histone deacetylase (HDAC) inhibitor (chidamide) effectively curbs lung cancer growth in vivo [39].

Autophagy serves as a cellular survival strategy by breaking down and recycling proteins, macromolecules, and organelles, in contrast to apoptosis, which leads to cell death. It plays a crucial role in clearing out malfunctioning mitochondria generated by reactive oxygen species during mitochondrial respiration. Drug-induced accumulation of these impaired mitochondria can enhance malignancy by amplifying oxidative stress. In a lung cancer mouse model, for example, removing the vital autophagy gene Atg7 has been shown to boost oxidative stress and speed up tumor cell growth [40,41].

4.2. Transcriptome and Epigenome

Epigenetics encompasses gene regulatory processes that do not involve DNA sequences, including DNA methylation, hydroxymethylation, and modifications to histone proteins. The transcriptome is intricately linked to epigenetics. For instance, changes in chromatin structure facilitate the epigenetic control of apoptosis and gene expression. Some long noncoding RNAs are involved in oncogenesis through the regulation of transcription, translation, and epigenetics [42].

A characteristic of DTP cells is their reversible biological ability, which is not determined by the genome, but rather by adaptable epigenomic regulation [19].

Exposure to EGFR-TKIs can boost the expression of the epigenetic modulator KDM5A, which is an H3K4 demethylase. Given that KDM5A inhibits the transcriptional activity of H3K4, its increased expression can contribute to late primary resistance. Conversely, KDM5 inhibitors like CPI-455 can increase H3K4 trimethylation levels, thereby overcoming the primary resistance [43].

4.3. Somatic and Genomic Changes

Among somatic mutations in lung cancer, driver oncogenes such as EGFR have intense oncogenic potential known as "oncogene addiction". Therefore, a single molecularly targeted drug produces significant therapeutic effects. On the other hand, some NSCLC patients who have not undergone EGFR-TKI treatment possess additional driver changes alongside EGFR mutations, which could partially account for the inherent primary resistance observed in certain individuals [44]. In those cases, cancer proliferation may depend on several oncogenes in addition to EGFR mutation. Consequently, EGFR oncogenic potential seems to be slightly weak, and EGFR-TKIs cannot be the most effective treatment for them.

The recent advancements in comprehensive genomic profiling have enabled us to gain insight into diverse mutations and concurrent genetic alterations other than EGFR mutations [45–47]. This section focuses on common concurrent genetic alterations (TP53, PIK3CA, and PTEN) in EGFR-mutated lung cancer patients.

4.3.1. TP53

p53 can induce cell cycle halting, senescence, and programmed cell death [48]. Alterations in the TP53 gene, which codes for p53, are detected in 35–55% of NSCLC patients and are strongly linked to smoking behaviors [49]. TP53 is the primary concurrent alteration in EGFR-mutant NSCLC patients, occurring in 55–65% of cases [45,50,51].

The TP53 status correlates with reduced effectiveness to EGFR-TKIs. In NSCLC cell lines with wild-type p53, gefitinib can induce apoptosis by upregulating Fas at the plasma membrane and reviving caspase activation, thus increasing TKI responsiveness. Conversely, it has been indirectly shown that gefitinib-induced apoptosis is diminished in cells with mutated p53, leading to primary resistance [52]. Notably, the prognostic impact depends on different types of TP53 mutations [53].

4.3.2. PIK3CA Mutation

PIK3CA is responsible for encoding the active component of PI3K, and its mutations can stimulate the PI3K/AKT pathway [54]. Mutations in PIK3CA are uncommon, occurring in roughly 2–5% of NSCLC cases. The most frequent mutations are E545K in exon 9 and H1047R in exon 20 [55–57].

PIK3CA mutations frequently appear alongside other driver mutations, notably EGFR and KRAS [55,58,59]. PI3KCA mutations are present in roughly 3.5% of patients with EGFR mutations and are associated with intrinsic primary resistance to EGFR-TKIs [60,61]. In a preclinical study, introducing an active PIK3CA p.E545K mutation into HCC827 cell lines harboring EGFR 19 deletion developed resistance to gefitinib [62]. Conversely, a recent study indicated that specific PIK3CA mutations influenced the outcomes in EGFR-mutant NSCLC patients undergoing EGFR-TKI treatment. Mutations in the p85 binding domain (R88Q, R108H, and K111E) are associated with better progression-free survival (PFS), while mutations in the kinase (Y1021H and H1047R), helical (E542K), and C2 (N345K) domains lead to reduced PFS [63]. Therefore, primary resistance to EGFR-TKIs would be affected by the simultaneous presence of specific PIK3CA mutations.

4.3.3. PTEN Alterations

Phosphatase and tensin homologs deleted on chromosome 10 (PTEN) act as tumor suppressors and play a role in various cellular activities, including cell proliferation, survival, growth, metabolism, migration, and apoptosis [64–66]. PTEN serves as a primary inhibitor of the PI3K/AKT pathway, and its inactivation is crucial for the onset and progression of lung cancer [67,68]. Loss of PTEN function, found in more than 40% of cases, can activate the PI3K/AKT pathway, accelerating tumor progression and reducing sensitivity to EGFR-TKIs in NSCLC patients [68–70]. PTEN mutations occur rarely, in 2–5% of NSCLC cases [71,72].

This is due to PTEN's role in regulating the endocytic trafficking of EGFR, a crucial mechanism for controlling EGFR signaling [73]. Following the binding of EGF (ligand) to its receptor, EGFR, the receptor-ligand combination is taken up by clathrin-coated vesicles. These vesicles then transport the complex to early endosomes for categorization [74,75]. The EGF/EGFR combination progresses to late endosomes via vesicle maturation and subsequently undergoes lysosomal fusion, leading to receptor degradation [76,77]. PTEN plays a pivotal role in facilitating the transition of ligand-bound EGFR from early endosomes to late endosomes for receptor degradation by regulating the phosphorylation of Rab7 and maturing late endosomes [78]. Therefore, PTEN inactivation can lead to the inhibition of EGFR signaling.

