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REVIEW

Circumventing Cancer Drug Resistance in the Era of Personalized Medicine

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ABSTRACT

All successful cancer therapies are limited by the development of drug resistance. The increase in the understanding of the molecular and biochemical bases of drug efficacy has also facilitated studies elucidating the mechanism(s) of drug resistance. Experimental approaches that can help predict the eventual clinical drug resistance, coupled with the evolution of systematic genomic and proteomic technologies, are rapidly identifying novel resistance mechanisms. In this review, we provide a historical background on drug resistance and a framework for understanding the common ways by which cancers develop resistance to targeted therapies. We further discuss advantages and disadvantages of experimental strategies that can be used to identify drug resistance mechanism(s).

Significance: Increased knowledge of drug resistance mechanisms will aid in the development of effective therapies for patients with cancer. We provide a summary of current knowledge on drug resistance mechanisms and experimental strategies to identify and study additional drug resistance pathways. *Cancer Discovery; 2(3); 214–26. ©2012 AACR.*

INTRODUCTION

Recent advances in targeted cancer treatments have spawned considerable optimism that knowledge of salient genetic or molecular features underpinning tumorigenesis and maintenance may enable sustained therapeutic control of many types of cancer. However, this optimism is tempered by the recognition that few if any new cures have yet been achieved by this knowledge. Indeed, patients with advanced cancer die because some or all of their tumor cells exhibit or develop resistance to available therapeutic avenues. The challenge of tumor drug resistance therefore represents a pervasive barrier that confounds the ultimate goal of cure or long-term control of metastatic cancer.

When patients with cancer relapse after an initial tumor response (or fail to benefit at the outset), the ensuing therapeutic decision often proceeds in a manner agnostic to the mechanistic basis for resistance. Not surprisingly, response rates in the setting of tumor progression/relapse are dismal. On the other hand, knowledge of specific resistance mechanisms can inform novel therapeutic approaches to counter this phenomenon, just as knowledge of key driver genes has guided the advent of new therapeutics capable of eliciting meaningful (if transient) initial tumor responses in patients with advanced malignancies.

Despite the considerable importance of tumor drug resistance to cancer morbidity and mortality, our understanding of resistance mechanisms—and plausible therapeutic avenues to intercept them—remains highly incomplete. Accordingly, this field of research has seen intense renewed interest as the clinical burden of resistance to targeted agents has increased. We outline the evolution from historical notions of tumor drug resistance toward current paradigms that are guiding the targeted therapeutic framework. We have placed a particular emphasis on resistance to kinase inhibitors, although the challenge of drug resistance extends to many other drug categories (e.g., cytotoxic, immunomodulatory, and hormonal agents). Similarly, although our main focus involves tumor cell autonomous resistance mechanisms, we recognize the important contribution of microenvironmental and germline factors to this clinical challenge. Nonetheless, many principles articulated here should prove generally applicable across the spectrum of anticancer agents and biologic contexts. Moreover, we discuss a range of experimental approaches that may be applied to the question of resistance and how these efforts may uncover future therapeutic combinations that may augment the magnitude and/or duration of clinical responses in many cancers.

CANCER DRUG RESISTANCE: EARLY STUDIES

In 1963, R.W. Brockman (a former Head of the Drug Resistance Section of the Southern Research Institute)

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completed an approximately 100-page book chapter describing in detail the knowledge of anticancer drug resistance mechanisms at that time. Drawing extensively from studies of nucleoside analogues, antifolate compounds, and alkylating agents, Brockman articulated an elegant biochemical framework for resistance: "Studies of resistance have, for the most part, been built on the hypothesis that resistant cells differ biochemically from the parent sensitive cells. Examples of such differences include decreased conversion of the inhibitor to an active form, increased degradation of the inhibitor, increased synthesis of the inhibited enzyme, decreased sensitivity of an enzyme system to an inhibitor (altered enzyme in the resistant cell), and decreased permeability of resistant cells to an inhibitor" (1).

Although contemporary genetic understanding and expanded breadth of inquiry may provide additional color to these observations, Brockman's summary remains a highly prescient synopsis of resistance mechanisms to many anticancer agents. In particular, Brockman highlighted the importance of pharmacokinetic alterations (specifically, altered drug metabolism), pharmacodynamic effects (e.g., refractoriness of the cellular pathway or "enzyme system" to therapeutic inhibition), and modifications (e.g., increased synthesis or intrinsic alterations) within the drug target itself. Implicit in this description is the notion that the enzyme target must also be essential for growth or viability of the cancer cell (the fact that the aforementioned drugs were aimed at the components of DNA synthesis underscores this assumption). These foundational postulates provided a basis for many seminal discoveries that emerged from early models of cancer drug resistance.

Unfortunately, the remarkable conceptual insights put forth by Brockman and others often proved doggedly elusive in clinical practice. To be sure, the near-ubiquitous resistance to single agents led to the dissemination of chemotherapeutic combinations, some of which engendered curative treatment of certain hematologic malignancies. A few chemotherapeutic combinations also increased the cure rates of solid tumors such as breast and colon cancer when given in adjuvant or neoadjuvant settings. However, the malignant variants that relapsed after initial therapy typically not only had acquired resistance to the index regimen, but also showed heightened cross-resistance to alternative regimens. Furthermore, the majority of human solid tumors exhibited primary (or *de novo*) resistance to many cytotoxic drugs and combinations.

These challenges led many investigators to probe the molecular basis for so-called multidrug resistance (MDR) starting in the early 1980s (2, 3). Shortly thereafter, Kartner and colleagues (4, 5) described a P-glycoprotein associated with MDR, which in turn led to the discovery of a large class of proteins (ABC transporters) that protect cells from toxin exposure. An assortment of studies found that expression of *MDR1*, the gene encoding P-glycoprotein, correlated with resistance to cytotoxic chemotherapy in several cancer types [for reviews, see Gottesman and Ling (6) and Gottesman and colleagues (7)].

Insights into the molecular basis for the MDR phenotype eventually gave rise to a series of clinical trials of MDR inhibitors such as verapamil, quinidine, or cyclosporine analogs to reverse the MDR phenotype (8–14). Although there were hints of improved tumor response and patient survival in some instances, enthusiasm for these clinical investigations had waned by the early 2000s (15), largely because a causal relationship between *MDR1* expression and chemotherapy resistance was never demonstrated conclusively, particularly in solid tumors. Thus, despite several decades of outstanding basic science, the translation of the classic drug resistance framework toward improved clinical benefit remained scant at the turn of the millennium.

The aforementioned body of work produced numerous pivotal insights pertaining to enzyme biochemistry, nutrient transport, DNA synthesis, and cellular metabolism in organisms ranging from mammals to tropical parasites. At the same time, several assumptions underlying early work may seem somewhat incomplete—at least in hindsight. For example, the rationale behind many resistance studies of folate antagonists, alkylating agents, and other cytotoxic drugs was that tumor cells should be intrinsically sensitive to these drugs because they were believed to grow more rapidly than normal cells. Also, early resistance paradigms often did not fully account for the spectrum of cellular pathways and effectors capable of directing hallmark tumorigenic processes that could also modulate treatment efficacy. The notion of distinguishing treatment-sensitive tumor subsets *a priori* based on biology or genetics remained in its adolescence. Thus, the early conceptual framework required additional evolution to impel meaningful translational progress toward overcoming cancer drug resistance.

A "TUMOR DEPENDENCY" FRAMEWORK FOR UNDERSTANDING RESISTANCE

In parallel to the efforts described, the recognition that most cancers arise and persist through the coordinated actions of oncogenes and tumor suppressor genes gained increasing momentum during the 1990s and early 2000s. Accordingly, the simplistic depiction of cancer as a collection of transformed cells that grow more rapidly than their normal counterparts gave way to more sophisticated notions of tumor cells that proliferate inappropriately and evade apoptotic signals through oncogenic dysregulation of specific cellular signaling pathways. The proliferation of comprehensive cancer genome characterization efforts both refined our understanding of key cellular networks and specified genetic nodal points around which tumors could arise and progress.

