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Authors

Sabnis, Amit J
Bivona, Trever G

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Principles of resistance to targeted cancer therapy: lessons from basic and translational cancer biology

Amit J. Sabnis[1,2,3] and **Trever G. Bivona**[2,3]

[1]Department of Pediatrics, University of California, San Francisco, San Francisco, CA, USA

[2]Department of Medicine, University of California, San Francisco, San Francisco, CA, USA

[3]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

Abstract

Identifying the genomic drivers of cancer has led to the clinical development of targeted therapies that strike at the heart of many malignancies. Nonetheless, many cancers outsmart such precision medicine efforts, and thus therapeutic resistance significantly contributes to cancer mortality. Attempts to understand the basis for resistance in patient samples and laboratory models has yielded two major benefits. One, more effective chemical inhibitors and rational combination therapies are now employed to prevent or circumvent resistance pathways. Two, our understanding of how oncogenic mutations drive cancer cell survival and oncogene addiction is deeper and broader, highlighting downstream or parallel cellular programs that shape these phenotypes. This review discusses emerging principles of resistance to therapies targeted against key oncogenic drivers.

Keywords

cancer; resistance; tyrosine kinase inhibitors; oncogene addiction

Resistance limits the success of precision medicine in cancer

Cancer is a major contributor to mortality worldwide [1]. It also serves as a model system to understand basic cell biologic principles such as the regulation of growth, survival, and migration. Newly developed cancer drugs provide chemical tools to probe these phenotypes, with the goals of improving both patient health and our understanding of cancer biology.

Small molecules that disrupt the function of individual aberrant proteins that drive cancer progression (herein referred to as targeted therapies) have revolutionized the practice of oncology [2]. Their development could only proceed after oncogenic drivers – causative

Correspondence to: Trever G. Bivona MD PhD (trever.bivona@ucsf.edu).

genetic mutations or biochemical signals that transform cells – were identified and rigorously tested in the laboratory. This review examines four broad resistance-inducing strategies employed by cancer cells: direct target reactivation, activation of signals upstream or downstream of oncogenes, engagement of parallel oncogenic pathways, and adaptive survival mechanisms. Illustrative examples are drawn from three diseases for which targeted therapy has shown tremendous promise, but for which maximal clinical success remains limited by therapeutic resistance: melanoma, non-small cell lung cancer (NSCLC), and prostate adenocarcinoma.

The promise and shortcomings of targeted therapies in cancer

Malignant melanoma.

Malignant melanoma is a cutaneous cancer with around 100,000 new diagnoses in the United States per year [3]. Over eighty percent of malignant melanomas harbor mutations within the mitogen activated protein kinase (MAP kinase; see Glossary) cascade, comprised of the central nodes of the small GTPase RAS, downstream serine/threonine kinases RAF, MEK, and ERK [4] (Figure 1). The most common such mutation is the *BRAF* p.V600E allele, present in 35-50% of melanomas [4]. Targeted inhibition of *BRAF* with the kinase inhibitors vemurafenib and dabrafenib elicited unprecedented tumor shrinkage in the advanced-stage setting and prolonged 6-month survival from 64% with chemotherapy to 84% [5, 6]. Subsequent disease progression was observed within seven months on average, and BRAF inhibitor monotherapy is not curative.

NSCLC.

NSCLC is a leading cause of cancer mortality [3]. The genetic drivers of NSCLC include activating mutations in *RAS* (predominantly *KRAS)*, as well as mutations or translocations involving various receptor tyrosine kinases (RTKs). These include the epidermal growth factor receptor (*EGFR)*, the RTK *ALK*, and others. RTKs signal through multiple downstream effectors, including the MAP kinase pathway, the polyinositol-3-kinase (PI3K) cascade and Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway (Figure 1). Tyrosine kinase inhibitors (TKIs) targeting *EGFR, ALK*, and other RTKs have been used for several years with significant improvement in progression free survival of patients with biomarker-positive, advanced stage disease [7–10]; their role in early-stage disease is currently being tested [11]. Half of advanced-stage *EGFR* mutant cancer patients treated with a TKI will have disease progression within one to two years depending on the specific agent used (compared to less than half a year for patients treated with conventional chemotherapy), and some will rapidly progress due to intrinsic resistance that prevents drugs from eliciting an initial anti-tumor response [2].

Prostate adenocarcinoma.

While prostate cancer can have an indolent course, the presence of metastatic disease once portended a median survival of only 5 years in a natural history study of men initially treated with prostatectomy [12]. Unlike malignant melanoma or NSCLC, prostate cancer has had an identified precision-therapy target since the 1960s: androgenic signaling [13]. Although the androgen receptor is not typically activated by somatic mutation, androgen signaling is

essential for prostate cancer cell survival. Androgen deprivation therapy improved the overall survival of patients with high risk, lymph node-involved disease [14]; following radical prostatectomy and lymphadenectomy, patients randomized to early androgen deprivation therapy had a median survival of 13.9 years, compared to 11.3 years in those receiving observation alone. Nonetheless, 20% of patients receiving early androgen deprivation therapy progressed within 5 years to so-called castration resistant prostate cancer (CRPC), wherein anti-androgen treatment is ineffective.

Thus, the treatment of cancer patients with targeted therapy has improved the quality and quantity of their life, but disease progression remains a major cause of mortality. The current challenge is to translate lessons learned from the development of resistance into an understanding of the nuanced “addiction” of cancer cells to their putative drivers [15], and in turn to develop approaches to forestall or treat molecular mechanisms of targeted therapy resistance. In the subsequent sections of this review, defined classes of resistance mechanisms are discussed in the context of melanoma, NSCLC, and prostate cancer.

On-target resistance: reactivating oncogenes

One of the earliest and most striking examples of successful targeted therapy in cancer was the use of the ABL-kinase inhibitor imatinib in the treatment of chronic myelogenous leukemia [16]. Early studies of patients who relapsed through imatinib treatment made the critical observation that BCR-ABL kinase activity was frequently restored in this setting [17]. This highlighted a fundamental mechanism of resistance to targeted therapy: direct restoration of the biologic function that was disrupted by the applied small molecule drug [18–20].