4.4. Cell of Origin

The cell of origin is the lowest level of the pyramid. As previously stated, lineage plasticity, including small-cell or squamous-cell lung cancer transformation, is found in this layer, which may be related to late primary resistance.

On the other hand, there are also some intrinsic primary resistance cases before EGFR-TKIs administration in this layer, including ALK rearrangement, T790M, and BIMdeletion polymorphism.

This section discusses the lineage plasticity and BIM deletion polymorphism.

4.4.1. Lineage Plasticity

Histologic transformation is dominated by small-cell and squamous-cell lung cancer transformation as late primary resistance.

As for the mechanisms of SCLC transformation, there are two hypotheses. One theory is that NSCLC can be histologically differentiated into SCLC by EGFR-TKIs exposure through the inactivation of p53 and RB1 [79]. The other theory is that both SCLC and NSCLC components exist simultaneously in the same tumor. Since EGFR-TKIs can reduce the NSCLC elements of tumors, SCLC elements can remain and become dominant [80].

It is also suggested that there could be transcriptomic level lineage plasticity involved in squamous-cell transformation. One patient who developed histologic transformation from adenocarcinoma at baseline was found to have chromosome 3q, harboring squamous lineage transcription factors TP63 and SOX2 [15].

4.4.2. BIM Deletion Polymorphism

BIM, also referred to as BCL2L11 or B-cell chronic lymphocytic leukemia/lymphoma-like 11, is a proapoptotic member of the Bcl-2 family that solely possesses BH3 domains. These domains of the BIM gene are essential for apoptosis triggered by EGFR-TKIs [81–85]. Since BIM is degraded by ERK signaling, EGFR-TKIs can inhibit the ERK signaling and increase the level of BIM protein, leading to cell apoptosis.

The BIM deletion polymorphism involves a 2903 bp fragment removal in the BIM gene's intron 2, producing an inactive BIM protein variant. This variant is missing the essential BH3 domain, compromising apoptosis related to EGFR-TKIs and leading to inherent primary resistance to these inhibitors [82]. This BIM deletion polymorphism is found in 12–16% of EGFR-mutant lung cancer patients [86,87], especially in East Asian individuals [82].

HDAC inhibitors trigger the hyperacetylation of histone on promoter regions, leading to the transcriptional activation of proapoptotic BH3-only genes like BIM [88]. Therefore, combining EGFR-TKI with an HDAC inhibitor could potentially revive the expression of the BIM protein with a BH3 domain, counteracting EGFR-TKI resistance [89]. Indeed, a phase I trial involving 12 patients who received a combination of gefitinib and vorinostat (an HDAC inhibitor) demonstrated an enhanced disease control rate of 83.3% and an extended PFS of 5.2 months [90].

5. Tumour Microenvironment (TME)

Exposure to drugs impacts the TME, which is linked to primary resistance against EGFR-TKIs. CAFs, a significant component of the TME, are known to contribute to drug resistance by releasing growth factors and chemokines [91]. For example, cancer-associated fibroblasts (CAFs) secrete hepatocyte growth factor (HGF) in response to EGFR tyrosine kinase inhibitors (TKIs), resulting in primary resistance in EGFR-mutant non-small cell lung cancer (NSCLC) cells. This is because HGF interacts with the MET receptor, subsequently reactivating the MAPK and PI3K/AKT signaling pathways [92,93].

Additionally, TAMs and MDSCs play a crucial role in contributing to primary resistance to EGFR-TKIs. Specifically, MDSCs that are positive for S100A9, capable of differentiating into TAMs, have been linked to a diminished response to EGFR-TKIs in NSCLC patients [94]. From a mechanistic perspective, S100A9 enhances ALDH1A1 expression and activates the retinoic acid (RA) signaling pathway, thereby promoting cancer proliferation. Using a pan-RAR antagonist can significantly reduce cancer growth [95].

Immunity

Drug exposure affects tumor and tumor-infiltrating immune cells. Programmed death-ligand 1 (PD-L1) expression in EGFR-mutant NSCLC cells can be downregulated by EGFR TKIs, leading to the lack of efficacy of PD-1/PD-L1 inhibitors in most EGFR-mutant NSCLC patients [96]. Similarly, EGFR-mutant NSCLC cell lines with higher PD-L1 expression are resistant to gefitinib. This is because that PD-L1 overexpression may stimulate epithelial–mesenchymal transition (EMT) by activating the TGF-β/Smad canonical signaling pathway [97].

AXL, one of the members of the receptor tyrosine kinase group, has been associated with innate resistance to programmed cell death protein-1 (PD-1) inhibition and suppression of proper antigen presentation a by major histocompatibility complex (MHC)-I [98]. Therefore, inhibiting AXL could mediate a favorable reprogramming of the immune-suppressive TME [99,100].

6. Discussion

Removing the primary resistance for EGFR-TKIs has become a topic of increasing interest. Previous studies have focused on individual cancer cells, elucidating the acquired resistance mechanisms and investigating the treatment strategies. However, cancer cells interact with the surrounding environment and find another signal pathway for proliferation as a loophole. Tumors can progress on their own and be assisted by the surrounding environment.

To elucidate primary resistance mechanisms, we should overview cancer dynamics, including tumor microenvironment, epigenetic changes, and commutation alterations. The recent development of comprehensive genomic and epigenomic profiling has allowed us to understand various changes leading to primary resistance.

Regarding primary resistance, we believe that it is generally divided into two mechanisms. One is that lung cancer already has resistant mechanisms prior to EGFR-TKI treatment, including somatic and genome changes and "cell of origin", known as "intrinsic primary resistance". The other mechanism is related to the TME, immunity, metabolism, transcriptome, and epigenome, causing what is known as late primary resistance. This may be linked to drug- DTP cells, which can evade drug toxicity by changing their biolog-

ical nature to a reversible slow proliferation state. This may reflect the clinical situation in which some patients initially appear to be improved, but develop resistance within six months.

