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The pharmacogenomics of drug resistance to protein kinase inhibitors

Nancy K. Gillis[1,2] and **Howard L. McLeod**[2,3]

[1]Eshelman School of Pharmacy, Center for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill, NC

[2]H. Lee Moffitt Cancer Center and Research Institute, DeBartolo Family Personalized Medicine Institute, Tampa, FL

[3]Xiangya Hospital, Central South University, Changsha, China

Abstract

Dysregulation of growth factor cell signaling is a major driver of most human cancers. This has led to development of numerous drugs targeting protein kinases, with demonstrated efficacy in the treatment of a wide spectrum of cancers. Despite their high initial response rates and survival benefits, the majority of patients eventually develop resistance to these targeted therapies. This review article discusses examples of established mechanisms of drug resistance to anticancer therapies, including drug target mutations or gene amplifications, emergence of alternate signaling pathways, and pharmacokinetic variation. This reveals a role for pharmacogenomic analysis to identify and monitor for resistance, with possible therapeutic strategies to combat chemoresistance.

Keywords

pharmacogenetics; pharmacogenomics; cancer; resistance; somatic mutations

Introduction

Cancer is a genetic disease that arises primarily from the accumulation of genetic changes in genes regulating cellular growth, proliferation, and survival. Gain of function alterations inducing hyperactivity of oncogenes or loss of function alterations leading to inactivation of tumor suppressor genes cause deregulation of cellular signaling, a fundamental trait of cancer cells. In healthy cells, homeostasis is conveyed via growth factors binding to cell surface receptors, primarily protein kinases, which then activate intracellular signaling pathways and regulate cell cycle progression (Hanahan and Weinberg, 2011). Deregulation

Corresponding Author: Howard L. McLeod, Pharm.D., H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, MRC-CANCONT, Tampa, FL 33612, Phone: 813-745-8435, Fax: 813-745-6525, Howard.McLeod@moffitt.org.

of these signals results in uncontrollable cellular proliferation, metabolism, survival and, ultimately, cancer. Somatic (acquired, or tumor) mutations lead to constitutive activation of these signaling pathways. For example, mutations in the B-raf proto-oncogene, *BRAF*, a serine/threonine kinase, cause constitutive signaling through the mitogen-activated protein kinase (MAPK) pathway and are commonly observed in melanoma, colorectal (CRC), and papillary thyroid cancers (Araya et al., 2016). Identification of the genes and pathways deregulated in cancer, such as *BRAF*, has led to a rapid increase in the design and approval of therapies targeting these genetic drivers of oncogenesis. Targeted anti-cancer therapies function by interfering with specific molecular alterations that regulate cellular signaling and drive tumor growth. By binding to or inhibiting a known molecular driver, targeted therapies interrupt the signaling pathways, causing cellular deregulation and leading to cancer cell apoptosis or cell death. For example, vemurafenib selectively inhibits *BRAF V600*-mutated cancer cells, abrogating MAPK-mediated signaling, preventing proliferation of *BRAF*-mutated cells, and ultimately resulting in apoptosis (Tsai et al., 2008).

To optimize the use of targeted therapies, the genetic alterations causing pathway deregulation in each patient's tumor must be identified. This is the modern concept of personalized cancer medicine (or precision medicine). Pharmacogenomics is the study of how genetic variations influence the response of an individual to drugs. In the context of cancer, there are two genomes relevant to predicting drug response or resistance: (1) germline, or inherited, genetics may affect drug exposure, potentially causing variability in efficacy and/or toxicity, and (2) somatic, or tumor, genetics are the acquired alterations that may initiate and perpetuate cellular deregulation. Generally, it is the somatic germline that is interrogated to identify alterations driving oncogenesis and to select targeted therapies. It should be emphasized that drug resistance phenomena continue to be a primary hindrance to curative chemotherapy of solid tumors and hematologic malignancies (Fletcher et al., 2016; Niewerth et al., 2015; Wicki et al., 2016). Hence, deciphering the molecular mechanisms underlying chemoresistance should enhance targeted individualized cancer medicine (Assaraf et al., 2014; Livney and Assaraf, 2013; Swanton et al., 2016).

Due to their critical role in regulating cellular signaling, > 20 protein kinase inhibitors (PKIs) have been developed and approved across a wide range of tumor types. Despite their overall high response rates, many patients for whom the drugs are indicated will not have any evidence of disease control, while others will have transient benefit followed by tumor growth. This lack of complete and durable responses is indicative of drug resistance phenomena. If the correct alteration driving tumor growth is not identified from the outset, intrinsic resistance may be observed. When the tumor cells evolve to overcome targeted inhibition, the patient develops acquired resistance and stops responding to therapies that were previously effective. In this review, we will elaborate on the various classifications of cancer drug resistance, provide examples of how pharmacogenomics plays a role in resistance to PKIs, and discuss possible therapeutic strategies to overcome cancer drug resistance.