This mechanism has also proven to be true in NSCLC patients. The most common resistance-inducing mutation arising in *EGFR* mutant NSCLC patients treated with first-generation TKIs is the so-called “gatekeeper” T790M mutation. Over half of 155 patients treated with erlotinib or gefitinib developed T790M mutations at the time of disease progression in a large single-institution prospective study [21]. This observation prompted the development of third generation EGFR inhibitors, which inhibit T790M-mutant EGFR that arises in the background of the canonical activating mutations such as in-frame deletions in exon 19 and L858R. The FDA-approved third generation EGFR TKI osimertinib has shown significant promise in NSCLC patients who progress after initial EGFR inhibitor therapy as well as in the treatment-naïve setting ([22]; reviewed in [23]). Osimertinib use in previously untreated *EGFR* mutant NSCLC had similar response rates when compared to first generation EGFR inhibitors (erlotinib or gefitinib); however, patients randomized to osimertinib had roughly two-fold improvements in both median progression free survival (18.9 versus 10.2 months) and duration of response (17.2 months versus. 8.5 months). This demonstrates tangible benefit to the upfront anticipation and treatment of a common resistance-promoting event (i.e. *EGFR* p.T790M).

Single amino acid substitutions are not the only mechanisms by which oncoproteins targeted by small molecules can be restored. High potency anti-androgens, such enzalutamide [24], permits effective disease control even in CRPC. Tellingly, a common mechanism of

enzalutamide resistance eliminates the drug-binding domain of AR through alternative splicing [25, 26]. This switch appears to be under the control of NF-κB signaling [27], highlighting how distinct signaling pathways can restore the target of precision therapy. *In vitro* analysis of melanoma [28] and NSCLC [29] cancer cell lines driven by the *BRAFV600E* mutation both identified stable expression of a splice variant, BRAFp61, which can restore BRAF signaling through enhanced dimerization in spite of RAF inhibitor treatment. Thus, alternate splicing is a recurrent means of target re-activation in the face of targeted inhibitors. Another mechanism of on-target resistance is increased expression of the targeted oncoprotein via transcriptional upregulation or genomic amplification. This has been observed in melanoma, NSCLC, and prostate cancer, and can be overcome by more potent inhibitors and/or downstream signaling blockade [30–32].

These examples highlight that restoring the biologic function of targeted oncoproteins is a critical mechanism by which cancer cells can overcome targeted therapy and the principle of so-called oncogene addiction.

Engagement of upstream and downstream effectors: rationale for serial blockade of oncogenic pathways

Restoration of an oncogenic signaling node may not be necessary if mutation of an alternative target can genetically re-activate the pathway, thereby uncoupling it from a particular drug target. Such resistance mechanisms can both clarify the molecular mechanism by which particular mutations drive cancer cells, as well as illuminate rational, concurrent targets for combined therapy to prevent the emergence of resistance.

The early use of BRAF inhibitors in *BRAFV600E* positive melanoma demonstrated that responses to the single agent were dramatic, but generally of short duration, with median progression free survival of 6-8 months typically [6, 33]. Efforts to characterize mechanisms of resistance to vemurafenib failed to identify second-site mutations in *BRAF*, despite great efforts to probe the gatekeeper threonine residue [34]. Restoration of MAP kinase signaling was nonetheless observed in resistant cell lines. Subsequent work identified multiple means of reactivating MAP kinase signaling, including mutation or amplification of *RAS*, whose gene product activates the BRAF kinase through relief of auto-inhibition, and/or loss of the tumor suppressor gene neurofibromatosis-1 (*NF1*), a negative regulator of RAS activation [35]. This finding suggested that blockade of alternative targets within the central MAP kinase pathway might restore therapeutic response (Figure 2A).

An unanticipated complication of BRAF inhibitor treatment provided further rationale to address resistance caused by MAP kinase reactivation. Up to 25% of patients treated with BRAF inhibitors developed keratoacanthomas or squamous cell carcinomas. Sequencing identified recurrent *RAS* mutations in these malignancies [36] just as reports detailed a biochemical mechanism by which BRAF inhibitors actually enhance MAP kinase signaling in cells that lack a BRAF *V600E* mutation through RAF homo- and heterodimerization [37]. Thus, a premalignant cell harboring a *RAS* mutation but no *BRAF* mutation would perceive increased MAP kinase signaling when exposed to such an inhibitor, and be positively selected for evolution into frank malignancy.

In light of all these findings, a number of clinical trials treated patients with combined BRAF and MEK inhibition with improved progression free survival (up to 10 months) and decreased incidence of second cutaneous malignancies from approximately 10-20% to 2-7% [33, 38, 39]. Interestingly, sequential therapy did not recapitulate the benefits of combination treatment [40], underscoring the importance of upfront combination therapies to circumvent predictable resistance pathways at the onset of treatment.

Receptor tyrosine kinases such as EGFR or ALK are competent to signal through multiple downstream pathways, and so the relevant downstream targets for a similar serial blockade strategy are not clear. In this situation, clinical and preclinical study can elucidate the wiring of oncogenic circuits to identify rationale combination therapies. As an example, *KRAS* amplification was identified in cell lines and patient samples that had developed resistance to the ALK inhibitor crizotinib [41], and inhibition of MAP kinase, but not the parallel PI3K or JAK/STAT pathways, was sufficient to restore crizotinib sensitivity (Figure 2B) in preclinical models. A convergence on MAPK signaling and effect of combined inhibition of the oncoprotein and MEK was also observed in *EGFR* mutant NSCLC, prompting a similar clinical trial strategy [42]. Further context-specific studies of resistance to targeted inhibitors are needed to identify “on-pathway” resistance mechanisms and learn more about the essential outputs of targetable oncoproteins.

Parallel pathways: convergent oncogenic inputs

Oncogenic signaling networks such as the MAP kinase cascade control a transcriptional program that alters cellular behavior by promoting proliferation, survival, and growth, for instance through the activity of ERK on transcription factor function [43]. These programs are not necessarily tied to single inputs, and activation of parallel signaling could bypass requirements for a specific oncogenic activity. Parallel signaling was one of the first described mechanisms of acquired resistance to first generation EGFR inhibitors in NSCLC [44] (Figure 3A). Oncogenic EGFR proteins activate the PI3K-Akt cascade to promote transformation, through heterodimerization with the kinase-impaired ERBB3 receptor [45]. Prolonged *in vitro* exposure to the EGFR TKI gefitinib resulted in cell lines that maintained ERBB3 phosphorylation and PI3K signaling independent of EGFR inhibition, suggesting a parallel route to ERBB3 activation. Focal amplification of the *MET* locus, encoding an RTK with key roles in embryonic development and organogenesis, was found to be responsible. Either genetic or pharmacologic inhibition of MET re-sensitized cells to EGFR TKI therapy, establishing a *bona fide* resistance mechanism that has subsequently been observed in additional clinical cohorts [46]. Amplification of other RTKs such as *HER2* can rescue cells from EGFR inhibition in a similar manner [47].