As for genomic profiling, it is difficult to interpret the information that can be retrieved from the analysis of hundreds of genes. And most studies applying comprehensive genome profiling primarily focus on capturing driver mutations and analyzing the abundant co-mutations. At the same time, copy number gains and other alterations are less considered. This observation should be further elucidated because these are related to primary resistance.

Notably, focusing on the interaction between a tumor and other surrounding components is necessary to elucidate the primary resistance related to DTP cells. Hence, it is essential to develop robust preclinical models and molecular research techniques, such as organoid models capable of replicating the genetic and histological features of the original patient tumors, and establishing the tumor microenvironment through coculturing with fibroblasts and immune cells [101]. Moreover, leveraging cutting-edge technologies for single-cell mapping of transcriptomic, epigenomic, or proteomic alterations can offer a deeper understanding and validation of DTP cell characteristics.

Comprehensive genome profiling can allow us to predict the primary resistance to EGFR-TKIs, while it may be difficult to release the resistance directly. In contrast, many diverse mechanisms have been identified for primary resistance related to DTP cells. It is an obstacle to translating them clinically, but it is a great challenge to conquer lung cancer. The clinical application of studies clarifying the characteristics of DTP cells can prevent the development of resistance and delay the emergence of resistance. Further research concerning primary resistance will elucidate the cancer evolution mechanisms, leading to the rescission of acquired resistance and achieving a complete eradication of lung cancer.

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References

1. Fitzmaurice, C.; Abate, D.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdel-Rahman, O.; Abdelalim, A.; Abdoli, A.; Abdollahpour, I.; Abdulle, A.S.M.; et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2017: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol.* **2019**, *5*, 1749–1768.
2. Lynch, T.J.; Bell, D.W.; Sordella, R.; Gurubhagavatula, S.; Okimoto, R.A.; Brannigan, B.W.; Harris, P.L.; Haserlat, S.M.; Supko, J.G.; Haluska, F.G.; et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **2004**, *350*, 2129–2139. [CrossRef] [PubMed]
3. Paez, J.G.; Jänne, P.A.; Lee, J.C.; Tracy, S.; Greulich, H.; Gabriel, S.; Herman, P.; Kaye, F.J.; Lindeman, N.; Boggon, T.J.; et al. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* **2004**, *304*, 1497–1500. [CrossRef] [PubMed]
4. Mok, T.S.; Wu, Y.L.; Thongprasert, S.; Yang, C.H.; Chu, D.T.; Saijo, N.; Sunpaweravong, P.; Han, B.; Margono, B.; Ichinose, Y.; et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **2009**, *361*, 947–957. [CrossRef] [PubMed]
5. Maemondo, M.; Inoue, A.; Kobayashi, K.; Sugawara, S.; Oizumi, S.; Isobe, H.; Gemma, A.; Harada, M.; Yoshizawa, H.; Kinoshita, I.; et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N. Engl. J. Med.* **2010**, *362*, 2380–2388. [CrossRef] [PubMed]
6. Rosell, R.; Moran, T.; Queralt, C.; Porta, R.; Cardenal, F.; Camps, C.; Majem, M.; Lopez-Vivanco, G.; Isla, D.; Provencio, M.; et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N. Engl. J. Med.* **2009**, *361*, 958–967. [CrossRef]