The current genomic framework thus carries important implications for both cancer treatment and tumor drug resistance. Genome characterization efforts have highlighted the importance of "driver" somatic alterations that activate crucial oncoproteins such as *RAS*, *EGFR*, *BCR-ABL*, and many others. In its fullest incarnation, driver genomic dysregulation gives rise to a pivotal tumor dependency: an unusual reliance of the cancer cell on a particular molecular pathway or module. "Oncogene addiction"—the excessive reliance within a cancer cell on a gain-of-function oncoprotein mutation for cell survival—represents perhaps the best known tumor-dependency mechanism (16). Similar addictions may result from tumor suppressor gene mutations [e.g., tumor dependence

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Table 1. Examples of tumor dependencies

	Alteration	Tumor type	Therapeutic agent
Oncogenes			
Receptor tyrosine kinase			
EGFR	Mutation/amplification (none)	NSCLC, GBM, colorectal	Gefitinib, erlotinib, cetuximab
KIT	Mutation	GIST, acral/mucosal melanoma	Imatinib, sunitinib
MET	Amplification	Gastric cancer	Crizotinib
ALK	Rearrangement	ALCL, NSCLC	Crizotinib
HER2 (ERBB2)	Amplification	Breast	Trastuzumab, lapatinib, others in development
Nonreceptor tyrosine kinases			
ABL	Translocation	CML	Imatinib, nilotinib, dasatinib
JAK2	Mutation	MPD	Ruxolitinib
Serine/threonine/lipid kinases			
BRAF	Mutation	Melanoma	Vemurafenib
PIK3CA	Mutation	Multiple	Many in development
Hormonal targets			
Estrogen receptor	Expression	Breast cancer	
Androgen receptor	Expression	Prostate cancer	
Metabolic			
Asparagine	Required for cell survival	ALL	L-asparaginase
Lineage			
MITF	Amplification	Melanoma	None
NKX2-1 (TTF1)	Amplification	Adenocarcinoma	None
SOX2	Amplification	Squamous cell cancer	None

Abbreviations: ALCL, anaplastic large cell lymphoma; ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; GBM, glioblastoma multiforme; GIST, gastrointestinal stromal tumor; MPD, myeloproliferative disorders; NSCLC, non-small cell lung cancer.

on dysregulated phosphatidylinositol 3 kinase (PI3K) pathway activation in the setting of *PTEN* inactivation]. The term "nononcogene addiction" has also been coined to describe dependencies that are not directly elaborated by somatic cancer gene alterations (17). Other types of tumor dependency with actual or potential clinical relevance include hormone dependency (e.g., breast and prostate cancer), lineage dependency (e.g., MITF-driven oncogenicity in melanoma), and metabolic dependency (e.g., L-asparagine in acute lymphoblastoid leukemia) (17, 18).

Three aspects of tumor dependencies have particular relevance to current models of tumor drug resistance. First, the fact that different dependencies are linked to distinct genetic alterations or cellular contexts undergirds the considerable heterogeneity observed in both clinical behavior and response to treatment—even within a given tumor linage. Second, such dependencies commonly denote an exquisite specificity; that is, the dependency may not become manifest unless a particular driver genetic alteration is present in the tumor cells. Third, effector proteins engaged by tumor dependencies may exhibit "druggability," meaning that they are vulnerable in principle to rational therapeutics that may be deployed from the existing repertoire or designed using standard drug discovery approaches.

Tumor dependencies driven by dominant oncogenes, hormones, or metabolites may prove vulnerable (sometimes impressively so) even to single-agent therapeutic regimens especially designed to intercept them. Prominent examples are shown in Table 1. Oncogene dependencies induced by *BCR-ABL*, *KIT,* and *EGFR* genetic alterations are well known and have provided decisive clinical proof of principle for the genome-based paradigm over the past decade, whereas other driver genetic events have been exploited more recently (e.g., *BRAF* and *ALK* alterations in melanoma and lung cancer, respectively) (19–23). However, clinical responses to single agents are invariably followed by the development of drug resistance, as noted previously. In other instances, tumors may fail to respond to targeted therapy despite carrying key driver events.

The tumor dependency framework thus highlights the distinction between *acquired* resistance—tumor progression in the face of ongoing treatment to which the tumor was initially sensitive—and *de novo* resistance—primary refractoriness to a therapy that should have been effective based

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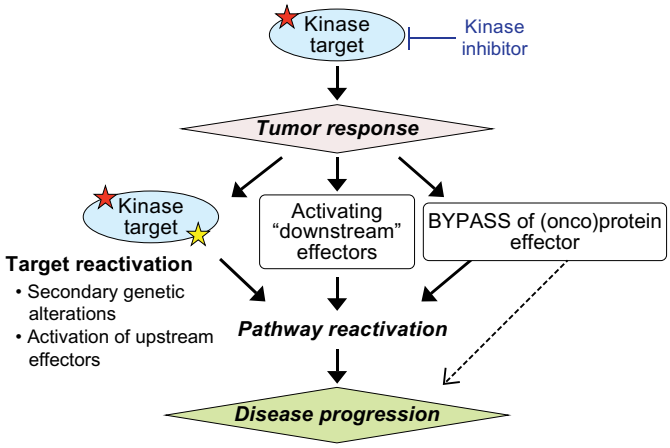

Figure 1. Mechanisms of acquired resistance to kinase inhibitors. Kinase inhibitors are effective clinical therapies in subsets of cancers, but resistance inevitably emerges. These resistance mechanisms can lead to reactivation of the target (i.e., through a secondary mutation), activation of upstream or downstream effectors, and/or activation of a bypass oncoprotein. All of these lead to reactivation of the critical signaling pathway for the specific kinase and clinically to cancer progression.

on the underlying biology or genetics. The emergence of a "gatekeeper" mutation within the BCR-ABL oncoprotein in relapsing chronic myelogenous leukemia (CML) cells treated with the ABL inhibitor imatinib constitutes an example of acquired resistance. Strictly speaking, such mutations are not "produced" by drug treatment; rather, they represent positive selection of rare cell subpopulations in which the drug resistance allele pre-exists (24). On the other hand, approximately 10% of $BRAF^{V600E}$ melanomas that show rapid disease progression on treatment with the RAF inhibitor vemurafenib (21, 25) represent examples of *de novo* resistance. Each of these resistance categories may in turn be distinguished from therapeutic "indifference," which refers to the expected lack of clinical efficacy when a therapy is used that is irrelevant to the dependencies therein. Altogether, this framework underpins an emerging treatment strategy that identifies druggable tumor dependencies *in situ*, applies rational therapeutics to counter these dependencies, and anticipates drug resistance mechanisms—many of which engage the same cellular effectors that comprise the index dependency module.

HALLMARKS OF RESISTANCE TO AGENTS THAT INTERCEPT KINASE-DRIVEN TUMOR DEPENDENCIES

Guided by the tumor dependency framework, mechanisms of resistance to anticancer agents have been analyzed extensively over the past decade with intensive focus on small-molecule kinase inhibitors. In the aggregate, these efforts have given rise to 3 main categories of resistance to targeted therapies, outlined in Figure 1 and summarized in Table 2. An important commonality across each of these mechanisms is the persistent activation of either the drug target itself or its critical downstream signaling pathway(s). Mechanisms that operate independently of the "index" driver pathway or module occur less commonly and remain poorly understood. A specific understanding of the nature and prevalence for each of these mechanisms in relation to specific drugs and their (oncoprotein) targets is essential to the design of effective therapeutic strategies that salvage or circumvent the acquisition of drug resistance.

Secondary Genetic Alterations in the Target (Onco)Protein

One of the most common drug resistance mechanisms involves additional genetic alterations within the target oncogene itself. This mechanism was first described in patients with CML treated with imatinib; however, so-called secondary somatic alterations have subsequently been detected in a wide variety of tumors from patients treated with different kinase inhibitors (26–30). Several lines of evidence have shown that these mutations exist at low levels before drug treatment and undergo positive selection during exposure to a clinically effective targeted agent (24, 31).