As *HER* and *MET* mutations can drive NSCLC independently of *EGFR* mutations [48], these switches are reasonable to anticipate. An example of more divergent resistance signaling comes from the study of prostate cancers that develop resistance to enzalutamide. Arora and colleagues identified robust induction of the glucocorticoid receptor, *NR3C1*, in resistant cell line models and tumor samples from enzalutamide-resistant patients [49]. Androgen and glucocorticoid receptors can share DNA binding sites [50]; thus, it seemed plausible that GR might “rescue” the expression of certain AR targets. Indeed, this was the

case: the transcriptional targets of AR and GR were found to overlap, and GR activation with dexamethasone was able to confer resistance to anti-androgens (Figure 3B). This finding was critical as dexamethasone premedication is given in some prostate cancer chemotherapy regimens. While dexamethasone has a role in the treatment of CRPC, its use with newer anti-androgen agents is now evaluated through a new lens [51].

Cell state transitions highlight new oncogenic pathways

Some of the more difficult mechanisms of targeted cancer therapy resistance to understand are those that eliminate dependence on the founding genetic identity of a cancer cell. To date, there are both genetically and phenotypically defined models that appear to “free” a cancer from prior pathway dependence. One genetic model is in the transformation of NSCLC into small cell carcinoma. Between 3 and 10% of patients who progress on EGFR inhibitors develop small cell histology [52, 53]. Genetic interrogation of a large cohort of samples undergoing this transition confirmed loss of the tumor suppressor *RBI* in all cases, supporting the notion that the transition to small cell histology is reflected in cancer cell genetics [54]. In a larger cohort, combined loss of *RBI* and the tumor suppressor *TP53*, presumably disrupting cell cycle regulation and DNA damage response respectively, was implicated in the development of small cell histology [55]. Further, combined inactivation was found to precede clinical detection of small cell transformation. Importantly, small cell transformation appears to suppress expression of *EGFR*, offering a plausible mechanism as to why *EGFR* inhibition is no longer effective (Figure 4). An analogous differentiation switch, neuro-endocrine differentiation (NED) is observed in the progression of CRPC [56]. These tumors are also enriched for genetic inactivation of *RBI* and *TP53* [57], and exhibit decreased expression of the androgen receptor [58].

Conversely, epithelial to mesenchymal transition (EMT) is a phenotypic change seen in multiple carcinomas that does not have a clear genetic basis. EMT has been observed in NSCLC and other epithelial cancers during targeted therapy [59] or conventional chemotherapy [60, 61]. Several drivers of the EMT transcriptional program have been identified, including *SNAI1*, *ZEB1*, and *TWIST1* [62]. These proteins repress the expression of canonical epithelial markers such as E-cadherin, and cooperate with TGF-α and WNT signaling to increase the expression of mesenchymal markers such as vimentin [63]. Thus, EMT is largely defined by transcriptional state rather than genetic alterations (Figure 5). Preclinical efforts to target mesenchymal signaling have shown some promise in restoring *EGFR* inhibitor sensitivity [64–66], but this approach has not proven successful in preliminary clinical testing [67].

An alternative therapeutic approach is to identify and target proteins that drive the growth of mesenchymal cancer cells. Preclinical screens identified activation of the receptor tyrosine kinase UFO, encoded by the gene *AXL*, in a number of NSCLC models with EMT [68], and follow up work identified *AXL* gene expression as a hallmark of EMT in patient samples, with inhibition of UFO being sufficient to enhance erlotinib sensitivity in mesenchymal NSCLC cell lines [69]. Of note, *AXL* overexpression is seen in subgroups of cancers resistant to MAPK inhibitors (in melanoma [70]), PI3K inhibitors (in head and neck and esophageal squamous cell carcinoma [71]), and perhaps even cytotoxic chemotherapy [72,

73]. Adaptive, pro-survival programs such as EMT may thus represent a broad and common pathway by which resistance emerges, making it an attractive therapeutic target.

More acute adaptive changes can also permit the survival of cancer cells in response to targeted therapy. These may be crucial to identify or anticipate early in treatment, as they do not require long periods of time or fixed genetic alterations to develop and could thus quickly limit the efficacy of targeted therapy [74]. Genetic screening identified survival signaling through the NF-kB pathway as a critical mediator of resistance to EGFR TKI treatment in NSCLC [75]. In contrast to acquired-resistance mechanisms that develop over weeks *in vitro*, engagement of NF-kB signaling was a rapid event that promoted the development of residual disease through nuclear localization of RELA within hours of EGFR inhibition [76]. In *BRAF* mutated NSCLC, as well as in other MAP kinase driven models of melanoma, thyroid, and colorectal cancer, a *YAP1* transcriptional program can enable cancer cell survival despite targeted therapy [77]. In a manner analogous to NF-κB signaling, the *YAPl* transcriptional program promotes the persistence of cancer cells despite MAP kinase inhibition.

The multiple factors highlighted in these cell state transitions may lead oncogene-addicted cells through a drug-tolerant “persister” state that can be a pool for the acquisition of genetic, resistance-causing mutations [78]. However, this persister state may be associated with its own therapeutic vulnerabilities [79, 80]. Understanding this process, as well as defining whether there is true convergence on one resistant state or many, remains both a challenge and an opportunity for the cancer resistance field.

Translating the mechanisms of therapy resistance into durable remission and cure

The challenge in transforming this wealth of preclinical and clinical data towards patient benefit lies in mapping multiple resistance mechanisms into space and time for individual patients. Resistance may be present at the time of initial diagnosis as a sub-clonal pool of drug-resistant kinase mutations and/or as co-occurring driver mutations [81], or arise as a consequence of therapy [78]. These two scenarios may require different strategies to mitigate their effects on tumor progression. In fact, resistance may adopt multiple paths in a single patient, which bulk DNA sequencing of individual tumor biopsy sites may fail to capture [82]. Thus, one liability in trying to treat resistance to targeted therapy is to assume a single patient/single resistance pathway model that slowly evolves over time [83] (Box 1).

Instead, increasing data support the notion that genetic heterogeneity enables the emergence of resistance through multiple pathways, which clinical trials must: (1) capture, ideally prior to disease progression, and (2) target through therapy modification, in the hopes of impacting patient survival (Figure 5).