7. Zhang, Y.; Wang, Z.; Hao, X.; Hu, X.; Wang, H.; Wang, Y.; Ying, J. Clinical characteristics and response to tyrosine kinase inhibitors of patients with non-small cell lung cancer harboring uncommon epidermal growth factor receptor mutations. *Chin. J. Cancer Res.* **2017**, *29*, 18–24. [CrossRef] [PubMed]
8. Rosell, R.; Carcereny, E.; Gervais, R.; Vergnenegre, A.; Massuti, B.; Felip, E.; Palmero, R.; Garcia-Gomez, R.; Pallares, C.; Sanchez, J.M.; et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* **2012**, *13*, 239–246. [CrossRef]
9. Zhou, C.; Wu, Y.L.; Chen, G.; Feng, J.; Liu, X.Q.; Wang, C.; Zhang, S.; Wang, J.; Zhou, S.; Ren, S.; et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. *Lancet Oncol.* **2011**, *12*, 735–742. [CrossRef]
10. Park, K.; Tan, E.H.; O'Byrne, K.; Zhang, L.; Boyer, M.; Mok, T.; Hirsh, V.; Yang, J.C.; Lee, K.H.; Lu, S.; et al. Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): A phase 2B, open-label, randomised controlled trial. *Lancet Oncol.* **2016**, *17*, 577–589. [CrossRef]
11. Wu, Y.L.; Cheng, Y.; Zhou, X.; Lee, K.H.; Nakagawa, K.; Niho, S.; Tsuji, F.; Linke, R.; Rosell, R.; Corral, J.; et al. Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): A randomised, open-label, phase 3 trial. *Lancet Oncol.* **2017**, *18*, 1454–1466. [CrossRef] [PubMed]
12. Soria, J.C.; Ohe, Y.; Vansteenkiste, J.; Reungwetwattana, T.; Chewaskulyong, B.; Lee, K.H.; Dechaphunkul, A.; Imamura, F.; Nogami, N.; Kurata, T.; et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N. Engl. J. Med.* **2018**, *378*, 113–125. [CrossRef] [PubMed]
13. Yu, H.A.; Arcila, M.E.; Rekhtman, N.; Sima, C.S.; Zakowski, M.F.; Pao, W.; Kris, M.G.; Miller, V.A.; Ladanyi, M.; Riely, G.J. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin. Cancer Res.* **2013**, *19*, 2240–2247. [CrossRef] [PubMed]
14. Oxnard, G.R.; Hu, Y.; Mileham, K.F.; Husain, H.; Costa, D.B.; Tracy, P.; Feeney, N.; Sholl, L.M.; Dahlberg, S.E.; Redig, A.J.; et al. Assessment of Resistance Mechanisms and Clinical Implications in Patients with EGFR T790M-Positive Lung Cancer and Acquired Resistance to Osimertinib. *JAMA Oncol.* **2018**, *4*, 1527–1534. [CrossRef] [PubMed]
15. Chua, K.P.; Teng, Y.H.F.; Tan, A.C.; Takano, A.; Alvarez, J.J.S.; Nahar, R.; Rohatgi, N.; Lai, G.G.Y.; Aung, Z.W.; Yeong, J.P.S.; et al. Integrative Profiling of T790M-Negative EGFR-Mutated NSCLC Reveals Pervasive Lineage Transition and Therapeutic Opportunities. *Clin. Cancer Res.* **2021**, *27*, 5939–5950. [CrossRef] [PubMed]
16. Engelhardt, H.; Böse, D.; Petronczki, M.; Scharn, D.; Bader, G.; Baum, A.; Bergner, A.; Chong, E.; Döbel, S.; Egger, G.; et al. Start Selective and Rigidify: The Discovery Path toward a Next Generation of EGFR Tyrosine Kinase Inhibitors. *J. Med. Chem.* **2019**, *62*, 10272–10293. [CrossRef] [PubMed]
17. Jackman, D.; Pao, W.; Riely, G.J.; Engelman, J.A.; Kris, M.G.; Jänne, P.A.; Lynch, T.; Johnson, B.E.; Miller, V.A. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *J. Clin. Oncol.* **2010**, *28*, 357–360. [CrossRef]
18. Marcoux, N.; Gettinger, S.N.; O'Kane, G.; Arbour, K.C.; Neal, J.W.; Husain, H.; Evans, T.L.; Brahmer, J.R.; Muzikansky, A.; Bonomi, P.D.; et al. EGFR-Mutant Adenocarcinomas That Transform to Small-Cell Lung Cancer and Other Neuroendocrine Carcinomas: Clinical Outcomes. *J. Clin. Oncol.* **2019**, *37*, 278–285. [CrossRef] [PubMed]
19. Sharma, S.V.; Lee, D.Y.; Li, B.; Quinlan, M.P.; Takahashi, F.; Maheswaran, S.; McDermott, U.; Azizian, N.; Zou, L.; Fischbach, M.A.; et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **2010**, *141*, 69–80. [CrossRef]
20. Hata, A.N.; Niederst, M.J.; Archibald, H.L.; Gomez-Caraballo, M.; Siddiqui, F.M.; Mulvey, H.E.; Maruvka, Y.E.; Ji, F.; Bhang, H.E.; Krishnamurthy Radhakrishna, V.; et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat. Med.* **2016**, *22*, 262–269. [CrossRef]
21. Raha, D.; Wilson, T.R.; Peng, J.; Peterson, D.; Yue, P.; Evangelista, M.; Wilson, C.; Merchant, M.; Settleman, J. The cancer stem cell marker aldehyde dehydrogenase is required to maintain a drug-tolerant tumor cell subpopulation. *Cancer Res.* **2014**, *74*, 3579–3590. [CrossRef] [PubMed]
22. Ramirez, M.; Rajaram, S.; Steininger, R.J.; Osipchuk, D.; Roth, M.A.; Morinishi, L.S.; Evans, L.; Ji, W.; Hsu, C.H.; Thurley, K.; et al. Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells. *Nat. Commun.* **2016**, *7*, 10690. [CrossRef]
23. Mikubo, M.; Inoue, Y.; Liu, G.; Tsao, M.S. Mechanism of Drug Tolerant Persister Cancer Cells: The Landscape and Clinical Implication for Therapy. *J. Thorac. Oncol.* **2021**, *16*, 1798–1809. [CrossRef]
24. Deeken, J.F.; Beumer, J.H.; Anders, N.M.; Wanjiku, T.; Rusnak, M.; Rudek, M.A. Preclinical assessment of the interactions between the antiretroviral drugs, ritonavir and efavirenz, and the tyrosine kinase inhibitor erlotinib. *Cancer Chemother. Pharmacol.* **2015**, *76*, 813–819. [CrossRef] [PubMed]
25. Chhun, S.; Verstuyft, C.; Rizzo-Padoin, N.; Simoneau, G.; Becquemont, L.; Peretti, I.; Swaisland, A.; Wortelboer, R.; Bergmann, J.F.; Mouly, S. Gefitinib-phenytoin interaction is not correlated with the C-erythromycin breath test in healthy male volunteers. *Br. J. Clin. Pharmacol.* **2009**, *68*, 226–237. [CrossRef] [PubMed]
26. Swaisland, H.C.; Ranson, M.; Smith, R.P.; Leadbetter, J.; Laight, A.; McKillop, D.; Wild, M.J. Pharmacokinetic drug interactions of gefitinib with rifampicin, itraconazole and metoprolol. *Clin. Pharmacokinet.* **2005**, *44*, 1067–1081. [CrossRef]

