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Acquired Resistance in Lung Cancer

Asmin Tulpule[1] and Trever G. Bivona[2]

[1]Division of Pediatric Hematology/Oncology, University of California, San Francisco, California 94143, USA

[2]Division of Hematology and Oncology, University of California, San Francisco, California 94143, USA; email: Trever.Bivona@ucsf.edu

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Abstract

The last decade has witnessed a transformation in the treatment of advanced-stage lung cancer from a largely palliative approach to one where long-term durable remissions and even cures might be within reach. In this review, we discuss the current state of oncogene-directed precision medicine therapies in lung cancer and focus on the major cause of mortality for lung cancer patients: acquired resistance. We consider the multifaceted resistance mechanisms tumors utilize, often simultaneously. We then present areas for future scientific and clinical investigation with an emphasis on population dynamics, early detection, combinatorial therapies targeting resistance mechanisms, and understanding the drug-tolerant persister state.

Lung cancer remains a major public health scourge and the number one cause of cancer-related mortality worldwide (Bray et al. 2018). Treatment as recently as the early 2000s consisted mainly of palliative chemotherapy with median survival times of six to eight months (Rapp et al. 1988). The explosion of molecular diagnostic techniques has led to a reorganization in how we classify lung cancer (and cancer, in general) from a purely histologic-based approach to one that now incorporates identification of the genetic drivers of the disease.

The term "oncogene addiction," first coined by I. Bernard Weinstein in 2002, was at the time a hypothesis that cancer cells depend on continued activity of their driver oncogene to maintain malignant potential (Weinstein & Joe 2006). The identification and subsequent targeted therapies directed at major oncogenes in lung cancer [e.g., *EGFR* (epidermal growth factor receptor; Lynch et al. 2004), *ALK* (anaplastic lymphoma kinase; Solomon et al. 2018), *ROS1* (C-ROS oncogene 1; Shaw et al. 2014), *BRAF* (Planchard et al. 2017), *MET* (Drilon et al. 2016)] led to a significant prolongation of overall survival (OS) and dramatic disease remissions in some patients, cementing the concept of oncogene addiction as central to precision medicine therapies.

However, virtually all patients with advanced-stage lung cancer ultimately develop acquired resistance and succumb to the disease. This review seeks to summarize the current understanding of acquired resistance in lung cancer and then explores approaches that move beyond oncogene addiction to address obstacles such as initial tumor volume, population dynamics, tumor heterogeneity, and drug-tolerant persister cell states that cooperate to limit long-term survival for these patients.

DRIVER ONCOGENES AND ACQUIRED RESISTANCE

Molecular testing in non-small-cell lung cancer (NSCLC) is now considered standard of care, and genome sequencing approaches have uncovered an ever-expanding list of oncogenic mutations (Lindeman et al. 2018). The most common oncogenic driver in lung cancer that is targetable by existing therapies [tyrosine kinase inhibitors (TKIs)] is activating mutations in *EGFR*, resulting in proliferative and oncogenic signals through the RAS-MAPK, PI3K-AKT-mTOR, and JAK/STAT pathways. The incidence of *EGFR* mutations varies significantly by country and ethnicity, from 10–20% in the United States to greater than 40% in parts of Asia (Midha et al. 2015). Other activating kinase mutations have been described for *BRAF* (Marchetti et al. 2011), *MET* (exon 14 skipping) (Awad et al. 2016), and *HER2* (Arcila et al. 2012). In addition to mutational drivers, several receptor tyrosine kinases (RTKs) undergo gene rearrangement to generate constitutively active kinases. This class of oncogenic drivers includes ALK (Soda et al. 2007), ROS1 (Rikova et al. 2007), and RET (Kohno et al. 2012). The common theme from the current list of targetable oncogenic drivers in NSCLC is that almost all of them represent somatic events that result in hyperactivation of a kinase and oncogene addiction in the tumor cells. Therefore, the specific targeted therapies (e.g., kinase inhibitors) have a significantly larger therapeutic window than traditional cytotoxic chemotherapy.

Response rates to targeted therapies vary by driver oncogene but range between 40 and 80%. For example, first-generation EGFR inhibitors including erlotinib and gefitinib demonstrated head-to-head superiority over traditional chemotherapy in terms of progression-free survival (PFS) in EGFR-mutant NSCLC, but interestingly did not improve OS likely due to rapid emergence of drug resistance (Mok et al. 2009, Rosell et al. 2012). Later-generation EGFR inhibitors demonstrate increased PFS and OS when compared with first-generation EGFR inhibitors (Soria et al. 2018), as well clear superiority over historical OS data for traditional chemotherapy. While most patients have either a partial response or stable disease as measured by RECIST (Response Evaluation Criteria in Solid Tumors) criteria, de novo or primary resistance remains a clinical