Classifications of drug resistance mechanisms important in cancer

Drug resistance can be defined as the lack of therapeutic benefit or response to a medication. In the cancer setting, drug resistance is apparent with an increase in tumor size or metastasis (i.e. disease progression). Various mechanisms of chemoresistance can result in lack of complete or durable response to cancer therapies (Fig. 1). An overview of the common classifications is provided below. It is important to note that a single pharmacogenomic biomarker may represent multiple mechanisms of drug resistance.

Pharmacological vs. biological resistance

Pharmacological resistance reflects inadequate drug exposure at the drug target, and can be caused by environmental factors (e.g., drug-drug interactions, non-compliance), germline pharmacogenomics (i.e., inter-individual variability in drug metabolism or pharmacokinetics) (Fig. 1A & 1C) as well as drug sequestration away from its target (Goler-Baron and Assaraf, 2011; Zhitomirsky and Assaraf, 2016). For example, addition of an antacid to alleviate gastroesophageal reflux caused by some PKI therapies will affect gastric absorption and exposure to the PKI, thereby leading to pharmacological resistance (Budha et al., 2012). Additionally, the observation that PKI-sensitive clones reemerge post-PKI discontinuation demonstrates the cytostatic, rather than cytotoxic, nature of some targeted therapies (Browning et al., 2013; Sequist et al., 2011). Therefore, inconsistent suppression of the drug target due to missed doses may lead to upregulation of the cancer-driving pathways and, ultimately, cancer progression. Biological resistance results from cancer cell evolution in the presence of adequate drug exposure (Fletcher et al., 2016; Liu et al., 2016; Niewerth et al., 2015). In the context of cancer, biological resistance can arise from somatic alterations in drug targets or pathways (Fig. 1B & 1C). Examples include genetic alterations in the drug target itself, activation of alternative signaling pathways (bypass tracks), alteration in signaling proteins downstream of the drug target, or phenotypic switch. Most known mechanisms of resistance to targeted cancer therapies are of the biological resistance subtype; hence, these will be the primary focus of this article.

Intrinsic vs. acquired resistance

Cancer drug resistance can also be classified based on timing during the course of treatment. Intrinsic resistance (also referred to as innate, inherent, or primary resistance) is the absence of discernible, even transitory beneficial effect from a medication. From the outset, there is neither cessation in tumor growth nor increase in survival benefits (Fig. 1D). Evidence of intrinsic resistance can be visualized in a waterfall plot, in which some patients fail to ever meet Response Evaluation Criteria in Solid Tumors (RECIST). For example, approximately 20% of *BRAF V600*-mutated melanoma patients do not respond to BRAF inhibitors (Hauschild et al., 2012), demonstrating intrinsic drug resistance. Mechanisms of intrinsic resistance can include germline or somatic alterations. Intrinsic resistance is a major challenge in cancer therapy, and it is important to elucidate the mechanisms conferring lack of therapeutic benefit. However, these mechanisms remain less well understood at this time.

Acquired or secondary resistance is the progression of disease after an initial benefit. In oncology, the tumor initially shrinks (responds) but eventually begins to increase in size

(Fig. 1E). While intrinsic drug resistance can be due to germline or somatic mutations, acquired resistance is most commonly attributed to somatic mutations (an exception would be a new drug-drug interaction due to changes in therapy). Mechanisms that result in acquired drug resistance include genetic alterations in the drug target, activation of bypass tracks, alteration in downstream signaling proteins, phenotypic switch, drug efflux, drug catabolism as well as drug compartmentalization (Camidge et al., 2014; Gridelli et al., 2014; Liu et al., 2016).

Germline pharmacogenomics as a mechanism of pharmacological resistance

Germline pharmacogenomics can affect one's inherent response to a medication or therapy. In the context of cancer, germline pharmacogenomics has most widely been associated with risk of developing adverse effects, rather than drug resistance (Hertz and Rae, 2015). Since adverse effects generally result from off-target (i.e., non-tumor) effects, it is logical that genetic variation in the germ cells throughout the body would confer risk to adverse events. However, due to the inherited nature of germline genetics, they may also play a role in one's initial response to therapy. In fact, there are a few well-studied examples of how germline variation can confer intrinsic resistance to anticancer medications (Table 1).

Germline variation in *TPMT* as a pharmacogenomics predictor of response and adverse events