27. Shibata, Y.; Chiba, M. The role of extrahepatic metabolism in the pharmacokinetics of the targeted covalent inhibitors afatinib, ibrutinib, and neratinib. *Drug Metab. Dispos.* **2015**, *43*, 375–384. [CrossRef]
28. Stopfer, P.; Marzin, K.; Narjes, H.; Gansser, D.; Shahidi, M.; Uttereuther-Fischer, M.; Ebner, T. Afatinib pharmacokinetics and metabolism after oral administration to healthy male volunteers. *Cancer Chemother. Pharmacol.* **2012**, *69*, 1051–1061. [CrossRef]
29. Wind, S.; Giessmann, T.; Jungnik, A.; Brand, T.; Marzin, K.; Bertulis, J.; Hocke, J.; Gansser, D.; Stopfer, P. Pharmacokinetic drug interactions of afatinib with rifampicin and ritonavir. *Clin. Drug Investig.* **2014**, *34*, 173–182. [CrossRef]
30. Ahluwalia, M.S.; Becker, K.; Levy, B.P. Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors for Central Nervous System Metastases from Non-Small Cell Lung Cancer. *Oncologist* **2018**, *23*, 1199–1209. [CrossRef]
31. Kosaka, T.; Tanizaki, J.; Paranal, R.M.; Endoh, H.; Lydon, C.; Capelletti, M.; Repellin, C.E.; Choi, J.; Ogino, A.; Calles, A.; et al. Response Heterogeneity of EGFR and HER2 Exon 20 Insertions to Covalent EGFR and HER2 Inhibitors. *Cancer Res.* **2017**, *77*, 2712–2721. [CrossRef]
32. Robichaux, J.P.; Elamin, Y.Y.; Tan, Z.; Carter, B.W.; Zhang, S.; Liu, S.; Li, S.; Chen, T.; Poteete, A.; Estrada-Bernal, A.; et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. *Nat. Med.* **2018**, *24*, 638–646. [CrossRef] [PubMed]
33. Robichaux, J.P.; Le, X.; Vijayan, R.S.K.; Hicks, J.K.; Heeke, S.; Elamin, Y.Y.; Lin, H.Y.; Udagawa, H.; Skoulidis, F.; Tran, H.; et al. Structure-based classification predicts drug response in EGFR-mutant NSCLC. *Nature* **2021**, *597*, 732–737. [CrossRef] [PubMed]
34. Warburg, O. On the origin of cancer cells. *Science* **1956**, *123*, 309–314. [CrossRef] [PubMed]
35. Molina, J.R.; Sun, Y.; Protopopova, M.; Gera, S.; Bandi, M.; Bristow, C.; McAfoos, T.; Morlacchi, P.; Ackroyd, J.; Agip, A.A.; et al. An inhibitor of oxidative phosphorylation exploits cancer vulnerability. *Nat. Med.* **2018**, *24*, 1036–1046. [CrossRef]
36. Hangauer, M.J.; Viswanathan, V.S.; Ryan, M.J.; Bole, D.; Eaton, J.K.; Matov, A.; Galeas, J.; Dhruv, H.D.; Berens, M.E.; Schreiber, S.L.; et al. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* **2017**, *551*, 247–250. [CrossRef] [PubMed]
37. Viswanathan, V.S.; Ryan, M.J.; Dhruv, H.D.; Gill, S.; Eichhoff, O.M.; Seashore-Ludlow, B.; Kaffenberger, S.D.; Eaton, J.K.; Shimada, K.; Aguirre, A.J.; et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* **2017**, *547*, 453–457. [CrossRef]
38. Nechushtan, H.; Hamamreh, Y.; Nidal, S.; Gotfried, M.; Baron, A.; Shalev, Y.I.; Nisman, B.; Peretz, T.; Peylan-Ramu, N. A phase IIb trial assessing the addition of disulfiram to chemotherapy for the treatment of metastatic non-small cell lung cancer. *Oncologist* **2015**, *20*, 366–367. [CrossRef]
39. Sun, Q.; Zhang, W.; Wang, L.; Guo, F.; Song, D.; Zhang, Q.; Zhang, D.; Fan, Y.; Wang, J. Hypermethylated CD36 gene affected the progression of lung cancer. *Gene* **2018**, *678*, 395–406. [CrossRef]
40. Guo, J.Y.; Teng, X.; Laddha, S.V.; Ma, S.; Van Nostrand, S.C.; Yang, Y.; Khor, S.; Chan, C.S.; Rabinowitz, J.D.; White, E. Autophagy provides metabolic substrates to maintain energy charge and nucleotide pools in Ras-driven lung cancer cells. *Genes Dev.* **2016**, *30*, 1704–1717. [CrossRef]
41. Strohecker, A.M.; Guo, J.Y.; Karsli-Uzunbas, G.; Price, S.M.; Chen, G.J.; Mathew, R.; McMahon, M.; White, E. Autophagy sustains mitochondrial glutamine metabolism and growth of BrafV600E-driven lung tumors. *Cancer Discov.* **2013**, *3*, 1272–1285. [CrossRef] [PubMed]
42. Ramilowski, J.A.; Yip, C.W.; Agrawal, S.; Chang, J.C.; Ciani, Y.; Kulakovskiy, I.V.; Mendez, M.; Ooi, J.L.C.; Ouyang, J.F.; Parkinson, N.; et al. Functional annotation of human long noncoding RNAs via molecular phenotyping. *Genome Res.* **2020**, *30*, 1060–1072. [CrossRef] [PubMed]
43. Vinogradova, M.; Gehling, V.S.; Gustafson, A.; Arora, S.; Tindell, C.A.; Wilson, C.; Williamson, K.E.; Guler, G.D.; Gangurde, P.; Manieri, W.; et al. An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells. *Nat. Chem. Biol.* **2016**, *12*, 531–538. [CrossRef] [PubMed]
44. Tetsu, O.; Hangauer, M.J.; Phuchareon, J.; Eisele, D.W.; McCormick, F. Drug Resistance to EGFR Inhibitors in Lung Cancer. *Chemotherapy* **2016**, *61*, 223–235. [CrossRef] [PubMed]
45. Hong, S.; Gao, F.; Fu, S.; Wang, Y.; Fang, W.; Huang, Y.; Zhang, L. Concomitant Genetic Alterations with Response to Treatment and Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Patients With EGFR-Mutant Advanced Non-Small Cell Lung Cancer. *JAMA Oncol.* **2018**, *4*, 739–742. [CrossRef] [PubMed]
46. Blakely, C.M.; Watkins, T.B.K.; Wu, W.; Gini, B.; Chabon, J.J.; McCoach, C.E.; McGranahan, N.; Wilson, G.A.; Birkbak, N.J.; Olivas, V.R.; et al. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. *Nat. Genet.* **2017**, *49*, 1693–1704. [CrossRef] [PubMed]
47. Barnet, M.B.; O'Toole, S.; Horvath, L.G.; Selinger, C.; Yu, B.; Ng, C.C.; Boyer, M.; Cooper, W.A.; Kao, S. EGFR-Co-Mutated Advanced NSCLC and Response to EGFR Tyrosine Kinase Inhibitors. *J. Thorac. Oncol.* **2017**, *12*, 585–590. [CrossRef] [PubMed]
48. Zilfou, J.T.; Lowe, S.W. Tumor suppressive functions of p53. *Cold Spring Harb. Perspect. Biol.* **2009**, *1*, a001883. [CrossRef]
49. Ma, X.; Le Teuff, G.; Lacas, B.; Tsao, M.S.; Graziano, S.; Pignon, J.P.; Douillard, J.Y.; Le Chevalier, T.; Seymour, L.; Filipits, M.; et al. Prognostic and Predictive Effect of TP53 Mutations in Patients with Non-Small Cell Lung Cancer from Adjuvant Cisplatin-Based Therapy Randomized Trials: A LACE-Bio Pooled Analysis. *J. Thorac. Oncol.* **2016**, *11*, 850–861. [CrossRef]
50. Kim, Y.; Lee, B.; Shim, J.H.; Lee, S.H.; Park, W.Y.; Choi, Y.L.; Sun, J.M.; Ahn, J.S.; Ahn, M.J.; Park, K. Concurrent Genetic Alterations Predict the Progression to Target Therapy in EGFR-Mutated Advanced NSCLC. *J. Thorac. Oncol.* **2019**, *14*, 193–202. [CrossRef]