One method to analyze treatment-emergent genetic changes is through acquisition of tumor tissue on therapy. For patients with metastatic NSCLC, who are often prescribed TKI as their initial treatment, a repeat biopsy can represent additional risk and cost with unproven benefit, and thus may be difficult to justify. An alternative approach is to instead offer

neoadjuvant targeted therapy to early-stage NSCLC patients for whom eventual surgery is standard-of-care [11]. Treatment-induced genetic evolution could be compared from time of biopsy to resection, suggesting possible treatment modification (discussed further below).

In depth genetic, epigenetic, and transcriptional assessments of solid tumors might also be gained through so-called “liquid biopsies,” the isolation and study of tumor-derived material from peripheral blood. This material might involve circulating tumor cells (CTCs) or cell free DNA (cfDNA) that is shed from tumors. Liquid biopsies have shown promise as biomarkers of response and as a way to identify possible resistance mechanisms early in treatment. For instance, in melanoma patients treated with immune checkpoint inhibitors, CTCs could be enriched through microfluidic sorting and then identified by digital PCR for a melanoma-specific RNA signature [84]. Patients with a reduction of these identified CTC-specific transcripts within 7 weeks of therapy had a one-year disease progression rate of only 15%, compared to 64% of patients whose CTC score increased. More exploratory studies in prostate cancer identified transcriptional signatures of WNT signaling in patients developing resistance to anti-androgen therapy [85], supporting the use of this technology to probe transcriptional changes that emerge on treatment. Similarly, analysis of cfDNA from NSCLC patients demonstrated that tumor genetic complexity increases with TKI therapy [83], creating a permissive pool from which resistance might emerge across distinct tumor clones. Detailed analysis of mixed responses to targeted therapy have, in fact, used cfDNA to define unappreciated molecular heterogeneity and explain why one metastatic site of disease responds to targeted therapy, while another progresses [86]. In summary, liquid biopsy offers a minimally invasive, longitudinal assessment of changes that may develop on targeted therapy, an integrative biological view of multiple different tumor metastases, and an important complement to tumor tissue-based sampling and analysis.

The next challenge is for clinical scientists to use this information to try and alter the course of therapeutic resistance and measure the subsequent outcome. Given the extensive heterogeneity in resistance mechanisms as discussed above, it will be important to quickly test and either accept or reject biomarker-treatment pairs as effective or ineffective in preventing disease progression. Adaptive trial designs [87] utilize Bayesian methods and interim assessment to do so, refining clinical trial models and treatment assignments based on emerging information from the trial in process. In the ongoing I-SPY-2 (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging And Molecular Analysis 2) trial in breast cancer, a fixed proportion of patients are randomized to a control arm, while randomization to other arms is based on (a) the patient’s categorization into 8 predefined biomarker categories, and (b) the Bayesian predictive probability, updated monthly, that a given treatment arm is superior to control for a given biomarker category. Drugs whose predictive probability of being successful for further phase 2 testing drops below 10% in all biomarker categories are triaged, thus opening an arm for newer drug testing [88].

How might adaptive trial designs combine with genetic assessments to forestall resistance? Half of *EGFR* mutant NSCLC patients treated with osimertinib will progress within one to two years, so they may be an ideal population to test biomarker-treatment pairs to extend progression-free survival. In one such model, early stage patients with biopsy-confirmed *EGFR* mutations will have a liquid biopsy at diagnosis and begin osimertinib therapy plus

another, randomized targeted agent; initial data will inform future randomization to specific arms if a liquid biopsy biomarker appears to be predictive of prolonged progression-free survival (for instance, a finding of amplification of Cyclin Dependent Kinase 4 (CDK4) might be preferentially treated with a CDK4 inhibitor). An important correlative endpoint might be the elimination of a given sub-clonal mutation on future liquid biopsies.

Moving forward, on-therapy tumor or liquid biopsy might identify a more established resistance mechanism, such as *MET* amplification, which would alter therapy to include a MET inhibitor plus osimertinib in lieu of the initial pair of agents (Figure 5). The analysis of such a trial will be complex, as will the choice of which biomarker-drug combinations to include and prioritize to align with accumulating preclinical data. Toxicity of combination therapy must be addressed, and additional studies of concurrent versus sequential treatment schedules are warranted. However, the potential benefit is high as this longitudinal, in-depth approach could permit eradication or longer-term control of the cancer's evolutionary path toward drug-resistant, lethal disease.

Concluding Remarks

In summary, the study of cancer drug resistance mechanisms has shed remarkable light on basic biologic tenets of disease, including the structural biology of protein kinases, the wiring of signal transduction, and the plasticity of transcriptional programs that control cellular survival. In the next chapter of this biological tale, investigators should focus on the deeper interrogation of individual cancer patients over multiple time points, for instance through liquid biopsy, to permit early detection of resistance mechanisms and precise and tailored intervention to forestall or treat resistance to targeted cancer therapies. These new strategies can offer more nimble and dynamic assessment and early intervention, as opposed to the current common approach of using a single agent until a patient has developed complete resistance as shown through clinical progression. Ongoing work remains for future clinical and basic investigation (see Outstanding Questions), as even with the approaches we propose for the next five to ten years of research, a universal cure for cancer through targeted treatment is likely to remain an elusive goal.

Glossary

AXL
a gene encoding the RTK UFO, which appears to be upregulated in a number of resistant cancer contexts. Its role in normal (i.e., nonmalignant) biology is as yet underexplored.

Bayesian model
a statistical model that incorporates both the prior probability of a given hypothesis being true based on earlier information and ongoing data that can support or refute that hypothesis, into a dynamic prediction of the likelihood of a hypothesis being true or not.

BRAF
one of three human rapidly accelerated fibrosarcoma (*RAF*) genes, which encodes a serine/threonine kinase that is activated by GTP-bound RAS and in turn activates MEK to activate ERK in the MAP kinase cascade.

Cell-free DNA (cfDNA)
released under varying conditions into the bloodstream, but appears to be particularly elevated in the setting of cancer as well as in pregnancy (wherein it is derived from the developing fetus). The mechanisms controlling cfDNA secretion are still being explored.

Epidermal growth factor receptor (EGFR)
also known as ERBB1; one of four human EGFR (HER) family members. It is a RTK involved in epithelial patterning that is mutationally activated in subsets of NSCLC and glioblastoma and overexpressed in head and neck epithelial tumors.

Epithelial-to-mesenchymal transition (EMT)
an observed phenotypic alteration whereby epithelial malignancies acquire a more mesenchymal histology and transcriptional profile. EMT is hypothesized to play a role both in cancer resistance and in metastatic potential.