Secondary mutations can impede the effects of a kinase inhibitor by altering contact points for drug binding or by perturbing the conformational state of the kinase (32). Among contact point mutations, the most therapeutically challenging is the so-called gatekeeper mutation (32). The gatekeeper is a conserved amino acid residue situated within the catalytic cleft of tyrosine kinases that determines the accessibility of a hydrophobic pocket critical to binding of many small-molecule tyrosine kinase inhibitors [TKI (33)]. Gatekeeper mutations have been detected from patients with a variety of TKI-resistant cancers after treatment with selective inhibitors. Examples include imatinib-, nilotinib-, and dasatinib-resistant CML ($T315I$); gefitinib- and erlotinib-resistant epidermal growth factor receptor (EGFR)–mutant non–small cell lung cancer [NSCLC ($T790M$)]; imatinib-resistant GIST (KIT^{T670I}); and crizotinib-resistant, ALK-rearranged NSCLC [ALK^{L1196M} (26–28, 30, 34)]. Despite this evolutionary convergence in target-oriented resistance to TKIs, the specific mechanism by which the gatekeeper mutation leads to drug resistance may vary. For example, the ABL^{T315I} gatekeeper mutation produces steric hindrance that disrupts imatinib binding (35), whereas the analogous $EGFR^{T790M}$ mutation increases the affinity of EGFR for adenosine-5′-triphosphate (ATP), thus rendering the EGFR kinase inhibitors gefitinib and erlotinib less effective in displacing this molecule (36).

Beyond the gatekeeper mechanism, many other secondary mutations have been reported that alter the conformation state of kinase drug targets. In general, these mutations

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Table 2. Main categories and examples of different acquired drug resistance mechanisms

Acquired resistance mechanism	Example
Secondary genetic alteration in drug target	
Mutation in drug contact residue	*ABL* T315I
Mutation in noncontact residue leading to altered conformation	*ABL* G250E
Mutation leading to increased ATP affinity	*EGFR* ^{T790M}; *ALK* ^{F1174L}
Amplification	*BCR-ABL*
Alternative spliced form	p61*BRAF* ^{V600E}
Bypass mechanism	
Activation of parallel signaling pathway	*MET* amplification (erlotinib resistance)
	COT or RTK overexpression (vemurafenib resistance)
Alterations in upstream or downstream effectors	
Upstream effector	*BRAF* amplification; selumetinib resistance
Downstream effector	*MEK1/2* mutation; vemurafenib resistance
	NRAS mutation, vemurafenib resistance
Pathway independent	
Epithelial–mesenchymal transition	EGFR inhibitor resistance
Changes in tumor microenvironment	JAK2 inhibitor resistance
Altered angiogenesis	EGFR inhibitor resistance

either promote adoption of an active conformation by the kinase or alter the flexibility of the P-loop that prevents conformational changes required for drug binding. This mechanism has been most extensively described and studied for imatinib, which binds the inactive conformation of ABL (32). Mutations that alter the conformational state of ABL are predicted to disfavor imatinib binding and, consequently, its efficacy. Secondary mutations in KIT that occur in the kinase activation loop (which likely promote the active conformation) are associated with resistance to the KIT inhibitors imatinib and sunitinib *in vitro* and a worse clinical outcome in patients treated with sunitinib (37–39). In contrast, mutations in the drug/ATP binding pocket of KIT, although common in patients with gastrointestinal stromal cell tumors (GIST) who develop imatinib resistance, are less often detected from *in vitro* studies or from patients with GISTs that develop sunitinib resistance (37–39). These differences may be related to the differential potencies of KIT inhibition between imatinib and sunitinib. More recently, 3 mutations in ALK (F1174L, C1156Y, and L1152R) were detected in patients with ALK-rearranged cancers that developed clinical resistance to the ALK inhibitor crizotinib (29, 30). All of these mutations are located outside the drug-binding region. Biochemical studies of the F1174L mutation reveal that it results in an increase in ATP affinity analogous to the EGFR^{T790M} drug resistance mutation (40).

Most small-molecule kinase inhibitors in clinical use fall into 1 of 2 categories: type I inhibitors (which occupy the ATP-binding pocket when the kinase assumes its active conformation) or type IIa inhibitors (which bind both the ATP-binding site and adjacent motifs that are revealed when the kinase resides in an inactive conformation). The EGFR inhibitor erlotinib represents a well-known type I kinase inhibitor, whereas the ABL/KIT/platelet-derived growth factor receptor (PDGFR) inhibitor imatinib is a classic type IIa inhibitor. On the other hand, inhibitors of the MEK serine–threonine kinase exemplify "type IIb" inhibitors: small molecules that occupy an allosteric pocket adjacent to the ATP-binding motif and are thus non-ATP competitive agents (33). *In vitro* mutagenesis studies have indicated that secondary MEK resistance mutations may involve hydrophobic-to-hydrophilic amino acid substitutions along the C-helix or at various other key positions within the allosteric pocket (41). In addition, a clinically observed *MEK1* mutation (*MEK1* P124L) may simultaneously modulate the negative regulatory affects of the recently described A-helix based on its predicted proximity to this N-terminal motif in 3-dimensional models (41).

Increased gene dosage has long been recognized as a potential means to engender resistance to enzymatic inhibitors, dating back to early "dominant genetics" studies in yeast and parasites (42, 43). Toward this end, amplification of the drug target has been identified as an additional somatic resistance mechanism in relapsing tumor cells. BCR-ABL amplification has been detected both *in vitro* and in imatinib-resistant CML specimens (26). In addition, amplification of the *EGFR*^{T790M} allele was detected in an *in vitro* model of resistance to the irreversible EGFR inhibitor PF299804 [dacomitinib (44)], and amplification of *BRAF* has been observed in an *in vitro* study of resistance to MEK inhibition (45, 46).

A novel drug resistance mechanism resulting from alternatively spliced form of an oncoprotein has been recently described. A 61-kDA variant of BRAF^{V600E} (p61BRAF^{V600E}), resulting from a splicing isoform and lacking the RAS binding domain, was

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detected in both vemurafenib *in vitro*-resistant cells and from resistant patient tumor biopsies (47). The p61BRAF^{V600E} produces enhanced dimerization with other RAF family members and resistance to vemurafenib but not MEK inhibitors (47).

Activation of Bypass Mechanisms

An increasingly recognized drug resistance mechanism occurs through engagement of a so-called bypass signaling module. This results in the activation of a critical downstream signaling effector—normally activated by the kinase and extinguished by a kinase inhibitor—through a parallel mechanism that is indifferent to the kinase-directed therapy. An illustrative example of bypass-mediated resistance has been described in EGFR-mutant NSCLC. Here, reactivation of PI3K/AKT signaling (a critical pathway augmented as a result of oncogenic RTK activation) results in drug resistance (48). Aberrant activation of PI3K/AKT signaling in the presence of the EGFR kinase inhibitor gefitinib can occur as a result of activation of MET (through either MET amplification or by its ligand hepatocyte growth factor [HGF]) or insulin growth factor-1 receptor (IGF-IR) signaling (49–53). In each of these examples, gefitinib is no longer able to turn off PI3K/AKT signaling as a means to inhibit tumor growth. Analogous bypass mechanisms may confer resistance to vemurafenib in melanomas harboring the *BRAF*^{V600E} mutation. *In vitro* and *in vivo* studies have provided evidence that upregulation of the COT kinase promotes sustained ERK activation by circumventing BRAF inhibition (54). Receptor tyrosine kinase dysregulation may constitute an additional RAF inhibitor bypass mechanism. Toward this end, PDGFRA and IGF-IR overexpression have been associated with resistance to vemurafenib (or related compounds) *in vitro* and *in vivo* (55, 56). Vemurafenib-resistant *BRAF*^{V600E} melanoma cells with IGF-IR overexpression exhibited sustained ERK phosphorylation that was dependent on both A-RAF and C-RAF overexpression, suggesting a target ortholog-dependent bypass mechanism (55, 56).