51. Nahar, R.; Zhai, W.; Zhang, T.; Takano, A.; Khng, A.J.; Lee, Y.Y.; Liu, X.; Lim, C.H.; Koh, T.P.T.; Aung, Z.W.; et al. Elucidating the genomic architecture of Asian EGFR-mutant lung adenocarcinoma through multi-region exome sequencing. *Nat. Commun.* **2018**, *9*, 216. [CrossRef]
52. Rho, J.K.; Choi, Y.J.; Ryoo, B.Y.; Na, I.I.; Yang, S.H.; Kim, C.H.; Lee, J.C. p53 enhances gefitinib-induced growth inhibition and apoptosis by regulation of Fas in non-small cell lung cancer. *Cancer Res.* **2007**, *67*, 1163–1169. [CrossRef] [PubMed]
53. Sabapathy, K.; Lane, D.P. Therapeutic targeting of p53: All mutants are equal, but some mutants are more equal than others. *Nat. Rev. Clin. Oncol.* **2018**, *15*, 13–30. [CrossRef]
54. Kang, S.; Bader, A.G.; Vogt, P.K. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 802–807. [CrossRef]
55. Kawano, O.; Sasaki, H.; Endo, K.; Suzuki, E.; Haneda, H.; Yukiue, H.; Kobayashi, Y.; Yano, M.; Fujii, Y. PIK3CA mutation status in Japanese lung cancer patients. *Lung Cancer* **2006**, *54*, 209–215. [CrossRef] [PubMed]
56. Yamamoto, H.; Shigematsu, H.; Nomura, M.; Lockwood, W.W.; Sato, M.; Okumura, N.; Soh, J.; Suzuki, M.; Wistuba, I.I.; Fong, K.M.; et al. PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res.* **2008**, *68*, 6913–6921. [CrossRef]
57. Spoerke, J.M.; O'Brien, C.; Huw, L.; Koeppen, H.; Fridlyand, J.; Brachmann, R.K.; Haverty, P.M.; Pandita, A.; Mohan, S.; Sampath, D.; et al. Phosphoinositide 3-kinase (PI3K) pathway alterations are associated with histologic subtypes and are predictive of sensitivity to PI3K inhibitors in lung cancer preclinical models. *Clin. Cancer Res.* **2012**, *18*, 6771–6783. [CrossRef] [PubMed]
58. Chaft, J.E.; Arcila, M.E.; Paik, P.K.; Lau, C.; Riely, G.J.; Pietanza, M.C.; Zakowski, M.F.; Rusch, V.; Sima, C.S.; Ladanyi, M.; et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. *Mol. Cancer Ther.* **2012**, *11*, 485–491. [CrossRef]
59. Scheffler, M.; Bos, M.; Gardizi, M.; König, K.; Michels, S.; Fassunke, J.; Heydt, C.; Künstlinger, H.; Ihle, M.; Ueckeroth, F.; et al. PIK3CA mutations in non-small cell lung cancer (NSCLC): Genetic heterogeneity, prognostic impact and incidence of prior malignancies. *Oncotarget* **2015**, *6*, 1315–1326. [CrossRef]
60. Li, S.; Li, L.; Zhu, Y.; Huang, C.; Qin, Y.; Liu, H.; Ren-Heidenreich, L.; Shi, B.; Ren, H.; Chu, X.; et al. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: A comprehensive mutation profiling from 5125 Chinese cohorts. *Br. J. Cancer* **2014**, *110*, 2812–2820. [CrossRef]
61. Ludovini, V.; Bianconi, F.; Pistola, L.; Chiari, R.; Minotti, V.; Colella, R.; Giuffrida, D.; Tofanetti, F.R.; Siggillino, A.; Flacco, A.; et al. Phosphoinositide-3-kinase catalytic alpha and KRAS mutations are important predictors of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in patients with advanced non-small cell lung cancer. *J. Thorac. Oncol.* **2011**, *6*, 707–715. [CrossRef] [PubMed]
62. Engelman, J.A.; Mukohara, T.; Zejnullahu, K.; Lifshits, E.; Borrás, A.M.; Gale, C.M.; Naumov, G.N.; Yeap, B.Y.; Jarrell, E.; Sun, J.; et al. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. *J. Clin. Investig.* **2006**, *116*, 2695–2706. [CrossRef] [PubMed]
63. Jin, Y.; Bao, H.; Le, X.; Fan, X.; Tang, M.; Shi, X.; Zhao, J.; Yan, J.; Xu, Y.; Quek, K.; et al. Distinct co-acquired alterations and genomic evolution during TKI treatment in non-small-cell lung cancer patients with or without acquired T790M mutation. *Oncogene* **2020**, *39*, 1846–1859. [CrossRef] [PubMed]
64. Di Cristofano, A.; Pandolfi, P.P. The multiple roles of PTEN in tumor suppression. *Cell* **2000**, *100*, 387–390. [CrossRef] [PubMed]
65. Li, J.; Yen, C.; Liaw, D.; Podsypanina, K.; Bose, S.; Wang, S.I.; Puc, J.; Miliaresis, C.; Rodgers, L.; McCombie, R.; et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **1997**, *275*, 1943–1947. [CrossRef] [PubMed]
66. Myers, M.P.; Stolarov, J.P.; Eng, C.; Li, J.; Wang, S.I.; Wigler, M.H.; Parsons, R.; Tonks, N.K. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9052–9057. [CrossRef] [PubMed]
67. Balsara, B.R.; Pei, J.; Mitsuuchi, Y.; Page, R.; Klein-Szanto, A.; Wang, H.; Unger, M.; Testa, J.R. Frequent activation of AKT in non-small cell lung carcinomas and preneoplastic bronchial lesions. *Carcinogenesis* **2004**, *25*, 2053–2059. [CrossRef] [PubMed]
68. Vazquez, F.; Sellers, W.R. The PTEN tumor suppressor protein: An antagonist of phosphoinositide 3-kinase signaling. *Biochim. Biophys. Acta* **2000**, *1470*, M21–M35. [CrossRef]
69. Yamamoto, C.; Basaki, Y.; Kawahara, A.; Nakashima, K.; Kage, M.; Izumi, H.; Kohno, K.; Uramoto, H.; Yasumoto, K.; Kuwano, M.; et al. Loss of PTEN expression by blocking nuclear translocation of EGR1 in gefitinib-resistant lung cancer cells harboring epidermal growth factor receptor-activating mutations. *Cancer Res.* **2010**, *70*, 8715–8725. [CrossRef]
70. Sos, M.L.; Koker, M.; Weir, B.A.; Heynck, S.; Rabinovsky, R.; Zander, T.; Seeger, J.M.; Weiss, J.; Fischer, F.; Frommolt, P.; et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Cancer Res.* **2009**, *69*, 3256–3261. [CrossRef] [PubMed]
71. Wang, F.; Diao, X.Y.; Zhang, X.; Shao, Q.; Feng, Y.F.; An, X.; Wang, H.Y. Identification of genetic alterations associated with primary resistance to EGFR-TKIs in advanced non-small-cell lung cancer patients with EGFR sensitive mutations. *Cancer Commun.* **2019**, *39*, 7. [CrossRef]
72. Wu, S.G.; Chang, Y.L.; Hsu, Y.C.; Wu, J.Y.; Yang, C.H.; Yu, C.J.; Tsai, M.F.; Shih, J.Y.; Yang, P.C. Good response to gefitinib in lung adenocarcinoma of complex epidermal growth factor receptor (EGFR) mutations with the classical mutation pattern. *Oncologist* **2008**, *13*, 1276–1284. [CrossRef] [PubMed]
73. Tomas, A.; Futter, C.E.; Eden, E.R. EGF receptor trafficking: Consequences for signaling and cancer. *Trends Cell Biol.* **2014**, *24*, 26–34. [CrossRef]