Gatekeeper residue
an amino acid in the ATP-binding pocket of kinases. Mutation of gatekeeper residues appears to widely block the activity of small-molecule kinase inhibitors through potentially multiple mechanisms including blocking access to the hydrophobic ATP-binding pocket, allosteric changes that increase intrinsic autophosphorylation kinase activity, and/or enhanced ATP affinity. Gatekeeper mutations associated with drug resistance include *EGFR* p.T790M and *BCR-ABL1* p.T315I.

Human epidermal growth factor receptors (HERs)
a broad family including *EGFR, HER2, ERBB3*, and *ERBB4*. Heterodimerization of all family members is possible and is required for any activity arising from the kinase domain-deficient ERBB3.

MET
a RTK whose ligand is hepatocyte growth factor (HGF) [also called scatter factor (SF)] that normally plays a role in embryogenesis and organ patterning. *MET* can be activated by amplification or mutation in cancer.

Mitogen-activated protein (MAP) kinase
the MAP kinase cascade is a signaling module activated when GTP-bound RAS recruits RAF through its Ras-binding domain (RBD). RAF is thereby activated and kicks off a cascade of serine/threonine kinase activation including MEK and ERK.

Nuclear factor kappa B (NF-κB)
a transcription factor formed as a homo- or heterodimer of subunits including RELA, RELB, and others. NF-κB activity is implicated in a variety of cell-biologic functions including antiapoptosis and inflammation.

RAS
the rat sarcoma oncogene is a small GTPase that cycles between an active, GTP-bound form and an inactive, GDP-bound form. When recruited to the cell membrane and active, RAS initiates signaling through multiple pathways to control the growth and survival of cells. The

human genome encodes three RAS isoforms: *NRAS, KRAS*, and *HRAS*. Mutations in one of these three genes are present in up to a third of all cancers.

RB1
a tumor-suppressor gene encoding the retinoblastoma (RB) protein, which binds to and sequesters E2F transcription factors, thus suppressing cell-cycle progression among other phenotypes.

Receptor tyrosine kinases (RTKs)
transmembrane proteins that bind to extracellular ligands, leading to the stimulation of their kinase activity. Activated RTKs can initiate intracellular signaling through multiple pathways.

TP53
a tumor suppressor that encodes the P53 protein, a master regulator of the DNA-damage response. Loss of *TP53* is common in cancer, and conversely, individuals with germline mutations in *TP53* are affected by a significant cancer-predisposition condition known as Li–Fraumeni syndrome.

Tyrosine kinase inhibitors (TKIs)
small molecules that block the autophosphorylation and activation of protein tyrosine kinases.

WNT
a signaling pathway discovered in *Drosophila* melanogaster, where its homolog *wingless* controls wing-bud development. In humans, the canonical *WNT* pathway is activated by extracellular WNT ligands that bind to the Frizzled family of transmembrane receptors, which in turn leads to the stabilization of beta catenin and its nuclear translocation to drive TCF/LEF transcription factor activity. WNT has been implicated in proliferation, migration, and cell fate or stemness.

Yes-associated protein 1 (YAP1)
a transcriptional cofactor for the TEAD transcription factors. It is normally activated by Hippo signaling and through its control of cell growth and antiapoptotic targets can help to control organ size in normal development in response to cell–cell contact signals. It also appears to drive resistance to targeted MAP kinase inhibitors in a number of contexts.

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Highlights

- Genetic and genomic studies of cancer cells have uncovered driving mutations that can in many cases be directly targeted to clinical benefit.
- The emergence of resistant cancer cells offers both an opportunity to dissect oncogenic and pro-survival cell biologic pathways, and a clear clinical need as resistance is a major cause of cancer mortality.
- Laboratory models and patient samples highlight resistance mechanisms that include (1) direct alteration of small molecule targets, (2) activation of upstream or downstream signaling nodes, (3) parallel signaling pathways to activate a common downstream pathway, (3) epigenetic or transcriptionally effected histologic transformation, or (4) increasingly understood adaptive signaling changes that alter transcriptional cell states to promote survival.
- Understanding, predicting, and circumventing resistance to targeted cancer therapy is now a major endeavor for translational cancer biologists to undertake.

Box 1:

Clinician's Corner.

- Although the proteins encoded by cancer-initiating oncogenes can be inhibited by small molecules, cancer cells often develop drug resistance.
- Resistance can pre-exist treatment or arise through many different mechanisms, making its early identification and treatment difficult.
- In some settings such as *BRAF* mutant melanoma, sequential targeting of resistance mechanisms after patients progress through initial treatment is clearly inferior to early combination therapy.
- The use of on-therapy biopsy or non-invasive "liquid biopsy" offers an opportunity to anticipate and identify resistance-inducing changes in cancers before they become dominant.
- New clinical trials must focus on testing the feasibility and benefit of early identification and therapeutic targeting of resistance-inducing events, to keep pace with and provide critical validation to preclinical discoveries.

Outstanding Questions.

- Given the plethora of resistance mechanisms, is there a general guiding principal that patients benefit most from upfront combination therapy, as is the case with BRAF and MEK inhibition in malignant melanoma?
- Can serial monitoring with increasingly sensitive genomic assays permit sequential, adaptive therapy changes in lieu of up-front one-size-fits all combinations?
- What are the drivers of epigenetic and transcriptional change that underlie epithelial to mesenchymal transition? More broadly, what common transcriptional pathways permit cancer cell survival in the face of targeted therapy, and how may they be directly inhibited?
- Can cancer cell plasticity be directly impeded to prevent the acquisition of resistance?
- What is the contribution of non-cell intrinsic mechanisms of targeted therapy resistance, such as microenvironmental signaling and immune evasion?