Germline variation in *TPMT*, the gene that encodes the thiopurine S-methyltransferase (TPMT) enzyme, is known to affect response to thiopurine drugs. Chemotherapeutic thiopurines include 6-mercaptopurine and 6-thioguanine, which are used in the treatment of pediatric and adult acute lymphocytic leukemia (ALL) (azathioprine is a prodrug of 6-mercaptopurine used in non-malignant conditions). Thiopurine drugs are inactive prodrugs that are bioactivated and metabolized via competing routes: (1) xanthine oxidase converts 6-mercaptopurine to an inactive metabolite, 6-thiouric acid; (2) hypoxanthine phosphoribosyltransferase (HPRT) converts thiopurines to activated nucleotide analogues, which can be incorporated into DNA or RNA, hence interfering with replication and transcription, resulting in cytotoxicity; and (3) TPMT inactivates thiopurines through methylation. The nucleotide analogues formed by HPRT are responsible for the efficacy and toxicity of thiopurine drugs, and insufficient TPMT activity results in upregulation of HPRT-mediated metabolism, conferring response. Patients with low TPMT activity have greater exposure to activated thioguanine nucleotides, resulting in the potential for greater efficacy, but also increased risk of severe toxicity (Lennard et al., 1997). This variation in activated nucleotide exposure is well-established as a predictor of treatment outcomes. Patients with *TPMT* loss-of-function variants have significantly lower rates of minimal residual disease positivity after receiving 6-mercaptopurine therapy when compared to wild type *TPMT* individuals (Stanulla et al., 2005). Increased risk of relapse has also been associated with wild type *TPMT* in children receiving 6-mercaptopurine, likely due to insufficient thioguanine nucleotide exposure, thus conferring a type of pharmacological resistance (Schmiegelow et al., 2009). Due to increased toxicity risk, decreased dosing for patients with *TPMT* variants has been recommended (Relling et al., 2011). However, it is unclear

how this may affect relapse rates (Levinsen et al., 2014; Relling et al., 2006). A recent study reported that patients with 6-mercaptopurine non-adherence were at a 2.7-fold increased risk of relapse when compared to patients with a mean drug adherence rate of 95% or greater ($p = 0.01$), further emphasizing the importance of continuous drug exposure and adherence as a means to avoid development of drug resistance phenomena (Bhatia et al., 2015).

Germline alterations in BIM as a predictor of intrinsic pharmacological resistance

A common variant in *BCL2L11* (also known as *BIM*), the gene that encodes the BCL2-like 11 protein, has been associated with intrinsic resistance to PKIs. *BIM* is a member of the B-cell CLL/lymphoma 2 (Bcl-2) family of genes and encodes a Bcl-2 homology domain 3 (BH3). BH3 activates cell death by either opposing the pro-survival members of the Bcl-2 family or by binding to the pro-apoptotic Bcl-2 family members and causing activation of their pro-apoptotic functions (Youle and Strasser, 2008). PKIs induce upregulation and stabilization of BIM through inhibition of the MAPK pathway, therefore, the activity of BIM is required for PKIs to induce apoptosis in kinase-driven cancers (Gong et al., 2007). Recently, a 2,903 bp germline deletion polymorphism in intron 2 of *BIM* was identified, which was associated with inferior responses to PKIs (i.e., imatinib, gefitinib, erlotinib, and afatinib) in chronic myeloid leukemia (CML), non-small cell lung cancer (NSCLC), and pediatric ALL patients (Lee et al., 2014; Ng et al., 2012; Soh et al., 2014). Functionally, this mutation results in alternative RNA splicing, leading to decreased production of BIM isoforms containing the essential BH3 domain.

Since its discovery, conflicting evidence of the ability of *BIM* variation to predict intrinsic resistance to PKIs has been documented (Chen et al., 2014; Cheng and Sawyers, 2012; Isobe et al., 2014). Two retrospective studies failed to observe an association between *BIM* genotype and response rates to PKIs in NSCLC patients (Lee et al., 2013; Lee et al., 2015a). However, a systematic review and meta-analysis of 951 patients supported the *BIM* deletion polymorphism as a predictor of shorter progression free survival (PFS) in NSCLC patients who were treated with PKIs (adjusted HR = 2.38, $p < 0.001$) (Nie et al., 2015). Another meta-analysis found that the *BIM* deletion polymorphism was associated with response rates (HR = 0.44, 95% CI = 0.27–0.7) and PFS (HR = 2.19, 95% CI = 1.7–2.8) in NSCLC, but not in CML (Ying et al., 2015). Further evidence indicating a lack of benefit or increased risk of harm in individuals carrying *BIM* deletions must be generated before this biomarker of intrinsic resistance can reasonably be implemented in clinical practice.

Methods to overcome BIM-related PKI resistance are already being explored. A preclinical study in NSCLC cell lines and xenograft models indicated that cells harboring the common *BIM* deletion had enhanced response to gefitinib when treated in combination with a histone deacetylase inhibitor, vorinostat (Nakagawa et al., 2013). Vorinostat functioned by increasing expression of BH3 in a dose-dependent manner, thus restoring sensitivity to tyrosine kinase inhibition. These findings further support the importance of *BIM* expression in PKI response and provide evidence to suggest that combination therapeutics may be a potential strategy to overcome this form of resistance.

Additional germline pharmacogenomic markers as predictors of drug resistance

One potential mechanism that can confer pharmacological resistance is decreased exposure at the drug target, which can result from drug-drug interactions or inter-individual genetic variability (Fig. 1A). There are a few well-established examples of germline genetics affecting exposure to anticancer therapies [reviewed in (Hertz and Rae, 2015)]. While outside the scope of this review, the importance of an established link between active drug exposure levels and clinical outcomes or adverse events must be noted. Drug exposure is predicted to affect drug efficacy or toxicity. However, discrete evidence must exist before clinical implementation is warranted (Gillis and Innocenti, 2014).