Another avenue though which bypass resistance mechanisms may operate involves modulation of feedback loops. Cell signaling cascades are commonly regulated by feedback inhibition of various network components. In particular, pharmacologic inhibition of an individual node within an oncogenic signaling pathway may result in relief of feedback inhibition at multiple upstream nodes. Several recent studies have documented this phenomenon and raise the possibility that such feedback modulation may contribute to *de novo* or acquired resistance. Examples include HER3 pathway upregulation in the setting of AKT inhibition (57), PI3K/AKT activation by TOR inhibitors (58), and augmentation of AKT signaling by MEK inhibitors (59).

Genomic Alterations Affecting Upstream or Downstream Effectors

A third category of acquired drug resistance involves genomic alterations that dysregulate signaling proteins acting either upstream or downstream of the target (onco)protein. To date, this type of drug resistance mechanism has been most extensively described for MEK and RAF inhibitors. In particular, activating mutations in *MEK1* or *NRAS* have been observed in tumors that progressed in the setting of vemurafenib treatment and were also shown to be sufficient to confer resistance to RAF inhibition *in vitro* (55, 60). Both mutations also lead to constitutive ERK signaling, even in the presence of RAF inhibition. In the case of *MEK1* mutations (which exemplify downstream effector dysregulation), MEK signaling becomes uncoupled from the inhibited BRAF oncoprotein (60). In contrast, oncogenic NRAS (illustrative of upstream effector dysregulation) activates ERK signaling through CRAF, thus bypassing BRAF inhibition in an ortholog-dependent manner (55).

In a recent study of erlotinib resistance in lung cancer, dysregulation of NF-κB signaling was implicated through a short hairpin RNA (shRNA) suppressor screen and supported indirectly by clinical data showing that such dysregulation was associated with decreased patient survival (61). NF-κB signaling has been postulated as a gating mechanism downstream of both MAP kinase and PI3 kinase signaling in EGFR-mutant lung cancer. Amplification of *BRAF* has been described as an alternative "upstream" mechanism of resistance to the MEK inhibitor AZD6244 (45, 46). This mechanism leads to increased MEK phosphorylation and consequently ERK activation, which no longer can be inhibited by AZD6244 (45, 46). In addition, an oncogenic PIK3CA mutation was sufficient to engender gefitinib resistance in *EGFR*-mutant cancer cells and has also been observed in an erlotinib-resistant EGFR-mutant tumor, thus suggesting that downstream effectors may ultimately become relevant in this context as well (62, 63).

Pathway-Independent Resistance Mechanisms

As noted, the most common and best understood mechanisms of resistance to kinase oncoprotein inhibitors involve sustained activation of the salient downstream signaling pathway. However, several studies have indicated that resistance may also arise even in the setting of sustained downstream pathway inhibition. For example, several *in vitro* studies, and evaluation of tumor specimens, of resistance to EGFR inhibitors in lung cancer showed that the epithelial–mesenchymal transition (EMT) may underpin an EGFR pathway-independent resistance process (63–65). The basis for EMT induction in this setting remains incompletely characterized; however, several recent reports have raised the possibility of a connection between EMT and the acquisition of stem/progenitor cell characteristics within heterogeneous tumor subpopulations [for a review, see Singh and Settleman (66)]. Given that drug resistance is often considered a hallmark feature of such stem-like cancer cell populations, a functional link between EMT and cancer stem cell biology presents an intriguing model worthy of additional investigation.

Studies of EGFR-mutant lung cancer cells that persist in the setting of erlotinib exposure have implicated a distinct chromatin state mediated by the histone demethylase KDM5A that results in resistance in a pathway-independent manner (67). On the clinical side, anecdotal reports of *BRAF*-mutant melanomas exhibiting resistance to RAF inhibition despite sustained MAP kinase inhibition have recently been reported, although the mechanisms of resistance in this setting remain to be determined. One possibility for these observations includes cancer-cell nonautonomous mechanisms of drug resistance mediated by the tumor microenvironment. Recent studies demonstrate that the tumor microenvironment can influence the efficacy

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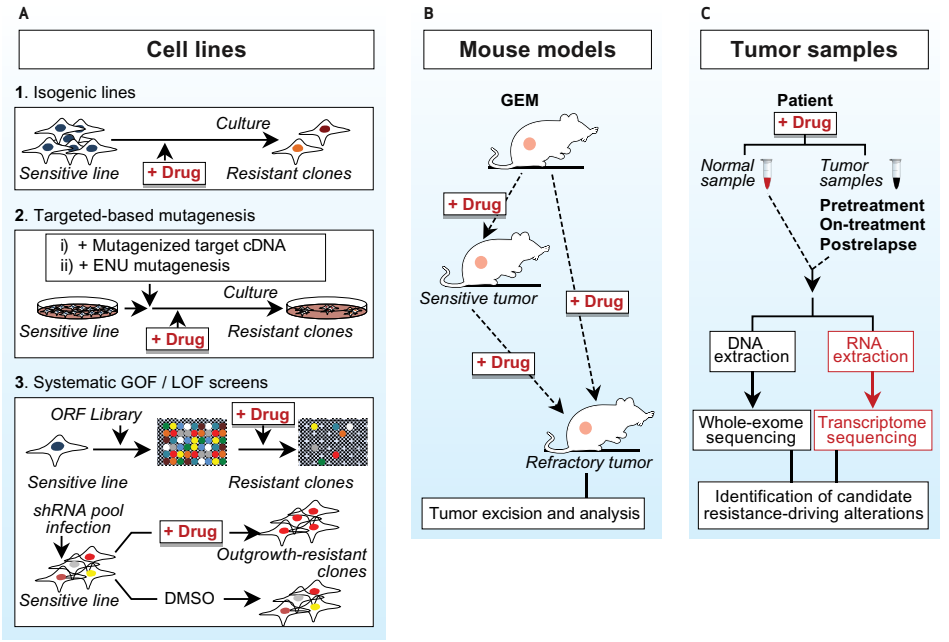

Figure 2. Experimental approaches to study cancer drug resistance. **A**, *in vitro* studies of resistance often use cell line models, such as 1) culturing of sensitive lines in the presence of drug until the appearance of resistant clones; 2) random mutagenesis of cDNAs that encode the target protein or N-ethyl N-nitrosourea (ENU) mutagenesis followed by drug selection; or 3) systematic gain-of-function (GOF) and loss-of-function (LOF) screens using open reading frame (ORF) and shRNA libraries. The resistant cell line clones that emerge are subjected to genomic/molecular studies as well as directed experiments that query the necessity and sufficiency of candidate resistance effectors. **B,** *in vivo* studies of resistance use xenografts or genetically engineered mouse (GEM) models. Here, the mouse harboring a (genetically defined) tumor is treated with a drug of interest until tumor regression is observed. Tumors that relapse are subjected to genomic and/or molecular characterization and follow-up experimental validation studies. **C,** the gold standard for characterization of resistance mechanisms involves in-depth characterization of patient tumor samples obtained before treatment and after relapse. Additionally, the acquisition of tumor specimens during treatment allows critical pharmacodynamic analyses to determine if the drug is achieving the expected target modulation (this may inform possible mechanisms of *de novo* resistance). Here, hypothesis-directed or unbiased genetic and molecular analyses are undertaken to identify candidate alterations linked to the acquisition of resistance. These studies are followed by experimental validation using *in vitro* and/or mouse models. Occasionally, it is possible to culture drug-refractory tumor cells obtained from relapsing patients (either as relapsing tissue or circulating tumor cells) and perform hypothesis-directed or unbiased studies of these cells (not shown).

of polo-like kinase and Janus-activated kinase 2 inhibitors (68, 69). These can be mediated by several different stromal secreted cytokines (69). HGF can be produced by tumor-derived stromal fibroblasts and cause resistance to EGFR kinase inhibitors (70). Altered tumor angiogenesis has also been demonstrated to lead to resistance to EGFR kinase inhibitors and to the anti-EGFR therapeutic antibody in preclinical models (71, 72). Continued studies elucidating the molecular basis for "pathway-independent" resistance to kinase inhibitors will have important implications for future therapeutic combinations in several genetically defined tumor subtypes. Furthermore, there is no reason to believe that "pathway-dependent" and "independent" mechanisms of drug resistance could not develop simultaneously. This underscores one of the challenges of developing effective clinical therapies to combat drug-resistant cancers.