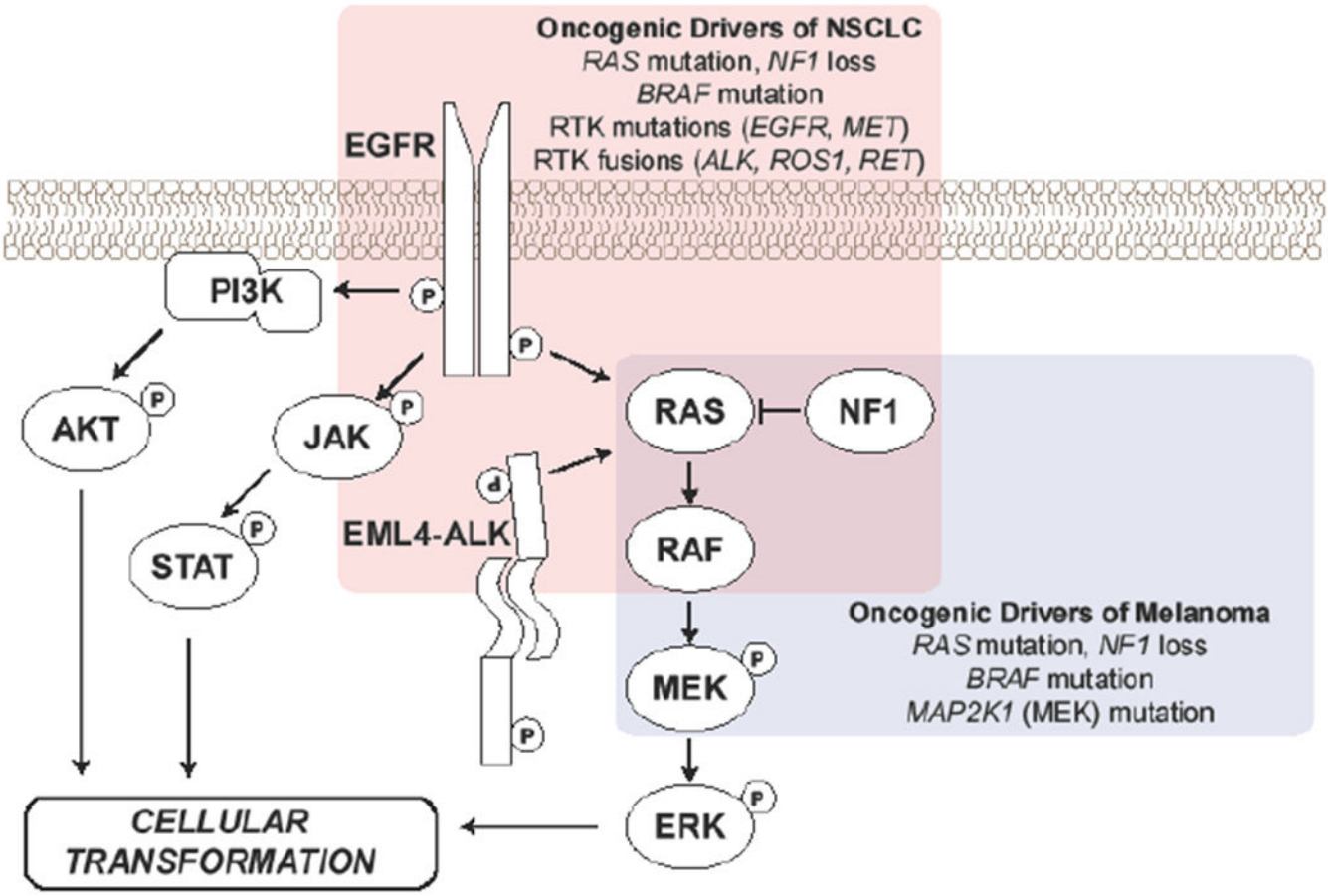

Figure 1. Drivers of malignant melanoma and non-small cell lung cancer.

A schematic of signaling pathways that promote oncogenic transformation when aberrantly activated by mutations in malignant melanoma (blue box) or non-small cell lung cancer (NSCLC; red box).

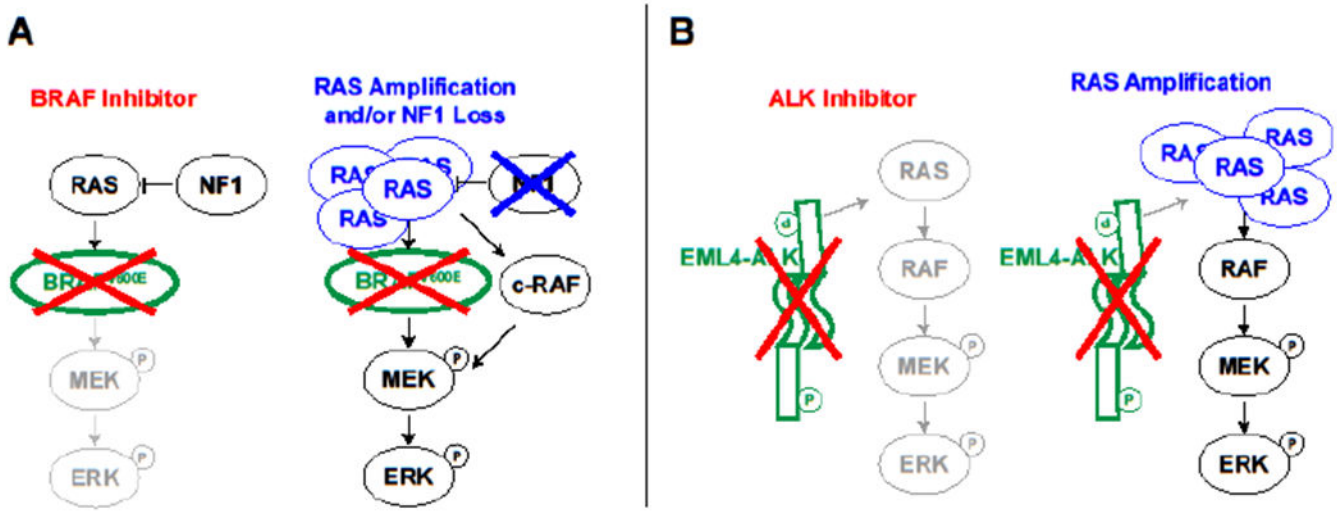

Figure 2. Upstream or downstream activation of signaling as a mechanism of targeted therapy resistance.

A) In melanoma cells treated with BRAF inhibitors, downstream Mitogen Activated Protein (MAP) kinase signaling is attenuated (grayed out MEK and ERK). Amplification of *RAS* or loss of its negative regulator *NF1* can restore MAP kinase signaling through parallel signal transduction using other RAF forms. B) In non-small cell lung cancer expressing the fusion oncogene *EML4-ALK*, ALK kinase inhibitors block activation of RAS from chimeric ALK proteins. Resistance can emerge through amplification of *RAS*, as in panel A, but in this case, it serves to activate MAP kinase downstream of the inhibited oncoprotein. Green: cancer-initiating mutant proteins; red, effects of targeted therapy; blue, resistance mechanisms.