Somatic pharmacogenomics as a mechanism of drug resistance

Somatic mutations result in upregulation of oncogenic pathways, and their effects can be inhibited with the use of targeted therapies. Since 2003, over 20 PKIs have been approved to target various somatic alterations across a broad range of cancer types (including hematologic and solid malignancies), and more than 20 additional PKIs are currently in clinical trials (CenterWatch, 2016). Because these drugs target protein kinases, somatic alterations in the targets or pathways may confer resistance or response (Table 1). Some well-established examples of somatic genetic drivers of resistance to PKIs are discussed below.

RAS status as a predictor of intrinsic resistance to EGFR inhibition in CRC

The epidermal growth factor receptor (EGFR or ErbB-1) is a transmembrane protein kinase that binds epidermal growth factor, inducing dimerization and autophosporylation, which signals downstream pathways (i.e., MAPK and PI3K/Akt) that mediate cellular proliferation and survival (Fig.2). The EGFR signaling pathway plays a pivotal role in tumor growth and progression in many cancer types, including glioblastoma, NSCLC, head and neck cancers, and CRC. EGFR is overexpressed in approximately 50% of CRC patients, and is associated with disease progression and poor prognosis (Siena et al., 2009). Anti-EGFR monoclonal antibodies, such as cetuximab and panitumumab, were hypothesized to be effective in colorectal tumors over-expressing EGFR, and were initially U.S. Food and Drug Administration (FDA) approved for that indication. However, as monotherapy, the response rates to cetuximab and panitumumab were only 10% and 30%, respectively, indicating potential intrinsic resistance (Jonker et al., 2007; Van Cutsem et al., 2007). Retrospective analysis of phase III clinical study data revealed differential response to anti-EGFR monoclonal antibodies dependent on Kirsten rat sarcoma viral oncogene (*KRAS*) homolog (Amado et al., 2008). When stratified by *KRAS* mutation status, response rate to panitumumab in wild type individuals was 17%, whereas 0% of individuals with mutant *KRAS* responded. Similarly, *KRAS* mutations were associated with resistance and decreased overall survival (OS) in patients receiving cetuximab (Lievre et al., 2006). These observations provided evidence for *KRAS* as a biomarker of intrinsic resistance to EGFR-targeted monoclonal antibodies.

KRAS is a member of the rat sarcoma virus (*RAS*) family of oncogenes, which also includes *HRAS* (Harvey rat sarcoma viral oncogene homolog) and *NRAS* (neuroblastoma RAS viral

oncogene homolog). Mutations in *RAS* genes lead to the constitutive activation of the MAPK pathway independent of *EGFR* status. Interestingly, in CRC, mutations in *KRAS* are significant enough to negate EGFR inhibition. A prospective-retrospective analysis of 1,183 patients who received either FOLFOX-panitumumab or FOLFOX alone revealed that mutation status in both *KRAS* and *NRAS* were predictive of response to panitumumab (HR for progression or death in *RAS* wild type 0.72, 95% CI 0.58–0.99, p= 0.0004) (Douillard et al., 2013). Recently, the American Society of Clinical Oncology released a provisional clinical opinion update recommending *NRAS* and *KRAS* mutation testing in CRC patients who are candidates for anti-EGFR monoclonal antibodies, supporting the significance of this biomarker as a predictor of intrinsic drug resistance (Allegra et al., 2016). Additional potential markers of intrinsic resistance have since been identified in models of *KRAS* wild-type patient xenografts, including *ERBB2* (*HER2*), *FGFR1*, *PDGFRA*, and *MAP2K1* (*MEK*); secondary mutations in *EGFR* at the site of cetuximab binding were identified in acquired resistance (Bertotti et al., 2015). Further studies demonstrating effects on patient outcomes are needed before clinical implementation of these novel biomarkers can be recommended.

EGFR status as a mediator of resistance to EGFR PKIs in NSCLC

Mutations in *EGFR* are one of the most common cancer drivers identified in NSCLC tumors. Approximately 15% of NSCLC patients in the U.S. will have an *EGFR* mutation, and the incidence is approximately 35% in patients of Asian descent (Shi et al., 2014b). In the U.S., *EGFR* mutations are most prevalent in females (17.9% vs. 8%, p= 0.002), non-smokers (42% vs. 6.6%, p < 0.001), and adenocarcinomas (15.6%) (Bauml et al., 2013). The mutations are typically located in exons 18 to 21 of *EGFR*, the region that encodes the catalytic tyrosine kinase domain. Approximately 90% of the mutations are short exon 19 deletions or the L858R point mutation in exon 21, which result in enhanced EGFR signaling (Sharma et al., 2007). Mutations in *EGFR* are predictive of response to EGFR inhibitors, such as erlotinib and gefitinib, in NSCLC. However, despite 70–80% response rates to EGFR PKIs, a majority of patients will acquire resistance after 10–12 months (Mitsudomi et al., 2010; Zhou et al., 2011). Additionally, absence of initial *EGFR* mutations confers lower overall response rates in NSCLC patients, a relative intrinsic resistance (Morgensztern et al., 2015; Yang et al., 2015).