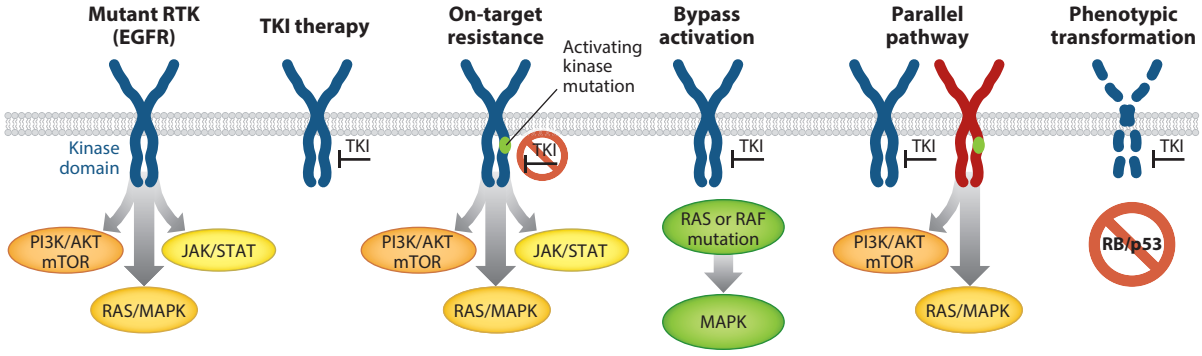

Figure 1

The signaling cascade of the classic oncogenic driver in non-small-cell lung cancer (NSCLC), mutant EGFR (epidermal growth factor receptor), and mechanisms of resistance to targeted therapy by tyrosine kinase inhibitors (TKIs). Oncogenic signaling downstream of mutant EGFR involves the RAS-MAPK, PI3K-AKT-mTOR, and JAK/STAT pathways. On-target resistance restores kinase function in the presence of TKIs, bypass signaling restores downstream input through activating mutations in, for example, RAS or RAF, and parallel pathway activation utilizes alternative receptor tyrosine kinases (RTKs) (*red*) to restore signaling input to the oncogene-addicted cell. Phenotypic transformation demonstrates a cell state transition and often downregulation of the oncogene.

challenge for a subset of patients. Examples of primary resistance to EGFR inhibitors include certain types of oncogenic mutations, such as *EGFR* exon 20 insertions that have reduced sensitivity to TKIs (Yasuda et al. 2013); the presence of concurrent genetic alterations, such as MET amplifications (Lai et al. 2019) or modifiers of response to TKIs such as low levels of proapoptotic proteins such as BIM (Costa et al. 2014); elevated basal levels of CRIPTO1 (Park et al. 2014); or high levels of NF-κB pathway activation (Blakely et al. 2015). The scope of primary resistance remains to be fully elucidated for other targeted therapies against ALK, ROS1, RET, and BRAF, although for BRAF in particular, the experience in metastatic melanoma suggests that the same types of alterations in the MAPK pathway (MAP2K1, MAP2K2, BRAF, and NRAS) that promote acquired resistance can also promote de novo resistance if present initially (Van Allen et al. 2014).

We focus in this review on acquired resistance, as it remains the major scientific and clinical challenge in lung cancer. Acquired resistance is traditionally divided into on-target and off-target mechanisms. "On-target" refers to mutations or other alterations in the target gene such as amplifications that allow the kinase to remain active even in the presence of an inhibitor. Off-target alterations are a much broader category and include activation of downstream or bypass survival programs, phenotypic transformation, and presence of codominant driver oncogenes (**Figure 1**).

ON-TARGET RESISTANCE PREDICTED BY STRUCTURE-FUNCTION STUDIES

The canonical on-target resistance mutation in NSCLC is the so-called gatekeeper *EGFR* T790M second-site mutation. This represents the dominant resistance mechanism (50–60% of patients) to first-generation EGFR-directed TKIs such as erlotinib or gefitinib (Pao et al. 2005, Sequist et al. 2011). The mutation results in a larger methionine side chain in the ATP binding pocket and steric clash on drug binding (Kobayashi et al. 2005). While a common theme in TKI resistance dating back to the very first kinase inhibitors used for chronic myeloid leukemia [*BCR-ABL* T315M mutations (Gorre et al. 2001)], the gatekeeper hypothesis may not represent the entire mechanism of resistance for EGFR; work in recent years has shown that *EGFR* T790M has increased ATP binding affinity and that the mutation may affect overall enzyme conformation (Yun et al. 2008).

In response to this dominant mode of resistance, third-generation EGFR-directed TKIs such as osimertinib were specifically designed to target the T790M resistance mutation through covalent binding to the ATP pocket (Finlay et al. 2014). Unsurprisingly, their use has been associated with new second-site resistance mutations, the best characterized of which is C797S, which occurs at the covalent binding site of osimertinib to prevent drug binding (Thress et al. 2015).

Interestingly, the spectra of resistance mutations for ALK, ROS1, MET, and HER2 are significantly more varied than for EGFR-mutant cancers, especially when considering first-generation EGFR inhibitors. Similar to EGFR, gatekeeper resistance mutations involving the ATP binding pocket in ALK (L1196M) (Choi et al. 2010) and ROS1 (L2026M) (McCoach et al. 2018) have been described in patients treated with the dual ALK/ROS1 inhibitor crizotinib, but overall they represent a significant minority of resistance events. Additional on-target mutations are multifaceted and include disruption of hydrophobic kinase-drug interactions (solvent front mutations) such as *ALK* G1202R (Chen et al. 2018) and *ROS1* G2032R (Awad et al. 2013) and activation loop mutants with allosteric effects (*MET* Y1230C) (Ou et al. 2017). Notably absent from this list of second-site resistance mutations is the serine/threonine kinase BRAF. While mechanisms of acquired resistance to BRAF inhibitors in NSCLC are not yet well defined, significant experience using RAF inhibitors in melanoma has not revealed secondary BRAF mutations as a major driver of resistance (Nazarian et al. 2010). BRAF oncogene amplification (Shi et al. 2012) and expression of alternative splice variants (Poulikakos et al. 2011) are observed in melanoma patients at progression and technically represent a mode of on-target resistance (since the oncogene can signal in the presence of the drug), although the predominant modes of resistance involve bypass signaling, as discussed below. Indeed, recent preclinical work has revealed roles for bypass track signaling, alternatively spliced BRAF, and the Hippo pathway effector YAP1 (Yes-associated protein 1) in RAF inhibitor treatment resistance in BRAF-mutant NSCLC models (Lin et al. 2014, 2015; Okimoto et al. 2016).