EXPERIMENTAL APPROACHES TO STUDY CANCER DRUG RESISTANCE

The gold standard for mechanistic characterization of tumor drug resistance involves detailed studies of tumor tissue obtained before treatment and after relapse together with experimental confirmation of candidate resistance effectors. However, such specimen collections may require many months or years to accrue and analyze at a quantity and quality sufficient to glean systematic insights. It has therefore proved useful to use experimental approaches capable of gaining knowledge of tumor drug resistance mechanisms in an anticipatory fashion (e.g., in advance of robust data sets from clinical collections). Multiple avenues have been developed for this purpose, each of which has certain advantages and limitations (Fig. 2). Their appropriate use requires a conceptual and methodologic framework that maximizes clinical pertinence while avoiding certain pitfalls that may lead to erroneous conclusions.

Laboratory investigation of tumor drug resistance should be guided by 4 fundamental questions: i) Is the candidate effector necessary to elaborate or sustain a resistance phenotype? ii) Is the candidate effector sufficient to confer resistance? iii) Does the candidate effector reactivate the salient downstream pathway? iv) Is the candidate effector dysregulated in drug-resistant clinical specimens?

Consideration of necessity and sufficiency is critical to assign causality to candidate resistance mechanisms, thus distinguishing *bona fide* effectors from associated epiphenomena. Understanding the extent to which the downstream pathway is sustained or reactivated provides gating

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mechanistic information that guides further experimental design (and future combinatorial therapeutic considerations, as described subsequently).

Studies of Isogenic Tumor Cells Generated *In Vitro*

One of the most common experimental approaches to study cancer drug resistance involves culturing of drug-sensitive cancer cell lines in the presence of the query drug until subpopulations emerge that proliferate avidly at high drug concentrations. This has been a very successful approach because many cancer cell lines that harbor a genomic alteration (mutation, amplification of genomic rearrangement) in the drug target are representative model systems and undergo apoptosis, an *in vitro* equivalent of tumor shrinkage observed in patients with cancer, after treatment with a kinase inhibitor. These inogenic, drug-resistant subpopulations are then compared with the drug-sensitive parental line to identify genetic, molecular, or biochemical differences that might account for the resistance phenotype. Such analyses usually begin with an interrogation of the cellular target and pathway inhibited by the drug and include sequencing of known drug targets, biochemical studies of target protein function (e.g., measurement of substrate phosphorylation if the target is a protein kinase), and determination of whether the resistance phenotype is reversible on drug discontinuation. It is also typical to extend these studies using unbiased omic approaches such as gene expression profiling (using microarrays or transcriptome sequencing), genomic studies (e.g., array comparative genomic hybridization or whole exome sequencing), or systematic protein-based studies (with phospho-antibody or reverse phase protein arrays).

When such studies are focused in scope, their interpretation often becomes straightforward. For example, characterization of *EGFR*, *MEK1/2,* and *ALK* genes in cell lines rendered resistant by stepwise selection to EGFR, MEK, or ALK inhibitors identified mutations in each gene that confer resistance through reduced drug binding (41, 73, 74). Similarly, interpretation of unbiased studies becomes clear when a clear target or pathway based alteration is revealed. For example, analysis of high-density single-nucleotide polymorphism arrays generated using isogenic *EGFR*-mutant NSCLC lines selected for resistance to gefitinib revealed focal *MET* amplification in several cases (49), thus pointing to an obvious bypass effector hypothesis. This resistance mechanism was first identified in drug-resistant cell lines and then validated in tumors from patients that had developed gefitinib or erlotinib resistance (49). However, characterization of resistant isogenic lines may become more complicated when no single dominant candidate effector mechanism is uncovered. In such cases, it is tempting to use enrichment-based analytical approaches to nominate candidate pathways or networks associated with resistance. Although often helpful, these approaches typically identify multiple candidate gene sets whose strength of statistical association can be influenced by the size of the gene set or confounded by adaptive or feedback-related changes that may not drive resistance *per se*. Choosing the best hypothesis for experimental follow-up is often a subjective process that may be further biased by prior knowledge (e.g., gravitation toward recognizable candidates) in a manner that hampers novel discovery.

In the absence of a clear resistance hypothesis (e.g., a new genetic alteration or "outlier" differential gene expression), follow-up studies in isogenic cell lines must rely on rigorous interrogation of the necessity and sufficiency of candidate effectors. When candidate effectors are ectopically expressed (or silenced) in the drug-sensitive parental lines, the extent to which the resulting pharmacologic IC_{50} shift (if any) recapitulates the IC_{50} of the resistant isogenic strains must be carefully considered. Conversely, the magnitude of IC_{50} reversal should be stringently assessed when putative gain-of-function effectors are silenced genetically (e.g., by RNA interference) or pharmacologically (e.g., using small-molecule inhibitors if available) in drug-resistant cell populations. In some isogenic studies, no single effector mechanism may be sufficient to fully recapitulate the magnitude of resistance present in the drug-resistant "daughter" populations; thus, multiple cellular perturbations may be necessary to resensitize resistant cells. Detailed attention to these issues should avoid the risk of over-interpreting effects that are significantly but not causally associated with the acquisition of drug resistance *in vitro*.

Target-Based Mutagenesis Approaches

The gain of secondary mutations within a target protein comprises a common mechanism of resistance to targeted agents, as described previously. Thus, several groups have used random mutagenesis to define a spectrum of mutations within the drug target that may confer resistance. Mutagenesis libraries (using an expression vector harboring the target cDNA) are generated using error-prone polymerase chain reaction techniques or by culturing the expression construct in "mutator" strains of *Escherichia coli* (75). These libraries are packaged into viral delivery systems and introduced into drug-sensitive cancer cell lines. The resulting populations are cultured in the presence of the targeted agent at concentrations that inhibit the parental cell line. Individual drug-resistant clones emerge as isolated colonies; after recovery of these clones, the target cDNA is characterized for mutations that may confer resistance. The random mutagenesis screening approach was pioneered through studies of imatinib resistance in CML (75). It has since been used to study target-based resistance to several other agents, including MEK and FLT3 inhibitors (41). A complementary approach exposes sensitive cell lines to mutagens such as N-ethyl N-nitrosourea (ENU) followed by drug selection and targeted sequencing. ENU mutagenesis screens have been used to identify resistance mutations to several kinase inhibitors, including imatinib, sunitinib, and nilotinib (38, 76). The advantages of these approaches are a relatively unbiased view of mutations within the target protein that may confer drug resistance. Such studies may anticipate future clinical findings, validate the cellular targets of small molecules, and facilitate the development of next-generation inhibitors whose effects are not blunted by the same mutations. The major disadvantage is the limited scope—usually restricted to the cDNA encoding the primary drug target.

Historically, individual resistant clones arising after mutagenesis screens required expansion and cDNA sequencing

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in parallel, which was tedious and labor-intensive. However, the advent of massively parallel sequencing has circumvented this limitation by enabling pooling and expansion of hundreds or thousands of clones followed by deep sequencing of the target cDNA (41). Indeed, massively parallel sequencing technology could eventually obviate the need for plasmid-based mutagenesis libraries altogether.

Systematic Gain- and Loss-of-Function Resistance Screens

The emergence of reagents that enable near genome-scale functional screens in mammalian systems has opened up powerful new genetic avenues. Application of systematic RNAi knockdown or open reading frame (ORF) expression-based studies to the question of cancer drug resistance has considerable appeal because these approaches are categorical in scope, unbiased, and functional as opposed to descriptive in nature. Furthermore, the use of selective small-molecule inhibitors and cell growth phenotypes (as opposed to loss of viability) allows robust signal-to-noise readouts for both screening and validation studies. Together, global functional screens could in principle define the universe of individual genes whose overexpression or silencing is sufficient to confer cellular resistance to many types of targeted agents.