Acquired resistance to EGFR inhibitors in NSCLC is complex and heterogeneous, but ultimately all mechanisms drive sustained signaling through downstream cancer pathways (e.g., MAPK or PI3K/Akt pathways). Known mechanisms of drug resistance include secondary genetic alterations in *EGFR*, upregulation of parallel signaling pathways, or phenotypic transformation (Fig. 2). The most common mechanism of acquired resistance in EGFR-positive NSCLCs is the single-nucleotide mutation T790M, which occurs in approximately 50–60% of acquired resistance cases (Sharma et al., 2007). Also known as the gatekeeper residue, substitution of bulky methionine at this position causes steric hindrance and prevents EGFR inhibitors from binding and eliciting their pharmacologic effect. Other *EGFR* mutations have been identified in patients with acquired resistance, but their frequencies are much lower (Fig. 3). Another common mechanism of acquired resistance to EGFR PKIs is amplification of the MET proto-oncogene (*MET*), a receptor

tyrosine kinase, which has been reported in up to 22% of resistant samples (Engelman et al., 2007). *MET* amplification drives resistance by inducing EGFR-independent phosphorylation of ERBB3 (HER3), which activates downstream PI3K/Akt signaling despite the presence of an EGFR inhibitor (Engelman et al., 2007). Amplifications of *ERBB2* (*HER2*) and *PIK3CA* have also been identified in patients with acquired resistance, similarly functioning to activate shared pathways independent of EGFR (Sequist et al., 2011; Yu et al., 2013). Phenotypic transformation as a mechanism of acquired resistance occurs when the histology of the tumor transitions to small cell lung cancer (SCLC) or from epithelial to mesenchymal (EMT) subtype, and occurs in approximately 14% and 5%, respectively (Sequist et al., 2011; Takegawa et al., 2016). Interestingly, transformed SCLCs retain the primary *EGFR* activating mutations, but do not carry T790M or *MET* amplification. Not much is known about the mechanism of histological transformation, but currently it is recommended that these patients receive standard SCLC treatments (Oser et al., 2015).

Numerous strategies are being investigated to overcome first generation EGFR inhibitor resistance due to secondary *EGFR* mutations or bypass track signaling. One strategy was to develop more potent inhibitors of EGFR. The FDA-approved second-generation EGFR inhibitor, afatinib, which irreversibly binds to and inhibits EGFR as well as HER2, HER3, and HER4. Despite its first-line indication and increased potency, afatinib has not demonstrated promise in the setting of acquired drug resistance. LUX-Lung 1, a phase 2b/3 trial of afatinib in patients who had progressed after treatment with an EGFR PKI, failed to meet its primary endpoint of OS (HR 1.08, 95% CI 0.86–1·35; p=0.74), and response rates were less than 10% (Miller et al., 2012). Demonstrating more promise than the second generation EGFR PKIs in combating acquired resistance are the third generation EGFR inhibitors: the recently approved osimertinib as well as rociletinib that is in clinical trials. Like the second-generation inhibitors, these are irreversible EGFR inhibitors. However, they have higher affinity for mutant *EGFR*, including T790M, than for wild type *EGFR*. In the pivotal phase 2 study of osimertinib in NSCLC patients who had progressed after treatment with a first generation PKI, response rates in T790M-positive patients were 61%. In a similar phase 1/2 study of rociletinib, patients with the T790M mutation had an objective response rate of 59% (Janne et al., 2015; Sequist et al., 2015).

Despite their therapeutic promise in acquired resistance to first generation EGFR PKIs, mechanisms of potential acquired resistance to third generation EGFR PKIs have already been reported. A study in patients who progressed on rociletinib reported that half of T790M-positive NCSLCs treated with rociletinib became T790-wild type at progression (Piotrowska et al., 2015). Loss of T790M was also observed in 27% patients who progressed on osimertinib, and 40% acquired a novel *EGFR* mutation, C797S, which is also resistant to rociletinib (Thress et al., 2015). A strategy that is being tested to overcome loss of T790M is combination therapy of an EGFR PKI with a monoclonal antibody that targets EGFR (e.g., NCT02496663). Recently, a preclinical study identified mutations and amplifications in *NRAS* and *KRAS* as mechanisms of acquired resistance to osimertinib (Eberlein et al., 2015). Due to their upstream signaling of MEK, alterations in *NRAS* and *KRAS* may confer response to MEK inhibitors. As such, clinical studies investigating combination therapy with third generation EGFR PKIs and MEK inhibitors are also underway (e.g., NCT02580708).