74. Vieira, A.V.; Lamaze, C.; Schmid, S.L. Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science* **1996**, *274*, 2086–2089. [CrossRef] [PubMed]
75. Goh, L.K.; Huang, F.; Kim, W.; Gygi, S.; Sorkin, A. Multiple mechanisms collectively regulate clathrin-mediated endocytosis of the epidermal growth factor receptor. *J. Cell Biol.* **2010**, *189*, 871–883. [CrossRef] [PubMed]
76. Brankatschk, B.; Wichert, S.P.; Johnson, S.D.; Schaad, O.; Rossner, M.J.; Gruenberg, J. Regulation of the EGF transcriptional response by endocytic sorting. *Sci. Signal.* **2012**, *5*, ra21. [CrossRef] [PubMed]
77. Futter, C.E.; Pearse, A.; Hewlett, L.J.; Hopkins, C.R. Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. *J. Cell Biol.* **1996**, *132*, 1011–1023. [CrossRef] [PubMed]
78. Shinde, S.R.; Maddika, S. PTEN modulates EGFR late endocytic trafficking and degradation by dephosphorylating Rab7. *Nat. Commun.* **2016**, *7*, 10689. [CrossRef]
79. Oser, M.G.; Niederst, M.J.; Sequist, L.V.; Engelman, J.A. Transformation from non-small-cell lung cancer to small-cell lung cancer: Molecular drivers and cells of origin. *Lancet Oncol.* **2015**, *16*, e165–e172. [CrossRef]
80. Sequist, L.V.; Waltman, B.A.; Dias-Santagata, D.; Digumarthy, S.; Turke, A.B.; Fidias, P.; Bergethon, K.; Shaw, A.T.; Gettinger, S.; Cosper, A.K.; et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci. Transl. Med.* **2011**, *3*, 75ra26. [CrossRef]
81. Youle, R.J.; Strasser, A. The BCL-2 protein family: Opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 47–59. [CrossRef]
82. Ng, K.P.; Hillmer, A.M.; Chuah, C.T.; Juan, W.C.; Ko, T.K.; Teo, A.S.; Ariyaratne, P.N.; Takahashi, N.; Sawada, K.; Fei, Y.; et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nat. Med.* **2012**, *18*, 521–528. [CrossRef]
83. Costa, D.B.; Halmos, B.; Kumar, A.; Schumer, S.T.; Huberman, M.S.; Boggon, T.J.; Tenen, D.G.; Kobayashi, S. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med.* **2007**, *4*, 1669–1679, discussion 1680. [CrossRef]
84. Cragg, M.S.; Kuroda, J.; Puthalakath, H.; Huang, D.C.; Strasser, A. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. *PLoS Med.* **2007**, *4*, 1681–1689, discussion 1690. [CrossRef]
85. Gong, Y.; Somwar, R.; Politi, K.; Balak, M.; Chmielecki, J.; Jiang, X.; Pao, W. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. *PLoS Med.* **2007**, *4*, e294. [CrossRef]
86. Ebi, H.; Oze, I.; Nakagawa, T.; Ito, H.; Hosono, S.; Matsuda, F.; Takahashi, M.; Takeuchi, S.; Sakao, Y.; Hida, T.; et al. Lack of association between the BIM deletion polymorphism and the risk of lung cancer with and without EGFR mutations. *J. Thorac. Oncol.* **2015**, *10*, 59–66. [CrossRef]
87. Cho, E.N.; Kim, E.Y.; Jung, J.Y.; Kim, A.; Oh, I.J.; Kim, Y.C.; Chang, Y.S. BCL2-like 11 intron 2 deletion polymorphism is not associated with non-small cell lung cancer risk and prognosis. *Lung Cancer* **2015**, *90*, 106–110. [CrossRef] [PubMed]
88. Xargay-Torrent, S.; López-Guerra, M.; Saborit-Villarroya, I.; Rosich, L.; Campo, E.; Roué, G.; Colomer, D. Vorinostat-induced apoptosis in mantle cell lymphoma is mediated by acetylation of proapoptotic BH3-only gene promoters. *Clin. Cancer Res.* **2011**, *17*, 3956–3968. [CrossRef] [PubMed]
89. Nakagawa, T.; Takeuchi, S.; Yamada, T.; Ebi, H.; Sano, T.; Nanjo, S.; Ishikawa, D.; Sato, M.; Hasegawa, Y.; Sekido, Y.; et al. EGFR-TKI resistance due to BIM polymorphism can be circumvented in combination with HDAC inhibition. *Cancer Res.* **2013**, *73*, 2428–2434. [CrossRef] [PubMed]
90. Takeuchi, S.; Hase, T.; Shimizu, S.; Ando, M.; Hata, A.; Murakami, H.; Kawakami, T.; Nagase, K.; Yoshimura, K.; Fujiwara, T.; et al. Phase I study of vorinostat with gefitinib in BIM deletion polymorphism/epidermal growth factor receptor mutation double-positive lung cancer. *Cancer Sci.* **2020**, *111*, 561–570. [CrossRef] [PubMed]
91. Meador, C.B.; Hata, A.N. Acquired resistance to targeted therapies in NSCLC: Updates and evolving insights. *Pharmacol. Ther.* **2020**, *210*, 107522. [CrossRef]
92. Wang, W.; Li, Q.; Yamada, T.; Matsumoto, K.; Matsumoto, I.; Oda, M.; Watanabe, G.; Kayano, Y.; Nishioka, Y.; Sone, S.; et al. Crosstalk to stromal fibroblasts induces resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors. *Clin. Cancer Res.* **2009**, *15*, 6630–6638. [CrossRef]
93. Straussman, R.; Morikawa, T.; Shee, K.; Barzily-Rokni, M.; Qian, Z.R.; Du, J.; Davis, A.; Mongare, M.M.; Gould, J.; Frederick, D.T.; et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature* **2012**, *487*, 500–504. [CrossRef]
94. Zhang, B.; Zhang, Y.; Zhao, J.; Wang, Z.; Wu, T.; Ou, W.; Wang, J.; Yang, B.; Zhao, Y.; Rao, Z.; et al. M2-polarized macrophages contribute to the decreased sensitivity of EGFR-TKIs treatment in patients with advanced lung adenocarcinoma. *Med. Oncol.* **2014**, *31*, 127. [CrossRef] [PubMed]
95. Biswas, A.K.; Han, S.; Tai, Y.; Ma, W.; Coker, C.; Quinn, S.A.; Shakri, A.R.; Zhong, T.J.; Scholze, H.; Lagos, G.G.; et al. Targeting S100A9-ALDH1A1-retinoic acid signaling to suppress brain relapse in EGFR-mutant lung cancer. *Cancer Discov.* **2022**, *12*, 1002–1021. [CrossRef] [PubMed]
96. Azuma, K.; Ota, K.; Kawahara, A.; Hattori, S.; Iwama, E.; Harada, T.; Matsumoto, K.; Takayama, K.; Takamori, S.; Kage, M.; et al. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. *Ann. Oncol.* **2014**, *25*, 1935–1940. [CrossRef] [PubMed]