While the general theme of on-target resistance is that drug selection imposes a selective pressure for the emergence of additional mutations that enable reengagement of the driver oncogene, there are specific details worth discussing. First, each kinase-drug pair has a unique structural relationship, and both structural predictions (Hauser et al. 2018) and in vitro mutagenesis screens have been utilized to predict resistance mutations, even prior to their clinical occurrence (Azam et al. 2003), allowing for the iteration of new compounds that had activity against those mutations. Second, it remains an open question why first-generation EGFR inhibitors have a dominant resistance mutation (T790M), whereas third-generation EGFR inhibitors and ALK, MET, and ROS1 inhibitors show a more diverse array of second-site mutations within the kinase and BRAF inhibitor resistance includes almost no second-site resistance mutations. This observation may relate to the tighter binding affinity or wild-type EGFR sparing properties of third-generation EGFR inhibitors or it could reflect different structural characteristics of the respective kinases (Patel et al. 2017). Moving forward, it will be important to understand the spectrum of resistance mutations for each kinase-drug pair, especially as more potent TKIs and more allosteric drugs are developed. It will also be critical to test whether minimizing on-target resistance simply shifts the balance toward off-target mechanisms of resistance.

OFF-TARGET RESISTANCE: MANY WAYS TO REPLACE AN ONCOGENE

Off-target resistance represents a daunting challenge because of the diverse and often multifactorial mechanisms responsible for tumor cell survival in the face of oncoprotein blockade. In the absence of target reengagement (e.g., on-target resistance), the dominant classes of resistance

include downstream pathway reactivation, activation of parallel survival pathways, and phenotypic transformation (**Figure 1**).

The classic example of downstream pathway reactivation comes from BRAF-mutant melanoma, where treatment with BRAF inhibitors did not result in BRAF second-site resistance mutations, but rather in amplification or activating mutations in RAS isoforms or loss of NF1, which restores downstream MAPK signaling (Van Allen et al. 2014, Whittaker et al. 2013). In melanoma, the addition of a second agent targeting the MAPK pathway (MEK inhibitor) significantly prolonged PFS and OS, demonstrating the vital importance of the MAPK pathway to survival of $BRAF^{V600E}$-driven melanoma (Robert et al. 2015). The experience in melanoma led to early phase clinical trials in NSCLC and FDA (US Food and Drug Administration) approval of combined BRAF and MEK inhibitor treatment for BRAF-mutant melanoma with impressive response rates (Planchard et al. 2017). The mechanisms of resistance to dual MAPK pathway blockade in NSCLC are poorly characterized at present, although MAPK reactivation has been described in individual patients (Rudin et al. 2013). It will be important to validate preclinical work on acquired BRAF inhibitor resistance in NSCLC models (Lin et al. 2014, 2015) as clinical specimens from this subset of patients become available through tumor and liquid biopsy protocols.

ALK-rearranged tumors also display a selective dependence on downstream RAS-MAPK signaling. KRAS amplification or downregulation of a negative MAPK input, the dual-specificity protein phosphatase 6 (DUSP6), was observed in several acquired resistance samples, and combinatorial blockade of ALK and the MAPK pathway (MEK inhibition), but not the PI3K-AKT and JAK-STAT pathways, delayed acquired resistance in cell line and animal models (Hrustanovic et al. 2015). The strategy of dual ALK/MEK inhibition is currently being tested in up-front clinical trials (**https://www.clinicaltrials.gov/** identifier NCT03087448). Similarly, acquisition of activating BRAF mutations has been described in a minority of EGFR-mutant patients at acquired resistance (Ohashi et al. 2012), although whether combination EGFR and MAPK pathway blockade will delay acquired resistance is currently being tested in clinical trials (NCT01859026).

PARALLEL PATHWAYS PRESERVE ONCOGENIC INPUTS

Oncogenic kinases are often competent to drive signaling through multiple pathways; for example, mutant EGFR, ALK, and ROS1 all activate the RAS-MAPK, PI3K-AKT, and JAK/STAT pathways to varying extents (Rotow & Bivona 2017). However, the key scientific and clinical questions in oncogene addiction are: What downstream signals are required for continued survival of the tumor and are there alternative ways of activating the same survival signals? One of the earliest discovered and well-characterized examples of parallel pathway activation is amplification of the MET kinase, which was detected at relapse in 5–10% of EGFR-mutant NSCLC patients treated with first-generation inhibitors (Engelman et al. 2007). MET amplification leads to increased sensitivity to hepatocyte growth factor signaling and increased flux through the PI3K-AKT-mTOR axis, which is thought to rescue cells from EGFR TKI blockade. Subsequent studies identified other mediators of parallel survival pathway activation in EGFR-mutant lung cancer, including HER2 amplification, FGFR3 amplification, PIKC3A mutations, and AXL overexpression (Le et al. 2018, Sequist et al. 2011, Yu et al. 2013, Zhang et al. 2012), all of which serve to reactivate downstream MAPK and PI3K signaling.