Therapeutic strategies targeting resistance mediated by upregulation of bypass signaling pathways are less established. Postulated strategies mainly consist of studies investigating dual inhibition of EGFR and known bypass tracks. In an ongoing phase 1b/2 clinical study of gefitinib plus a novel MET inhibitor (INC280) in patients who had progressed on a first-line EGFR PKI, partial responses were only observed in 6/41 (15%) individuals (Wu et al., 2014). A phase 1b study investigated concurrent inhibition of HER2 and EGFR with the combination of afatinib (dual HER2/EGFR inhibitor) and cetuximab in NSCLC patients who had progressed on gefitinib or erlotinib, and overall response rate (ORR) was 29%, and PFS was 4.7 months (Janjigian et al., 2014). A phase 2 clinical trial combining erlotinib with a PI3K inhibitor (BKM120) is currently ongoing in patients who acquired resistance to erlotinib (NCT01487265); the combination of afatinib and sirolimus is also being studied to overcome resistance due to mTOR (mechanistic target of rapamycin, a serine/threonine kinase) activation (NCT00993499), a downstream component of the PI3K/Akt pathway. These preliminary results suggest that dual inhibition of multiple signaling pathways may confer response in some individuals. However, an understanding of which individuals will benefit and durability of response is crucial.

Acquired resistance to ALK inhibitors

Anaplastic lymphoma kinase (*ALK*) is a receptor tyrosine kinase that is normally expressed in the nervous system and plays an important role in brain development. *ALK* rearrangements, mutations, and amplifications have been identified in numerous tumor types including anaplastic large cell lymphoma, neuroblastoma, and NSCLC. Chromosomal rearrangements resulting in gene fusions are the most common type of *ALK* alterations. Clinically, *ALK* alterations are known to be most actionable in NSCLC, where the most common rearrangement observed is echinoderm microtubule associated protein like 4 (*EML4)-ALK* (Soda et al., 2007). Multiple variants of *EML4-ALK* have been reported; however, they all encode the same cytoplasmic portion of *ALK*, but have different *EML4* truncations (Choi et al., 2008). Fusions of *ALK* with other genes have also been described (e.g., *KIF5B-ALK*, *TFG-ALK*, and *KLC1-ALK)*, but occur at much lower frequencies (Shaw and Engelman, 2013). These fusion proteins mediate ligand-independent dimerization of ALK, and like *EGFR* mutations, result in constitutive activation and downstream signaling through the MAPK and PI3K/Akt pathways (Soda et al., 2007). Interestingly, *ALK* gene arrangements are largely mutually exclusive with *EGFR* or *KRAS* mutations (Gainor et al., 2013). Cancers harboring *ALK* rearrangements, classified as ALK positive, derive clinical benefit from ALK PKI therapies.

Crizotinib is a first generation ALK PKI, which also inhibits the MET and ROS proto-oncogenes (*MET* and *ROS1*), which encode receptor tyrosine kinases. Crizotinib first received accelerated FDA approval for ALK-positive NSCLC in 2011 based on durable, objective response rates of 61% in a single-arm phase 1 study, and is now recommended first-line in ALK-rearranged NSCLC (Camidge et al., 2012). Crizotinib inhibits ALK phosphorylation and signal transduction through G_1-S phase cell cycle arrest and induction of cellular apoptosis (Christensen et al., 2007). Despite high response rates (74% in the first-line phase 3 study), resistance to crizotinib develops 10–12 months after therapy initiation (Solomon et al., 2014).

As observed with EGFR inhibitors, acquired resistance to crizotinib is heterogeneous and complex (Fig. 3). Mechanisms of resistance that have been reported include somatic alterations (amplification and/or mutation) in *ALK*, activation of alternative signaling pathways, and genetic alterations in other important oncogenes. A case of phenotypic neuroendocrine transformation was recently reported in a patient who developed acquired resistance to crizotinib (Caumont et al., 2016). Unlike that observed in EGFR inhibitor resistance, only ~one-third of patients with crizotinib resistance harbor an *ALK* mutation, and there are numerous mutations that can drive resistance. Two of the most commonly observed mutations are L1196M and G1269. Amino acid 1196 is considered the gatekeeper residue of *ALK* and, similar to *EGFR*, it controls access to the active site; therefore, the bulky substitution of methionine causes steric hindrance, impeding crizotinib binding (Choi et al., 2010). Individual mutations have been shown to confer variable degrees of crizotinib resistance (Heuckmann et al., 2011; Katayama et al., 2012). Activation of alternative signaling pathways, or bypass tracks, can occur via genomic or non-genomic mechanisms. These alterations lead to constitutive activation of redundant downstream pathways, rendering crizotinib incapable of suppressing tumor growth. Non-genomic mechanisms of resistance include increased phosphorylation of other tyrosine kinases (e.g., EGFR, IGF-1R, and Src); genomic alterations include mutations in *EGFR*, *c-KIT*, *MAPK*, and *KRAS* (Isozaki et al., 2015) (Fig. 3).