Several proof-of-principle studies of pooled RNAi suppressor screens that identify genes whose knockdown confers resistance have been performed. Notable examples include the demonstration that *CDK10* and *PTEN* silencing may cause resistance to tamoxifen and trastuzumab, respectively, in breast cancer (77, 78). As analytical tools improve and the scope of such studies expands, many additional validated effectors should emerge from genome-scale loss-of-function resistance efforts.

On the gain-of-function side, overexpression-based studies (or those that engender increased gene dosage) have long been used to query drug resistance in model organisms (so-called dominant genetics) as described previously. Recently, a related approach was applied to the study of resistance to selective RAF inhibition in melanoma. An arrayed, kinome-wide ORF expression library was introduced into $BRAF^{V600E}$ melanoma cells that were highly sensitive to the RAF inhibitor PLX4720 [an analogue of vemurafenib (54)]. This effort identified COT as a novel kinase that directs robust resistance to RAF inhibition. Of note, several other kinases also scored as "hits" in this study, including C-RAF and 3 receptor tyrosine kinases (54). C-RAF is the key resistance effector downstream of mutant NRAS, which has been observed in some vemurafenib-resistant clinical specimens; receptor tyrosine kinases as a class have also been implicated in clinical resistance to RAF inhibition, as noted previously (55). Importantly, most of these kinases conferred resistance through sustained extracellular signal-regulated kinase/mitogen-activated protein kinase activation, thus affirming the paramount importance of this hallmark melanoma tumor dependency in the resistance phenotype. Overall, systematic functional screens hold considerable promise to define a spectrum of resistance mechanisms, many of which may have immediate clinical relevance.

Studies of Resistance in Genetically Engineered Mouse Models

Although the aforementioned *in vitro* approaches offer powerful and scalable avenues for resistance characterization, they are limited to tumor cell autonomous mechanisms and cannot interrogate effects of the microenvironment in the context of an intact organism. Accordingly, several groups have endeavored to model resistance using genetically engineered mouse models that form autochthonous cancers. For example, RNAi-mediated silencing of *TOP2A*, which encodes the topoisomerase 2 enzyme, induced resistance to doxorubicin (a topoisomerase inhibitor) in a murine lymphoma model (79). On the other hand, knockdown of *TOP1* (which encodes topoisomerase 1) conferred resistance to camptothecin [a topoisomerase 1 inhibitor (79)].

More recently, resistance to PI3K inhibitors was investigated in a mammary tumor model in which *PIK3CA* H1047R, which encodes an oncogenic PI3K variant commonly found in many epithelial tumors, was rendered under the control of a doxycycline-inducible promoter (80). Induction of mutant PIK3CA caused mammary tumor formation, as expected; however, many of these tumors failed to regress on removal of doxycycline or after treatment with PI3K inhibitors. Thus, mutant PI3K was required for the genesis but not the maintenance of some tumors; in other words, they exhibited *de novo* resistance to PI3K inhibition. Detailed genetic and molecular studies revealed that some of these tumors had acquired *MYC* amplification, whereas others had acquired MET upregulation. These studies may inform ongoing clinical trials of PI3K inhibitors. Chronic treatment studies of genetically engineered mouse model of *EGFR*-mutant NSCLC have also been performed with erlotinib (81). The drug-resistant tumors develop both $EGFR^{T790M}$ and *MET* amplification, drug resistance mechanisms also found in patients with cancer (27, 28, 49). These examples illustrate the power of endogenous tumor models to uncover pivotal insights into drug resistance mechanisms. In the future, the use of patient-derived xenografts for preclinical studies of drug resistance may emerge as a powerful murine counterpart to the genetically engineered models described previously.

Genomic and Molecular Studies of Drug-Resistant Clinical Tumors

As noted previously, the gold standard for any resistance study is confirmation that a given mechanism is relevant to patients with cancer. Thus, the characterization of human tumors that relapse after exposure to anticancer agents has become an area of intense research activity. Toward this end, 2 overarching avenues may be pursued: candidate driven studies, in which a specific gene or mechanism identified *in vitro* is queried in human tumors, or unbiased studies, in which omic or related large-scale technologies are applied to these samples. Ideally, these investigations should use tumor specimens that are both patient-matched and lesion-matched, because rather subtle changes in gene or protein expression may need to be measured. Regarding the latter, frozen material is preferable to archival tissue, particularly when phospho-protein studies are interrogated. Indeed, measurement of protein phosphorylation often represents

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a crucial component of clinical resistance studies; such assays are usually required to determine if the relevant effector pathway has become constitutively activated despite drug treatment.

Thus far, relatively few whole genome/exome or transcriptome studies of pre- and postrelapse tumors have been published. A targeted massively parallel sequencing study focusing on approximately 140 known cancer genes identified a somatic *MEK1* mutation in a BRAF-mutant melanoma patient who relapsed after a dramatic response to vemurafenib treatment (60). This mutation was evident in the postrelapse sample but not in the pretreatment counterpart. Going forward, it will undoubtedly be of great interest to perform both whole exome and transcriptome sequencing using pretreatment, postrelapse, and matched normal DNA from many patients treated with targeted anticancer therapeutics. However, these discovery-oriented efforts must be coupled with detailed functional follow-up studies and/or parallel preclinical efforts to validate the resistance mechanisms operant in each case—paying special attention to issues of necessity, sufficiency, and effects of such perturbations on the index tumor dependency and downstream pathway. This aspect could pose a formidable challenge if multiple resistance associated mutations and differentially regulated genes are identified in a given patient—an outcome that seems likely when genome-scale technologies are applied. In the future, an intersection of omic results from clinical specimens with validated resistance genes identified through systematic functional studies performed *in vitro* (as described previously) may prove fruitful in designating high-priority resistance effectors operant in patients with cancer.

TOWARD THE DEVELOPMENT OF NEW THERAPEUTIC REGIMENS TO OVERCOME TUMOR RESISTANCE

It is expected that the elucidation of *de novo* and acquired resistance mechanisms arising in the setting of "targetable" tumor dependencies will direct the development of rational therapeutic combinations (elaborated in the ensuing section). Here, the goal is to increase the magnitude and/or duration of clinical response when such combinations are administered as initial therapy or to achieve effective salvage therapy in the setting of relapse after the initial regimen.

Knowledge of the specific mutational mechanisms of drug resistance has been revealing in the identification and study of alternative kinase inhibitors. For example, dasatinib, which binds the active conformation of ABL, is effective against those imatinib-resistant mutations that lead to a protein conformational change (32, 76, 82). Clinical support for this observation emerged from a phase III randomized clinical trial of treatment-naïve patients with CML. Here, dasatinib was associated with a significantly greater rate of complete cytogenetic response and major molecular responses compared with imatinib (83).

An alternative strategy is to develop next-generation selective inhibitors of the target (onco)protein. For example, nilotinib, which also binds the inactive conformation of ABL, represents an advance over imatinib in that it retains activity against many imatinib resistance mutations that alter the conformation of ABL (76, 82). In addition, nilotinib is associated with a significantly greater rate of major molecular response compared with imatinib in treatment-naïve patients with chronic-phase CML (84). Analogous findings have been observed in patients with GIST treated with imatinib or sunitinib (39, 85).

Mutations affecting the gatekeeper residue (e.g., T315I), a drug contact point, results in a high degree of resistance both *in vitro* and clinically to imatinib, dasatinib, and nilotinib (32, 82). Structural insights into this resistance mutation have aided in the design of ABL kinase inhibitors in which the T315I mutation does not lead to a steric hindrance. These include AP24534 and HG-7-85-01, which are effective against CML models harboring T315I and are currently undergoing clinical development (86, 87). Irreversible EGFR inhibitors, which covalently bind Cys 797 of EGFR, are effective in preclinical models harboring the gatekeeper T790M mutation (27, 88, 89). The irreversible nature of drug binding allows for greater occupancy at the ATP-binding site, thus overcoming increased ATP affinity imparted by the T790M mutation (36). Clinically, covalent EGFR inhibitors have not been particularly effective against gefitinib/erlotinib-resistant tumors harboring the $EGFR^{T790M}$ mutation (90). This is likely the result of their lack of potency against T790M and concurrent inhibition of wild-type EGFR, which limits the ability to achieve sufficient concentrations to effectively inhibit $EGFR^{T790M}$. Mutant selective EGFR inhibitors that are effective against T790M mutation have recently been identified and may be able to overcome the limitations of the current-generation inhibitors (91).