"Kinome rewiring" describes this general resistance mechanism by which an oncogenic input is compensated for via upregulation of signaling through alternative RTKs (Duncan et al. 2012). Interestingly, there appears to be a conserved set of growth and proliferative signals that cancers utilize, and so there is substantial overlap between mechanisms of resistance. For example, in both ALK and ROS1 patients with acquired crizotinib resistance, reactivation of wild-type EGFR

signaling by either EGFR mutations or overexpression of the receptor or ligand (Davies et al. 2013, McCoach et al. 2018) leads to rescue of MAPK signaling, which is the crucial downstream pathway for ALK and most ROS1-rearranged tumors (Neel et al. 2018). Similarly, oncogenic RET rearrangements are found in osimertinib-resistant EGFR-mutant samples (Piotrowska et al. 2018). The key question in each of these examples is whether these alterations are necessary and sufficient for resistance to TKI therapy and whether combinatorial therapies, such as dual blockade of EGFR and MET (Wu et al. 2018), can reverse and delay acquired resistance.

There are two important points relating to acquired resistance and bypass/parallel pathway activation that are worth discussing here. The first is that our understanding of acquired resistance largely comes from DNA sequencing analysis, which identifies mutations or copy number changes that emerge after progression on TKI therapy. However, these analyses cannot capture transcriptional, translational, or epigenetic events that may regulate or promote acquired resistance. The second related point is that acquired resistance is no longer viewed as a binary event—e.g., cells either die in the face of the drug or acquire mutations that render cells resistant (Foo & Michor 2014). Increasingly, it has become evident that treatment with targeted agents imposes a gradient of selection and results in a drug-tolerant or so-called persister population of residual cells that are not truly resistant [e.g., increased IC50 (half-maximal inhibitory concentration) to the drug], and this population serves as a reservoir for the emergence of cells with acquired resistance (Hata et al. 2016). These populations have a recognizable clinical correlate in that most patients treated with targeted therapy will have periods of stable residual disease and only later will develop progressive disease on therapy, which corresponds to tumor regrowth due to acquired resistance.

It is often assumed that transcriptional or other epigenetic changes can create a drug-tolerant population, but true acquired resistance requires a heritable DNA-based genetic change. Whether early adaptive events contribute to the diversity of mechanisms driving acquired resistance remains to be clarified. For example, activation of YAP1 and the NF-κB pathway are adaptive responses to EGFR TKI therapy, and high levels of pathway activation correlate with poor outcomes in patients (Bivona et al. 2011, Blakely et al. 2015, Lin et al. 2015, Noguchi et al. 2014). It is well established in other contexts like development that epigenetic and chromatin configurations can represent stable heritable cell states (Moazed 2011); below we introduce the concept of cell fate transitions (often without a clear genetic driver) that mediate acquired resistance. Therefore, the question of whether, for example, acquired resistance can develop from the clonal outgrowth of high-YAP1-expressing cells without an additional genetic event is worthy of investigation, as it would significantly increase the complexity and diversity of resistance mechanisms and pave the way for more nuanced resistance biomarkers and therapeutic targets.

CELL FATE TRANSITIONS

Thus far we have covered resistance mechanisms that fundamentally conform to the concept of oncogene addiction—e.g., there is a set of proliferative inputs that a cancer cell requires for continued survival and resistance mechanisms involve reactivating or substituting those inputs. In this framework, cell fate transitions represent a distinct class of acquired resistance where the original driver oncogene and its downstream signaling pathways appear to be fully dispensable at the emergence of resistance. The best-documented example is in EGFR-mutant NSCLC treated with TKIs, wherein 4–14% of patients with acquired resistance undergo histologic transformation from adenocarcinoma to small-cell lung cancer (SCLC) (Sequist et al. 2011, Yu et al. 2013). Small cell transformation involves obligate RB and p53 loss, along with loss of EGFR expression in some cases, which explains the acquired resistance to TKI therapy (Niederst et al. 2015).

DNA sequencing of small cell transformation reveals that the EGFR mutation remains present (though not expressed), suggesting a clonal evolution from adenocarcinoma to SCLC. Multiple cases of small cell transformation have now been reported for ALK-rearranged NSCLC treated with the ALK inhibitor alectinib (Fujita et al. 2016, Hobeika et al. 2018), suggesting that these cell fate transitions are not unique to EGFR mutant cases. The underlying biology of what drives cell fate switching and how that enables cancer cells to escape oncogene addiction remain poorly understood.

Another example of cell fate switching involves the epithelial-to-mesenchymal transition (EMT), which is less genetically defined. EMT refers to phenotypic transformation from a polarized epithelial cell restrained by its basement membrane attachments to the extracellular matrix to a mesenchymal phenotype with increased invasion and migration capacity (Dongre & Weinberg 2019). EMT is thought to be a key feature of both regional and hematogenous metastatic spread, which is the major cause of treatment failure and cancer mortality across all cancers. While some features of EMT are routinely described (such as increased vimentin expression and loss of cadherin expression), a more comprehensive understanding of the molecular programs that govern EMT is still lacking. In NSCLC, the transition is governed by a set of transcription factors [TWIST (Hui et al. 2013) and ZEB (Zhang et al. 2016)] and the kinase AXL (Zhang et al. 2012), but whether this molecular program results in a stable cell state of acquired resistance and how this phenotypic change alleviates oncogene dependence in the tumor cell remain areas of active investigation.