Multiple potential strategies exist to combat crizotinib resistance. The most well established second-line therapeutic option in patients who progress after receiving crizotinib is second-generation ALK inhibitors. Ceritinib, an FDA-approved second-generation ALK inhibitor, is 20-fold more potent against ALK than crizotinib and also inhibits IGF-1R (insulin-like growth factor 1 receptor). In a phase 1 clinical study, among the 80 patients who had received crizotinib previously, there was a 56% response rate to ceritinib in patients with various *ALK* resistance mutations (Shaw et al., 2014). A preclinical study suggests that some crizotinib-resistance mutations (F1174C and G1202R) may not be sensitive to ceritinib and, in fact, may be mechanisms of acquired resistance to ceritinib as well (Friboulet et al., 2014). Alectinib, another second-generation ALK inhibitor with efficacy in patients who progressed on crizotinib received FDA-approval December 2015 (Shaw et al., 2016b). Interestingly, acquired mutations in *ALK* (I1171N and F1245C), have been reported to confer resistance to alectinib, but are susceptible to ceritinib (Kodityal et al., 2016; Ou et al., 2015). Interestingly, acquired resistance to lorlatinib (via *ALK L1198F)*, a third-generation ALK inhibitor in clinical trials, has been reported to enhance crizotinib binding and resensitize resistant tumors (Shaw et al., 2016a). Findings such as these may be important when considering sequencing of therapy in resistant patients.

Strategies to overcome crizotinib resistance due to bypass track signaling are less well studied. However, rational combination therapies have been postulated. For example, a preclinical study in ALK-positive NSCLC cell lines with acquired IGF-1R upregulation demonstrated improved efficacy of combined ALK (crizotinib) and IGF-1R inhibition (OSI-906) (Lovly et al., 2014). In the case of EGFR-mediated mechanism of acquired resistance, combination therapy with an ALK and EGFR inhibitor has been suggested to be effective (Katayama et al., 2012; Yamaguchi et al., 2014). Other plausible combinations include ALK inhibition concurrently with MEK or Src inhibitors. Heat shock protein 90

(HSP90) inhibitors have also demonstrated efficacy against ALK-sensitive and ALK-mutant NSCLCs (Katayama et al., 2012; Sang et al., 2013). HSP90 is a molecular chaperone that aids in proper folding of specific target proteins; ALK fusion proteins are substrates of HSP90. The combination of crizotinib plus HSP90 inhibitors is currently in clinical trials (NCT01712217).

Acquired resistance to BCR-ABL inhibitors in hematologic malignancies

Imatinib is an inhibitor of the *BCR-ABL* fusion gene, also referred to as the Philadelphia chromosome, that is characteristic of CML and presents at lower frequencies in ALL and acute myeloid leukemia (AML). BCR-ABL is a constitutively active tyrosine kinase that functions by binding ATP and transferring a phosphate group to tyrosine residues to activate downstream signaling molecules. Imatinib competitively binds to the kinase pocket of BCR-ABL, inhibiting phosphorylation and downstream signaling (Savage and Antman, 2002; Signorovitch et al., 2014; Waller, 2014). The majority of CML patients will achieve clinically significant responses to imatinib. A complete and durable cytogenetic response is achieved in up to 80% of newly diagnosed patients and approximately 60% of patients with chronic-phase CML (Druker et al., 2006; Kantarjian et al., 2002). However, up to 27% of patients have been shown to develop resistance and relapse (Press et al., 2007).

Acquired resistance to imatinib occurs as a consequence of reactivation of BCR-ABL signaling, which can be due to *BCR-ABL* amplification, elimination of imatinib from the cell by multidrug efflux transporters, or, most commonly, by the development of *BCR-ABL* mutations (Gorre et al., 2001). The acquired mutations in *BCR-ABL* that have been associated with resistance to imatinib and other BCR-ABL inhibitors, such as nilotinib and dasatinib, are located in 12 key positions of *ABL1* (Abelson murine leukemia viral oncogene, homolog 1). These result in amino acid substitutions that change the BCR-ABL binding site, altering the ability of the PKI to bind and inhibit downstream signaling (Zabriskie et al., 2014). The most commonly observed *BCR-ABL* mutation conferring acquired resistance is T315I, followed by E255K/V. Interestingly, multiple acquired mutations in *BCR-ABL* have been identified, and the degree of imatinib (and other BCR-ABL PKI) resistance is dependent on the location of the point mutation within the BCR-ABL binding site (Shah et al., 2002). Strategies to overcome acquired resistance to BCR-ABL PKIs are currently being explored.

The second generation PKIs, such as nilotinib and dasatinib, were designed with a higher affinity for BCR-ABL in attempts to combat imatinib resistance. While they are able to overcome some mutations observed in acquired imatinib resistance, they are ineffective against the common T315I mutation (Zabriskie et al., 2014). In 2012, a third generation BCR-ABL PKI, ponatinib, was approved. Ponatinib is unique in that it is a high affinity pan-BCR-ABL inhibitor with activity in T315I-positive CML. Despite its effectiveness against the acquired T315I mutation, emergence of compound mutations in *BCR-ABL* have been identified as conferring differential resistance to ponatinib (Zabriskie et al., 2014). Complex screening of resistance mutations and sensitivity to BCR-ABL PKIs is necessary to optimize therapy selection at the time of disease progression.