The recognition that drug resistance mutations across different kinases lead to similar effects (e.g., promoting active conformation) or occur at a shared amino acid residue (e.g., gatekeeper mutations) may be useful in the design and selection of future kinase inhibitors. Thus, during drug screening and lead optimization, the prioritization and development of potent kinase inhibitors that bind the active conformation and/or those least affected by the presence of a gatekeeper mutation should be considered. Whether such agents are clinically more effective (and/or more toxic) or lead to longer durations of treatment will need to be determined through clinical trials.

Resistance that results from activation of a parallel signaling pathway requires a combination therapeutic approach rather than an alternative kinase inhibitor like those resulting from a secondary mutation. The EGFR inhibitor gefitinib in combination with a MET inhibitor effectively overcomes resistance mediated by MET signaling in EGFR-mutant cancers both *in vitro* and *in vivo* (49, 51). Similarly, the combination of PDGFR or IGF-IR inhibitors with MEK inhibitors or the combination of MEK and BRAF inhibitors may be an effective clinical treatment for patients who develop acquired resistance BRAF inhibitors (55, 56). For several bypass and upstream effector mechanisms described for resistance to BRAF-mutant melanoma, the combination of BRAF and MEK inhibition is an effective strategy to treat and/or to prevent the emergence of these different forms of drug resistance (47, 54). Clinical trials evaluating the combination of MEK and BRAF inhibitors are currently underway. Clinical development of combinations of kinase inhibitors will however likely encounter challenges. Because the clinical dose

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of many kinase inhibitors is determined based on toxicity (maximum tolerated dose), rather than target inhibition, the combination of 2 or 3 inhibitors is likely to result in enhanced toxicity that may limit clinical development. For example, the recommended phase II doses of the combination of the multikinase inhibitor XL184 and erlotinib were 125 mg and 50 mg, respectively (92). Both are below the single-agent doses for XL184 and erlotinib. Clinical trials and trial designs will thus also need to evolve to effectively treat drug-resistant cancers.

It is likely that a combination of multiple targeted therapies, analogous to the clinical success of combination chemotherapy in diseases such as lymphoma, will be necessary to effectively prevent and/or treat drug-resistant cancers. The theoretical number of therapeutic combinations is vast; thus, new preclinical paradigms are needed to prioritize high-yield combinations and define the genetic or molecular contexts in which they would most likely be efficacious. Such efforts will require expanded collaboration between academia and industry so that the appropriate resources and innovation may be brought to bear on this challenge. Concomitantly, the types of experimental approaches described previously will need refinement and validation with respect to the avenues most likely to be predictive of clinical relevance. The path to durable control of many cancer subtypes will likely be traveled by dedicated, multidisciplinary teams of preclinical and clinical experts that are resourced to both conduct and interpret the ensuing clinical trials with rigorous translational and analytical science. Ultimately, the knowledge of cancer drug resistance gained through such efforts holds considerable promise to improve the lives of many patients with cancer.

Disclosure of Potential Conflicts of Interest

L.A. Garraway: research support and consultant/advisory role (Novartis); ownership interest (Foundation Medicine). P.A. Janne: ownership interest (Gatekeeper Pharmaceuticals); consultant/advisory role (Roche; Genentech; AstraZeneca; Pfizer; Boehringer Ingelheim; Abbot Molecular); royalties (Dana-Farber Cancer Institute–owned intellectual property on EGFR mutations licensed to LabCorp).

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REFERENCES

1. Brockman RW. Mechanisms of resistance to anticancer agents. Adv Cancer Res 1963;7:129–234.
2. Blade J, Feliu E, Rozman C, Estape J, Milla A, Montserrat E. Cross-resistance to alkylating agents in multiple myeloma. Cancer 1983;52:786–9.
3. Shoemaker RH, Curt GA, Carney DN. Evidence for multidrug-resistant cells in human tumor cell populations. Cancer Treat Rep 1983;67:883–8.
4. Kartner N, Riordan JR, Ling V. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. Science 1983;221:1285–8.
5. Kartner N, Shales M, Riordan JR, Ling V. Daunorubicin-resistant Chinese hamster ovary cells expressing multidrug resistance and a cell-surface P-glycoprotein. Cancer Res 1983;43:4413–9.
6. Gottesman MM, Ling V. The molecular basis of multidrug resistance in cancer: the early years of P-glycoprotein research. FEBS Lett 2006;580:998–1009.
7. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002;2:48–58.
8. Belpomme D, Gauthier S, Pujade-Lauraine E, Facchini T, Goudier MJ, Krakowski I, et al. Verapamil increases the survival of patients with anthracycline-resistant metastatic breast carcinoma. Ann Oncol 2000;11:1471–6.
9. Warner E, Hedley D, Andrulis I, Myers R, Trudeau M, Warr D, et al. Phase II study of dexverapamil plus anthracycline in patients with metastatic breast cancer who have progressed on the same anthracycline regimen. Clin Cancer Res 1998;4:1451–7.
10. Rowinsky EK, Adjei A, Donehower RC, Gore SD, Jones RJ, Burke PJ, et al. Phase I and pharmacodynamic study of the topoisomerase I-inhibitor topotecan in patients with refractory acute leukemia. J Clin Oncol 1994;12:2193–203.
11. Wishart GC, Bissett D, Paul J, Jodrell D, Harnett A, Habeshaw T, et al. Quinidine as a resistance modulator of epirubicin in advanced breast cancer: mature results of a placebo-controlled randomized trial. J Clin Oncol 1994;12:1771–7.
12. Tsimberidou A, Estey E, Cortes J, Thomas D, Faderl S, Verstovsek S, et al.Gemtuzumab, fludarabine, cytarabine, and cyclosporine in patients with newly diagnosed acute myelogenous leukemia or high-risk myelodysplastic syndromes. Cancer 2003;97:1481–7.
13. List AF, Kopecky KJ, Willman CL, Head DR, Persons DL, Slovak ML, et al. Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia: a Southwest Oncology Group study. Blood 2001;98:3212–20.
14. Gruber A, Bjorkholm M, Brinch L, Evensen S, Gustavsson B, Hedenus M, et al. A phase I/II study of the MDR modulator Valspodar (PSC 833) combined with daunorubicin and cytarabine in patients with relapsed and primary refractory acute myeloid leukemia. Leuk Res 2003;27:323–8.
15. Garraway LA, Chabner B. MDR1 inhibition: less resistance or less relevance? Eur J Cancer 2002;38:2337–40.
16. Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heal of cancer. Science 2002;297:63–4.
17. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell 2009;136:823–37.
18. Garraway LA, Sellers WR. Lineage dependency and lineage-survival oncogenes in human cancer. Nat Rev Cancer 2006;6:593–602.
19. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001;344:1038–42.
20. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347:472–80.
21. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010;363:809–19.
22. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693–703.
23. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.