We have outlined a broad set of on- and off-target mechanisms of acquired resistance to targeted therapy in NSCLC. Our summary has focused on cell-autonomous mechanisms of resistance, but the emerging role of the tumor microenvironment and immune cells in modifying responses to therapy should be noted (for more details, see the excellent review by Altorki et al. 2019). None of these resistance mechanisms occurs in isolation; in fact, tumors are highly heterogenous such that multiple resistance mechanisms can and do emerge in parallel during acquired resistance (Jamal-Hanjani et al. 2017). For example, co-occurrence of *EGFR* T790M and *MET* amplification (Bean et al. 2007) or small cell transformation (Suda et al. 2015) has been observed in individual patients with acquired resistance to EFGR inhibitors. Additionally, alterations in other survival or homeostatic pathways (e.g., cell cycle, β-catenin signaling, DNA repair) are commonly observed at acquired resistance (Blakely et al. 2017, Michels et al. 2019), but whether these in fact contribute to resistance remains less clear. The importance of tumor heterogeneity is highlighted from clinical trials with EGFR-mutant NSCLC and first-generation TKIs, where *EGFR* T790M is the dominant mechanism of resistance. Use of the *EGFR* T790M–targeting drug osimertinib after progression on first-generation EGFR TKIs yielded a PFS of 9.9 months (Ahn et al. 2019), compared to 17.2 months when osimertinib was tested in the up-front setting (Soria et al. 2018). This observation demonstrates the heterogeneous polyclonal nature of acquired resistance even in circumstances with a predominant clone (e.g., *EGFR* T790M). Additionally, recent studies have suggested that resistance mutations such as *EGFR* T790M may often exist as subpopulations and not as the predominant clone in the entire resistant tumor (Le et al. 2018). This genetic heterogeneity poses a significant obstacle to long-term durable remissions because of the need to eradicate minimal residual disease.

HOW DO WE COMBAT ACQUIRED RESISTANCE AND ACHIEVE LONG-TERM CURES?

It is worth considering the subset of patients with NSCLC who do not develop acquired resistance. Patients with early stage disease (small lung tumors without any regional spread) have five-year

survival rates above 50% with surgical resection alone or adjuvant cytotoxic chemotherapy; many patients are long-term survivors with minimal therapy (Cronin et al. 2018). These survival rates are predicted to improve further with the use of targeted therapies in the adjuvant (or neoadjuvant) setting given the dramatic responses in advanced-stage disease, but multiple ongoing clinical trials are testing this question directly (Nagasaka & Gadgeel 2018). Unfortunately, only 10–15% of NSCLC patients are detected at early stages since routine screening is not currently done and symptoms prompting medical attention typically occur with more advanced and larger disease burdens.

What accounts for the large differences in survival between early and late stage patients in lung cancer (and almost all solid tumors)? From a biologic standpoint, there are differences between early and late stage disease, including increasing genomic complexity with the acquisition of new mutations, greater tumor heterogeneity, and a more metastatic phenotype (Blakely et al. 2017, Cancer Genome Atlas Res. Netw. 2014). Complex mathematical modeling of population dynamics has been employed to predict the development of acquired resistance and can inform our understanding of differential outcomes in early versus late stage disease (Lavi et al. 2012, Sun et al. 2016). Fundamentally, these models depend on two key inputs: total tumor burden (i.e., number of cells) and a combined cell killing rate that incorporates rates of cell death, proliferation, and frequency of acquired resistance mutations. Our focus so far has been on decreasing rates of acquired resistance, but by way of a highly simplified example, below we illustrate the dramatic effect of initial tumor burden on durable disease remissions.

Assuming a binary model in the face of the drug (acquired resistance or cell death) and a composite rate of 1 in 10,000 initial tumor cells acquiring resistance mutations, how does initial tumor burden impact the percentage of patients who eradicate all disease? At a starting tumor burden of 1,000 cells, the probability of having zero cells with acquired resistance is 90%, whereas for a tumor burden of 10,000 cells the probability drops to 36%, and increasing the tumor burden to 100,000 cells (just a 100-fold increase) drops the probability of eradicating all disease to essentially zero (0.004%). This simple example does not incorporate the many complexities of population dynamics such as intermediate response to drug (drug-tolerant persisters), tumor heterogeneity, de novo resistant clones, tumor-initiating potential of individual tumor cells (cancer stem cells), and timing of response to targeted therapies (Eigenmann et al. 2017). Yet fundamentally we propose that strategies to reduce the initial tumor burden need to be a part of the solution to achieve long-term cures in NSCLC.

EARLY DETECTION OF NON-SMALL-CELL LUNG CANCER

For the majority of NSCLC patients, early detection by either imaging or molecular techniques lacks feasibility using current technologies. Yet by first principles, it seems self-evident that identifying patients prior to the development of large tumor burdens and incurable metastatic, advanced-stage NSCLC will save lives. However, this remained an open question prior to 2010, as multiple clinical trials using chest X-ray and sputum cytology failed to demonstrate any survival benefit (Fontana et al. 1991), and the risks associated with overdiagnosis are now well characterized, including the medical risks of pursuing false positives, the significant costs to the health care system, and patient harm relating to overdiagnosis (Patz et al. 2014). The definitive trial to date is from the US National Lung Screening Trial, which randomized a high-risk population (55–74 years with 30 pack-years) to low-dose chest CT (computed tomography) versus chest X-ray annually for two years. The results were notable for increased percentage of early stage diagnoses in the CT arm and a 20% reduction in mortality at six-year follow-up (Aberle et al. 2011). Though there was no control arm in the trial per se, early unpublished data from the NELSON trial, which

included a true control arm, suggest similar benefits in terms of earlier stage of diagnosis and mortality reduction from chest CT screening (De Koning et al. 2018).