97. Zhang, Y.; Zeng, Y.; Liu, T.; Du, W.; Zhu, J.; Liu, Z.; Huang, J.A. The canonical TGF-β/Smad signalling pathway is involved in PD-L1-induced primary resistance to EGFR-TKIs in EGFR-mutant non-small-cell lung cancer. *Respir. Res.* **2019**, *20*, 164. [CrossRef]
98. Terry, S.; Abdou, A.; Engelsen, A.S.T.; Buart, S.; Dessen, P.; Corgnac, S.; Collares, D.; Meurice, G.; Gausdal, G.; Baud, V.; et al. AXL Targeting Overcomes Human Lung Cancer Cell Resistance to NK- and CTL-Mediated Cytotoxicity. *Cancer Immunol. Res.* **2019**, *7*, 1789–1802. [CrossRef]
99. Aguilera, T.A.; Rafat, M.; Castellini, L.; Shehade, H.; Kariolis, M.S.; Hui, A.B.; Stehr, H.; von Eyben, R.; Jiang, D.; Ellies, L.G.; et al. Reprogramming the immunological microenvironment through radiation and targeting Axl. *Nat. Commun.* **2016**, *7*, 13898. [CrossRef]
100. Aguilera, T.A.; Giaccia, A.J. Molecular Pathways: Oncologic Pathways and Their Role in T-cell Exclusion and Immune Evasion-A New Role for the AXL Receptor Tyrosine Kinase. *Clin. Cancer Res.* **2017**, *23*, 2928–2933. [CrossRef]
101. Huo, K.G.; D'Arcangelo, E.; Tsao, M.S. Patient-derived cell line, xenograft and organoid models in lung cancer therapy. *Transl. Lung Cancer Res.* **2020**, *9*, 2214–2232. [CrossRef] [PubMed]