Published OnlineFirst February 28, 2012; DOI: 10.1158/2159-8290.CD-12-0012

24. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, et al. Dynamics of chronic myeloid leukaemia. Nature 2005;435:1267–70.
25. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364:2507–16.
26. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876–80.
27. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786–92.
28. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:1–11.
29. Sasaki T, Okuda K, Zheng W, Butrynski J, Capelletti M, Wang L, et al. The neuroblastoma-associated F1174L ALK mutation causes resistance to an ALK kinase inhibitor in ALK-translocated cancers. Cancer Res 2010;70:10038–43.
30. Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734–9.
31. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2002;2:117–25.
32. Sherbenou DW, Druker BJ. Applying the discovery of the Philadelphia chromosome. J Clin Invest 2007;117:2067–74.
33. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer 2009;9:28–39.
34. Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 2006;24:4764–74.
35. Gorre ME, Ellwood-Yen K, Chiosis G, Rosen N, Sawyers CL. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. Blood 2002;100:3041–4.
36. Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 2008;105:2070–5.
37. Heinrich MC, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, et al. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. J Clin Oncol 2008;26:5352–9.
38. Guo T, Hajdu M, Agaram NP, Shinoda H, Veach D, Clarkson BD, et al. Mechanisms of sunitinib resistance in gastrointestinal stromal tumors harboring KITAY502-3ins mutation: an in vitro mutagenesis screen for drug resistance. Clin Cancer Res 2009;15:6862–70.
39. Gajiwala KS, Wu JC, Christensen J, Deshmukh GD, Diehl W, DiNitto JP, et al. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. Proc Natl Acad Sci U S A 2009;106:1542–7.
40. Bresler SC, Wood AC, Haglund EA, Courtright J, Belcastro LT, Plegaria JS, et al. Differential inhibitor sensitivity of anaplastic lymphoma kinase variants found in neuroblastoma. Sci Transl Med 2011;3:108ra14.
41. Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ, et al. MEK1 mutations confer resistance to MEK and B-RAF inhibition. Proc Natl Acad Sci U S A 2009;106:20411–6.
42. Rine J, Hansen W, Hardeman E, Davis RW. Targeted selection of recombinant clones through gene dosage effects. Proc Natl Acad Sci U S A 1983;80:6750–4.
43. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, et al. Mefloquine resistance in *Plasmodium falciparum* and increased PFMDR1 gene copy number. Lancet 2004;364:438–47.
44. Ercan D, Zejnullahu K, Yonesaka K, Xiao Y, Capelletti M, Rogers A, et al. Amplification of EGFR T790M causes resistance to an irreversible EGFR inhibitor. Oncogene 2010;29:2346–56.
45. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. Sci Signal 2010;3:ra84.
46. Little AS, Balmanno K, Sale MJ, Newman S, Dry JR, Hampson M, et al. Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK1/2 inhibitors in colorectal cancer cells. Sci Signal 2011;4:ra17.
47. Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature 2011;480:387–90.
48. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res 2008;14:2895–9.
49. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.
50. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932–7.
51. Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 2010;17:77–88.
52. Yano S, Wang W, Li Q, Matsumoto K, Sakurama H, Nakamura T, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res 2008;68:9479–87.
53. Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest 2008;118:2609–19.
54. Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA, et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature 2010;468:968–72.
55. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, et al Melanomas acquire resistance to B-RAF (V600E) inhibition by RTK or N-RAS upregulation. Nature 2010;468:973–7.
56. Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK, et al Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell 2010;18:683–95.
57. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell 2011;19:58–71.
58. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 2006;66:1500–8.
59. Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, et al. Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res 2009;69:565–72.
60. Wagle N, Emery C, Berger MF, Davis MJ, Sawyer A, Pochanard P, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. J Clin Oncol 2011;29:3085–96.
61. Bivona TG, Hieronymus H, Parker J, Chang K, Taron M, Rosell R, et al. FAS and NF-kappaB signalling modulate dependence of lung cancers on mutant EGFR. Nature 2011;471:523–6.
62. Engelman JA, Mukohara T, Zejnullahu K, Lifshits E, Borras AM, Gale CM, Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. J Clin Invest 2006;116:2695–706.
63. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.

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64. Suda K, Tomizawa K, Fujii M, Murakami H, Osada H, Maehara Y, et al. Epithelial to mesenchymal transition in an epidermal growth factor receptor-mutant lung cancer cell line with acquired resistance to erlotinib. J Thorac Oncol 2011;6:1152–61.
65. Yao Z, Fenoglio S, Gao DC, Camiolo M, Stiles B, Lindsted T, et al. TGF-beta IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer. Proc Natl Acad Sci U S A 2010;107:15535–40.
66. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 2010;29:4741–51.
67. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141:69–80.
68. McMillin DW, Delmore J, Negri J, Ooi M, Klippel S, Miduturu CV, et al. Microenvironmental influence on pre-clinical activity of polo-like kinase inhibition in multiple myeloma: implications for clinical translation. PLoS One 2011;6:e20226.
69. Manshouri T, Estrov Z, Quintas-Cardama A, Burger J, Zhang Y, Livun A, et al. Bone marrow stroma-secreted cytokines protect JAK2(V617F)-mutated cells from the effects of a JAK2 inhibitor. Cancer Res 2011;71:3831–40.
70. Wang W, Li Q, Yamada T, Matsumoto K, Matsumoto I, Oda M, et al. Crosstalk to stromal fibroblasts induces resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors. Clin Cancer Res 2009;15:6630–8.
71. Viloria-Petit A, Crombet T, Jothy S, Hicklin D, Bohlen P, Schlaeppi JM, et al. Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies in vivo: a role for altered tumor angiogenesis. Cancer Res 2001;61:5090–101.
72. Naumov GN, Nilsson MB, Cascone T, Briggs A, Straume O, Akslen LA, et al. Combined vascular endothelial growth factor receptor and epidermal growth factor receptor (EGFR) blockade inhibits tumor growth in xenograft models of EGFR inhibitor resistance. Clin Cancer Res 2009;15:3484–94.
73. Ogino A, Kitao H, Hirano S, Uchida A, Ishiai M, Kozuki T, et al. Emergence of epidermal growth factor receptor T790M mutation during chronic exposure to gefitinib in a non small cell lung cancer cell line. Cancer Res 2007;67:7807–14.
74. Katayama R, Khan TM, Benes C, Lifshits E, Ebi H, Rivera VM, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proc Natl Acad Sci U S A 2011;108:7535–40.
75. Azam M, Latek RR, Daley GQ. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. Cell 2003;112:831–43.
76. Bradeen HA, Eide CA, O'Hare T, Johnson KJ, Willis SG, Lee FY, et al. Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. Blood 2006;108:2332–8.
77. Iorns E, Turner NC, Elliott R, Syed N, Garrone O, Gasco M, et al. Identification of CDK10 as an important determinant of resistance to endocrine therapy for breast cancer. Cancer Cell 2008;13:91–104.
78. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007;12:395–402.
79. Burgess DJ, Doles J, Zender L, Xue W, Ma B, McCombie WR, et al. Topoisomerase levels determine chemotherapy response in vitro and in vivo. Proc Natl Acad Sci U S A 2008;105:9053–8.
80. Liu P, Cheng H, Santiago S, Raeder M, Zhang F, Isabella A, et al. Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. Nat Med 2011;17:1116–20.
81. Politi K, Fan PD, Shen R, Zakowski M, Varmus H. Erlotinib resistance in mouse models of epidermal growth factor receptor-induced lung adenocarcinoma. Dis Model Mech 2010;3:111–9.
82. O'Hare T, Eide CA, Deininger MW. Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. Blood 2007;110:2242–9.
83. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2010;362:2260–70.
84. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med 2010;362:2251–9.
85. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006;368:1329–38.
86. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell 2009;16:401–12.
87. Weisberg E, Choi HG, Ray A, Barrett R, Zhang J, Sim T, et al. Discovery of a small-molecule type II inhibitor of wild-type and gatekeeper mutants of BCR-ABL, PDGFRalpha, Kit, and Src kinases: novel type II inhibitor of gatekeeper mutants. Blood 2010;115:4206–16.
88. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 2008;27:4702–11.
89. Engelman JA, Zejnullahu K, Gale CM, Lifshits E, Gonzales AJ, Shimamura T, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. Cancer Res 2007;67:11924–32.
90. Miller VA, Hirsh V, Cadranel J, Chen Y-M, Park K, Kim S-W, et al. Phase IIB/III double-blind randomized trial of afatinib (BIBW2992, an irreversible inhibitor of EGFR/HER1 and HER2) + best supportive care (BSC) versus placebo + BSC in patients with NSCLC failing 1–2 lines of chemotherapy and erlotinib or gefitinib (LUX-LUNG 1). Ann Oncol 2010;21:LBA1.
91. Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. Nature 2009;462:1070–4.
92. Wakelee HA, Gettinger SN, Engelman JA, Janne PA, West HJ, Subramaniam DS, et al. A phase Ib/II study of XL184 (BMS 907351) with and without erlotinib (E) in patients (pts) with non-small cell lung cancer (NSCLC). J Clin Oncol 2010;28:Abstract 3017.

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