The current recommendation in the United States is that low-dose chest CT be utilized for screening high-risk individuals (a grade B recommendation) (Moyer 2014). Importantly, these screening modifications to high-risk individuals would impact only about 10% of the annual lung cancer deaths. It remains unknown whether extrapolation from this high-risk population to surveillance imaging for all asymptomatic patients can meaningfully affect OS given the expected shift toward lower incidence and higher false positive rates and whether the potential benefit is large enough to justify the significant costs. We propose that proactive imaging-based surveillance should continue to be an important area of clinical investigation that is revisited as new and efficacious therapies and imaging techniques are developed, especially given the clear evidence that screening can help detect earlier-stage disease.

A possible adjunctive approach to imaging-based surveillance is the use of liquid biopsies, which are increasingly utilized to monitor residual disease in lung and many other cancers (Chaudhuri et al. 2017). These tests enable the detection of circulating tumor DNA (ctDNA) shed by tumor cells and specifically assess for the presence of classic genetic drivers of NSCLC and other cancers (e.g., EGFR, BRAF, ALK, ROS1). A few cautionary notes are worth mentioning here regarding the potential utility of ctDNA for early detection. First, the amount of ctDNA is proportional to tumor volume, and through careful serial ctDNA analysis of early stage NSCLC patients, Abbosh et al. (2017) determined that a tumor volume of 10 cm^3 is needed to obtain a plasma variant allele frequency of 0.1%. Therefore, technological advances will be required to detect lower allele frequencies corresponding to early stage nodules that can be detected by CT (a nodule with a diameter of 5 mm has a volume of 0.065 cm^3). Second, multiple studies have cataloged the presence of classic oncogenic (KRAS) and tumor-suppressor alterations (p53) (Gormally et al. 2006), as well as clonal hematopoiesis, in the peripheral blood of healthy volunteers who did not go on to develop cancer (Genovese et al. 2014). The questions of how to interpret and follow up ctDNA tests in asymptomatic patients and whether the number of false positives will prove too large for effective and efficient early stage disease screening will need to be tested rigorously prior to widespread adoption.

COMBINATION THERAPIES TO REDUCE RATES OF ACQUIRED RESISTANCE

Improved strategies for early detection may ultimately yield less disseminated disease and lower initial tumor burdens, but to meaningfully address acquired resistance, combination therapies designed to forestall predictable mechanisms of acquired resistance will be essential. The question of how best to combine or sequence targeted therapies will benefit from mathematical models and empirical data on the resistance mechanisms actually arising in a given patient (McCoach & Bivona 2019). On-target resistance is now largely anticipated based on structural modeling of mutations within the kinase that abrogate or alter drug binding. Monotherapy with later-generation TKIs have improved outcomes in terms of PFS and OS in EGFR- (Soria et al. 2018) and ALK-driven NSCLC (Peters et al. 2017), presumably because of increased potency, increased activity against a known resistance mechanism in the case of osimertinib (*EGFR* T790M), and increased central nervous system penetration. However, an inevitable set of on-target resistance mutations to these TKIs are now emerging at acquired resistance, as outlined above. The development of allosteric inhibitors of EGFR and other targets offers a complementary approach to combat on-target resistance (Lu et al. 2018), as these inhibitors engage with sites other than the ATP binding pocket of the kinase and therefore are likely to have nonoverlapping resistance profiles with

current first- and third-generation EGFR inhibitors. It remains to be determined whether combination approaches or sequential utilization of allosteric inhibitors or inhibitors with nonoverlapping resistance profiles (e.g., erlotinib and osimertinib) will prove more effective in combating the emergence of on-target resistance.

Similarly, combinatorial therapies targeting the oncogene and downstream pathway activation or bypass track activation are being tested in clinical trials. Above we discussed the most dramatic success of this approach in BRAF-mutant tumors, where the dominant resistant mechanism is downstream MAPK reactivation and robust clinical trial data in melanoma (Robert et al. 2015) and emerging results in NSCLC (Planchard et al. 2017) demonstrate the benefit of dual blockade with a BRAF and downstream MEK inhibitor compared to monotherapy. Similar approaches are being tested in numerous clinical trials comparing monotherapy to dual blockade of bypass resistance mechanisms (EGFR inhibitors combined with either MET, AXL, or PI3K inhibitors; ALK + MEK inhibitors; and HER2 + mTOR inhibitors) (Rotow & Bivona 2017). Whether upfront combination therapy (or sequential therapy) can delay acquired resistance and improve OS remains uncertain, especially given that multiple mechanisms of resistance are present in a single tumor. For example, does dual EGFR and MET blockade delay acquired resistance in patients, as was observed in the preclinical models (Engelman et al. 2007) and early phase trials (Wu et al. 2018), or does it simply lead to acquired resistance via other mechanisms and not meaningfully impact patient survival? It will be of crucial importance to identify the molecular drivers of acquired resistance to combination therapies to inform future trials.