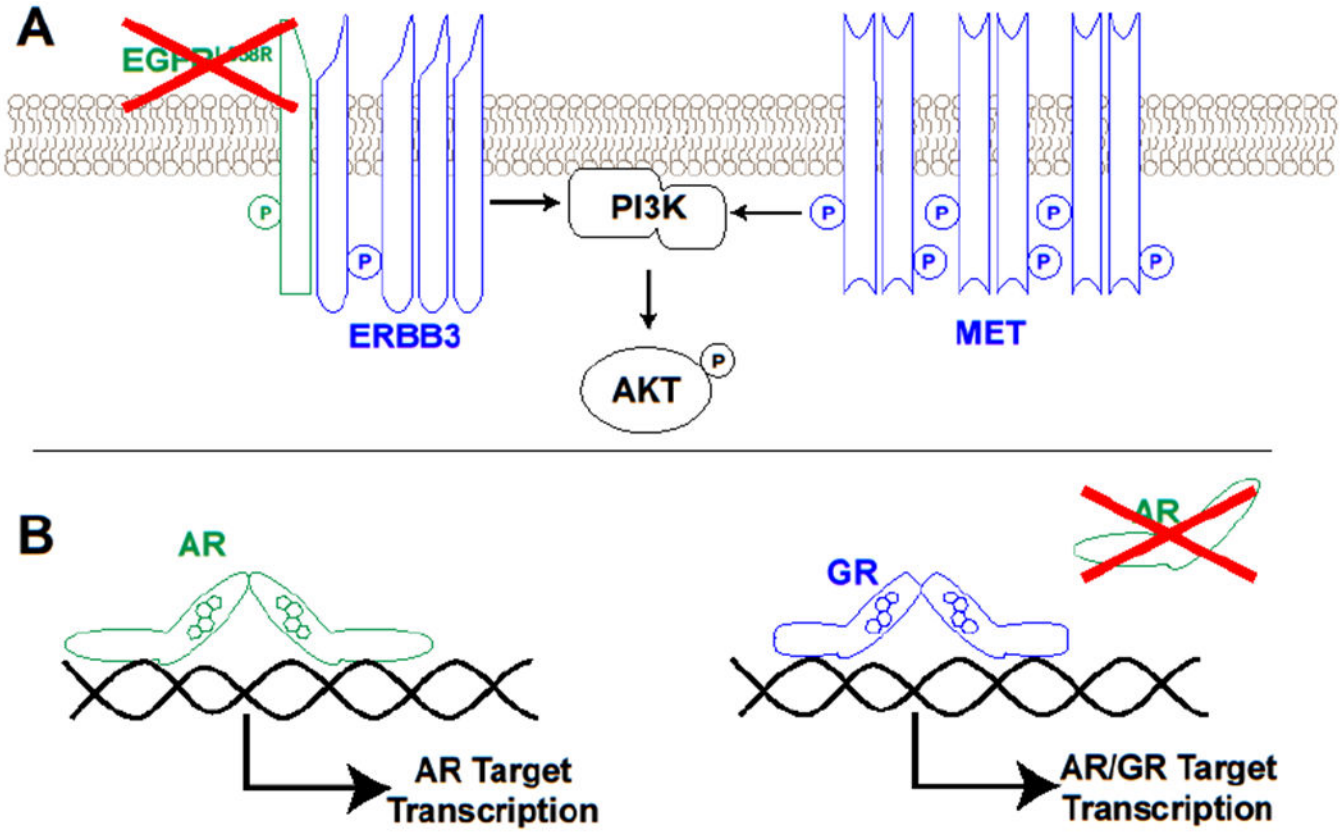

Figure 3. Parallel pathways to restore oncogenic signaling.

A) inhibition of EGFR in *EGFR* mutant non-small cell lung cancer can lead to amplification of *ERBB3* or *MET*. Both of these receptor tyrosine kinases can then restore PI3K-AKT signaling independently of EGFR, conferring resistance. B) Androgen receptor (AR) normally drive a tumorigenic transcriptional program upon binding to androgens. Androgen receptor inhibitors may prevent AR from executing this program, but glucocorticoid receptor can compensate to restore the transcription of a number of common target genes, thus overcoming AR antagonists. Colors are coded as in Figure 2.