UNDERSTANDING RESIDUAL DISEASE: TUMOR HETEROGENEITY AND DRUG-TOLERANT PERSISTERS

Strategies designed to reduce rates of acquired resistance need to contend with an intermediate population of cells between TKI-sensitive and TKI-resistant cells known as drug-tolerant persisters. Clinically, most patients display an incomplete response to therapy resulting in a residual tumor volume that remains radiographically stable on continued TKI therapy for months prior to the emergence of true resistant clones and enlarging (progressive) disease (**Figure 2**). Both the population dynamics and the underlying biology of this drug-tolerant population remain poorly understood (Salgia & Kulkarni 2018). First defined by Sharma et al. (2010), drug-tolerant cells are thought to represent a slow-growing subpopulation with reversible drug tolerance, altered chromatin dynamics, and a dependency on IGF-1R and GPX4 (Hangauer et al. 2017). In theory, therapies designed to reduce the frequency of drug-tolerant persisters or directly target them could ultimately decrease rates of acquired resistance by shrinking the viable residual disease tumor pool. This strategy has parallels to the use of local control measures (surgical resection or radiotherapy) in oligometastatic disease, which has been shown to prolong PFS in smaller studies (Gomez et al. 2016).

Our current understanding of the mechanistic basis of drug tolerance relies predominantly on in vitro studies using patient-derived cell lines or xenograft models where TKI titration can allow for selection of drug-tolerant persistence. The original description of the drug-tolerant state suggested a necessary interaction between IGF-1R signaling and the histone-demethylating activity of KDM5A, linking signaling pathways and kinome rewiring with semistable epigenetic changes (Sharma et al. 2010). Recent studies have implicated stress response pathways induced in response to TKI therapy as an early adaptive response that may underlie the development of drug tolerance. For example, the NF-κB (Blakely et al. 2015) and TNF (Gong et al. 2018) pathways are upregulated within hours of exposure to TKI in EGFR-mutant cell line models, and dual inhibition of EGFR and these pathways delays acquired resistance. EMT and AXL activation has also been

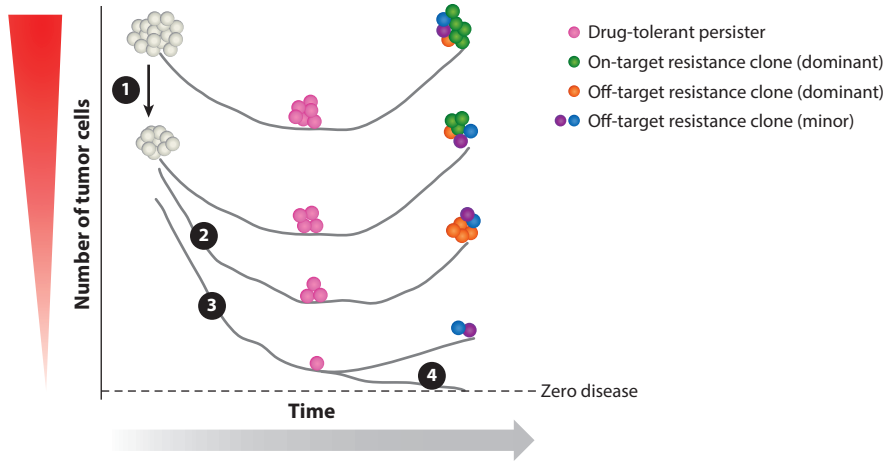

Figure 2

Population dynamics in acquired resistance. The top curve displays a typical clinical response to TKI (tyrosine kinase inhibitor) treatment with incomplete tumor shrinkage, residual disease, and emergence of acquired resistance mutations. (❶) Strategies for early detection decrease initial tumor volume. (❷) Next-generation TKI or combination TKI can eliminate a dominant on-target mutation at acquired resistance (e.g., *EGFR* T790M). (❸) Combination therapies to target bypass mechanisms of resistance reduce dominant off-target resistance clones. (❹) Orthogonal therapies (chemotherapy, immunotherapy, radiotherapy, and surgery) to treat residual disease enable tumor eradication and long-term cures.

implicated (Taniguchi et al. 2019, Viswanathan et al. 2017, Zhang et al. 2012). The connection between these adaptive responses and the heterogenous development of drug tolerance in small subsets of the initial tumor population is the subject of ongoing study.

An important goal of future work should be obtaining clinical samples of residual disease from patients to understand the biology of drug persistence and determine how faithfully the cell line–based models, on which the current understanding of mechanisms of drug tolerance and tumor cell persistence is entirely based, reproduce the clinical reality. Moreover, tissue biopsies combined with newly emerging single-cell sequencing approaches will allow for a more accurate serial characterization of the heterogeneity of the tumor at multiple stages (diagnosis, residual disease, and at acquired resistance), as will studies of the tumor microenvironment and immune cells, which are challenging to study with clinically accurate precision in murine models. There are risks associated with residual disease tumor rebiopsies. Thus, we propose integrating liquid biopsy–based circulating biomarker analyses such as ctDNA or epigenetic profiling (Shen et al. 2018) with neoadjuvant trials followed by surgical resection of residual disease. It will be important to understand the chronology of when classic acquired resistance mutations such as *EGFR* T790M or C797S emerge (or if they are present at small frequencies initially) during treatment. Whether a true drug-tolerant population can be defined and prospectively isolated from human patients also needs to be established in order to effectively target this population therapeutically.

ORTHOGONAL THERAPIES TO COMBAT ACQUIRED RESISTANCE: IMMUNOTHERAPY AND CHEMOTHERAPY

A large percentage of NSCLC patients have either no defined driver mutation (25%) or a lesion that is not currently druggable (e.g., RAS mutations present in approximately 30% of NSCLCs) (Cancer Genome Atlas Res. Netw. 2014). Focused research to identify drug targets and appropriate

therapies in those subtypes will certainly be an important piece of improving survival in lung cancer. In this last section, we discuss two orthogonal approaches, immunotherapy and traditional cytotoxic chemotherapy, that may play an important role in achieving long-term cures for NSCLC patients.

Immune checkpoint inhibitors are increasingly being tested in NSCLC, in both the up-front and relapsed settings. Nivolumab and pembrolizumab, both programmed death ligand 1 (PD-1) inhibitors, have shown modest but significant OS benefits in the relapsed setting compared to traditional chemotherapy (Herbst et al. 2016, Horn et al. 2017). The data for monotherapy in the up-front setting for advanced disease remain unclear given the contradictory results of two large clinical trials (Carbone et al. 2017, Reck et al. 2019b), though an OS benefit has been demonstrated in combination with cytotoxic chemotherapy (Gandhi et al. 2018, Socinski et al. 2018). The response profile of immune checkpoint inhibitors, at least in monotherapy, differs significantly from that of TKI therapy—only 20–40% of patients display even a partial response, with the remaining displaying either short-lived stable disease or frank progression. However, among the patients that do respond, durable remissions up to five years have been observed (Gettinger et al. 2018), suggesting that for a subset of patients immunotherapy can have dramatic benefits.

Future research must focus on the key unanswered question in immunotherapy: How can we identify patients who are predicted to benefit from these therapies? High expression of the PD-1 ligand, PD-L1, initially showed some promise in stratifying responders versus nonresponders, but ultimately appears to be a poor biomarker for response, at best (Havel et al. 2019). Tumor mutational burden is also weakly correlated with response, except for the clear data emerging from mismatch repair–deficient tumors, which have a log-fold increase in mutational burden and significantly higher response rates (Rizvi et al. 2015). Mechanisms of acquired resistance to immunotherapy-based treatment regimens remain to be defined in lung cancer.

Interestingly, oncogenic kinase-driven cancers (e.g., EGFR, ALK) appear to have inferior response rates to immune checkpoint inhibitors, although this has not been proven in prospective trials (Gainor et al. 2016). Going forward, it will be vitally important to obtain clinical biopsies and build better lab-based models of immune checkpoint inhibitor response and resistance in order to understand the determinants of sensitivity and ultimately acquired resistance to these important therapies. This is particularly true in the light of recent initial clinical data showing that some NSCLC patients with kinase-driven cancers may benefit from combined immunotherapy and anti-VEGF therapy (Reck et al. 2019a).

Finally, cytotoxic chemotherapy, which prior to 2004 was the only systemic therapy available to lung cancer patients, should remain part of a combination therapy approach, especially for younger patients who can tolerate the side effects. Adjuvant chemotherapy remains the standard of care for early stage resected patients and for advanced-stage patients without a known molecular driver. Pemetrexed and carboplatin, or other platinum-based doublets such as cisplatin plus gemcitabine or paclitaxel, have demonstrated small PFS and OS benefits compared to placebo, with response rates of 20–30% (Rapp et al. 1988, Sandler et al. 2000, Schiller et al. 2002). Unlike immunotherapy approaches, these responses are typically not durable and rapid chemotherapy resistance develops, with a two-year OS rate of approximately 10%. Cytotoxic chemotherapy used in combination with immune checkpoint inhibitors (Gandhi et al. 2018), angiogenesis inhibitors (Reck et al. 2009), and sequential but not continuous TKI (Herbst et al. 2005, Wu et al. 2013) has demonstrated improvements in PFS and OS, although the details of how best to combine these agents and in what sequence remain to be determined and may need to be highly individualized. Similar to immunotherapy, the determinants of response to cytotoxic chemotherapy and the mechanisms of acquired resistance to cytotoxic chemotherapy are overall poorly understood and should be the subject of additional studies. For example, patients with deficiencies in DNA

cross-link repair display increased sensitivity to platinum-based chemotherapy in other tumor types (Ceccaldi et al. 2015), and sequencing studies have identified multiple alterations in DNA repair enzymes, including BRCA1/2 in NSCLC, that might predict response to chemotherapy (Jordan et al. 2017).

Cytotoxic chemotherapy and immunotherapy in combination with either TKI or sequential therapy provide, in theory, orthogonal treatment approaches to address the large challenge of residual disease and acquired resistance. Both therapies have at least the potential to decrease total cell number and have nonoverlapping mechanisms of acquired resistance compared to TKIs. Therefore, identifying patients who a priori are likely to benefit from these therapies will be crucial, as will be the careful sequencing of therapies based on response and real-time analysis of residual or resistant clones.

SUMMARY

The remarkable success of targeted kinase inhibitors in lung cancer has also revealed the multitude of ways cancer cells can show plasticity and develop resistance to targeted drugs, often simultaneously in a single tumor and across different tumor sites in an individual patient. The goal of complete disease eradication in NSCLC is a lofty one and will require significant scientific and clinical advances on multiple fronts. Early detection is seldom included in discussions of acquired resistance but may prove central to identifying tumors with lower cell number and less aggressive and heterogenous features. Real-time understanding of drug-tolerant populations of cells in residual disease will be essential both to detect resistance mechanisms early and to enable adaptive treatment changes. Finally, rational combination therapies aimed at preventing known mechanisms of resistance, along with use of orthogonal agents such as immunotherapy and cytotoxic chemotherapy, will be needed to attempt to eradicate all residual disease to enable long-term cures.

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