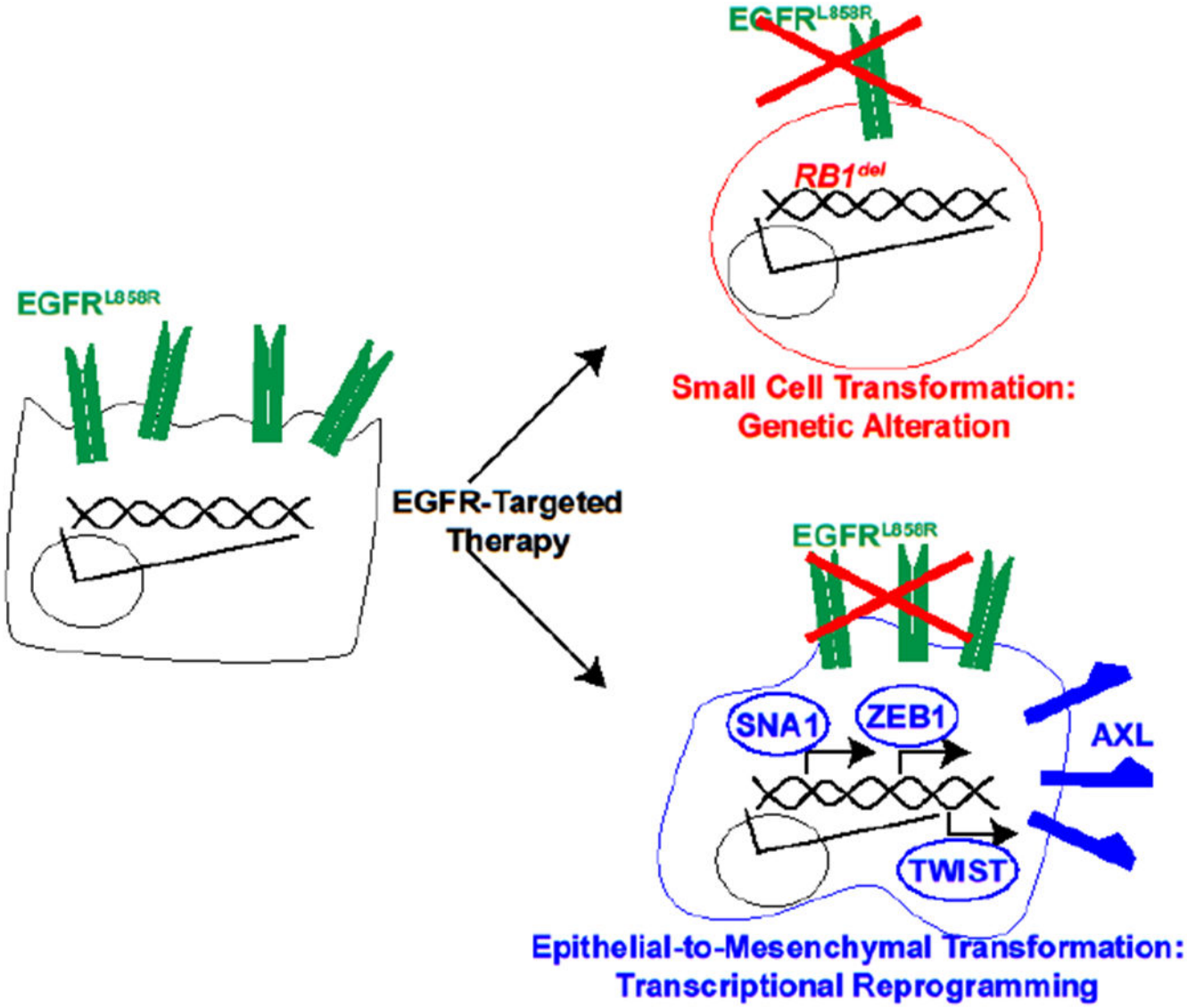

Figure 4. Genetic and non-genetic reshaping of cancer cell lineage.

EGFR mutant non-small cell lung cancer can adapt through either *RB1* loss and small cell transformation (top), or transcriptional reprogramming to promote epithelial-to-mesenchymal transformation (EMT; bottom). Both states promote EGFR inhibitor resistance, through downregulation of the mutant receptor, upregulation of alternative RTKs such as UFO, encoded by *AXL*, and other as yet undefined mechanisms. Colors are coded as in Figure 2.

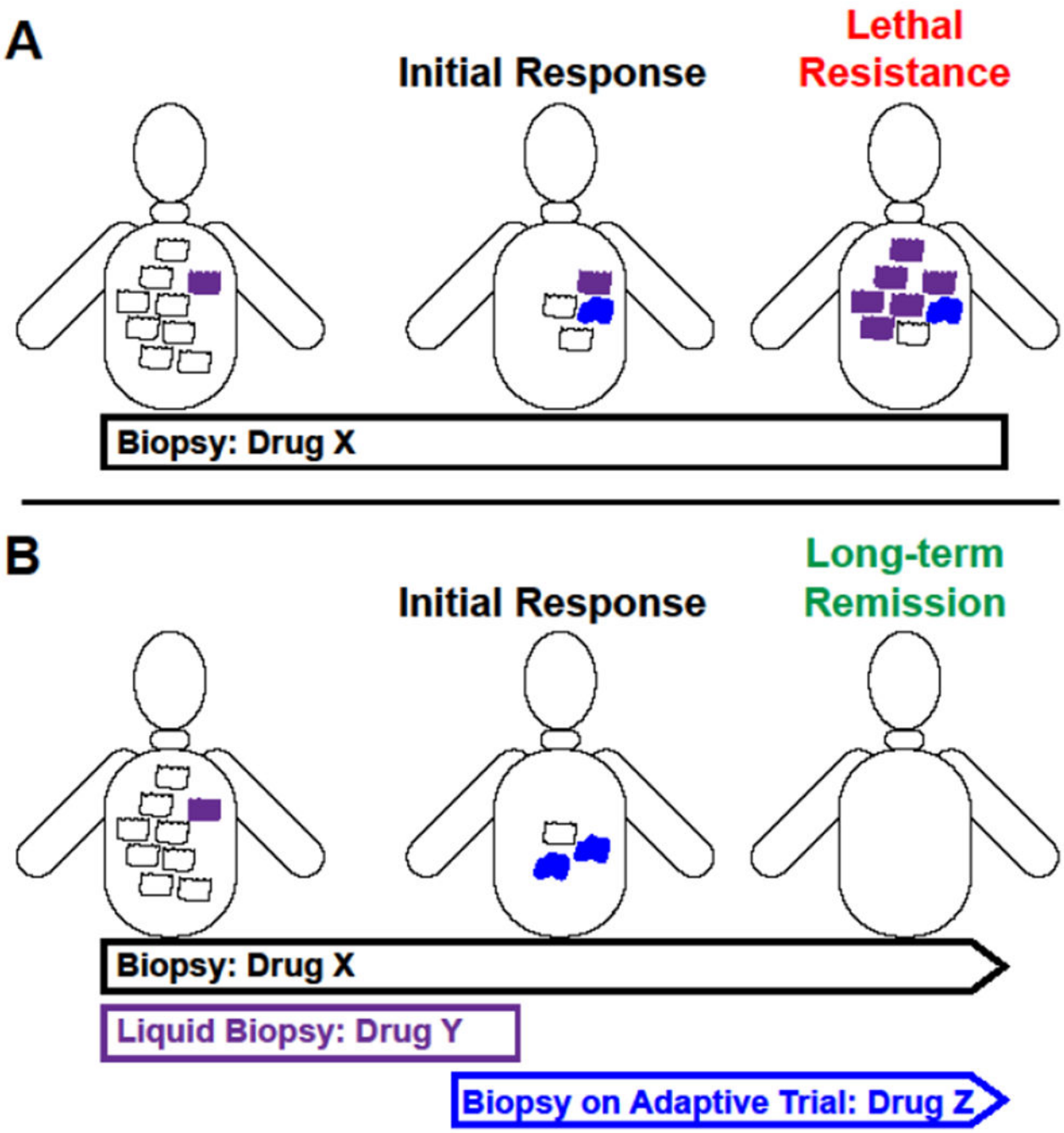

Figure 5. Strategies to overcome clinical acquired resistance in cancer.

A) Schematic of the current clinical approach: a biopsy may identify a driver mutation that is in the majority of cancer cells (black outline), but miss pre-existing resistance pathways (purple) and permit the outgrowth of others (blue). The result is a transient response that invariably gives rise to lethal resistance. B) Newer approaches may alter this trajectory; liquid biopsy might identify the heterogeneity that gives rise to early or later drug resistance, permitting combination therapy at diagnosis (black + purple arrows). Biopsy or resection of diseased tissue during therapy may permit the identification of a new resistant clone or

demonstrate resolution of a prior one. An adaptive clinical trial design would permit testing of biomarker-driven, tailored therapy (black + blue arrows) on an individual patient level.