Acquired resistance to BCR-ABL inhibitors in solid tumors

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract, characterized by positive staining for *KIT* (the v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene). Approximately 95% of GISTs express *KIT*, 80% have mutations in *KIT*, and 10% have mutations in the platelet-derived growth factor receptor alpha (*PDGFRA*) (Joensuu et al., 2013). Wild type GISTs lack *KIT* expression as well as *KIT* and *PDGFRA* mutations; alterations identified in wild type patients include mutations in *BRAF, RAS, NF1*, and succinate dehydrogenase deficiency. Imatinib is recommended first-line in GIST due to its inhibition of KIT and PDGFRA. Furthermore, *KIT* and *PDGFRA* mutations are associated with response to imatinib, with 84% of mutation-positive patients achieving a partial response compared to 0% of wild type patients (Heinrich et al., 2003). Despite high initial response rates, approximately 80% of GIST patients will develop imatinib resistance within 12–36 months (Joensuu et al., 2013).

As observed in hematologic malignancies, acquired imatinib resistance in GIST is most commonly due to secondary alterations in the drug targets. However, because the oncogenic targets differ, so do the resistance alterations. A retrospective analysis of tumors from patients who progressed on the phase 2 B2222 imatinib trial indicated that 22 of 33 (67%) patients with acquired resistance had secondary mutations in *KIT* or *PDGFRA* (Heinrich et al., 2006). Interestingly, secondary *KIT* mutations were only observed in patients with primary *KIT* mutations, and the secondary *PDGFRA* mutation was identified in a patient with a primary *PDGFRA* mutation. The acquired mutations were all located within the ATP-binding pocket of KIT (e.g., T670I, the gatekeeper mutation) or the activation loop, thus inhibiting imatinib binding and inactivation of KIT-mediated signaling.

Current treatment strategies for acquired resistance to imatinib in GIST focus on more potent inhibition of KIT and PDGFRA. Sunitinib, a multi-targeted PKI with activity against KIT, PDGFRA, VEGF, and numerous other tyrosine kinases, is currently approved second-line in patients with imatinib-resistant disease. Sunitinib has activity against all resistant genotypes, but response rates are significantly higher in wild-type GIST ($p= 0.04$) and patients with mutations in the ATP-binding domain ($p= 0.0005$) when compared to patients with alterations in the activation loop (Heinrich et al., 2008). Efficacy of the third-line treatment, regorafenib, may be due to sufficient activity in patients with acquired alterations in the activation loop of KIT (George et al., 2012). Despite their increased specificity for resistant disease, phase 3 clinical trial response rates to sunitinib and regorafenib were only 7% and 4.5%, and time to progression was 27 and 17 weeks, respectively (Demetri et al., 2013; Demetri et al., 2006). These low response rates suggest that the majority of imatinib-resistant GISTs may be entirely resistant to KIT inhibition, require more potent inhibitors, or may require alternative inhibition strategies.

Mutations and amplifications of *KIT* are also observed in melanomas (~3%), with higher frequencies in mucosal (39%), acral (36%), and chronically sun-damaged (28%) subtypes, suggesting possible benefit from imatinib (Curtin et al., 2006). A phase 2 trial of imatinib in patients with *KIT*-positive metastatic melanoma demonstrated potential efficacy, with 53.5% of the patients achieving a response and a 6-month PFS rate of 36.6% (Guo et al., 2011). Another phase 2 trial identified differential response rates between melanoma patients with

KIT mutations versus *KIT* amplification (54% vs. 0%, respectively) (Hodi et al., 2013). Retrospective data from patients with *KIT*-mutated melanoma demonstrated potential efficacy of sunitinib; of 4 evaluable patients, 3 (75%) responded to sunitinib (1 complete remission and 2 partial responses) (Minor et al., 2012). Additional studies support use of sunitinib in mucosal and acral subtypes of melanoma, but failed to show an association between *KIT* status and response (Buchbinder et al., 2015; Mahipal et al., 2012). Despite clinical benefit and relatively high initial response rates, most patients eventually progress on these therapies.

Similar to hematologic malignancies and GIST, the current strategy to overcome acquired resistance to KIT inhibition in melanoma is the use of more potent inhibitors. Nilotinib, the BCR-ABL PKI used in chemoresistant CML, also has activity against KIT, PDGFR, DDR, and several other protein kinases, with greater potency than imatinib. Recently, a phase 2 study of niltonib demonstrated potential efficacy in patients with acquired resistance to prior KIT inhibitors. The primary endpoint, 4-month disease control, was achieved in 27% of resistant patients (95% CI 8% – 56%), and two partial responses (18.2%, CI 3% – 47%) were observed (Carvajal et al., 2015). A similar phase 2 study of nilotinib in *KIT*-positive melanomas in a Korean population failed to meet its primary endpoint of response rate (overall response rate was 16.7%). However, 6 of the 7 responses observed occurred in patients with *KIT* mutations only (24% response rate in *KIT*-mutated melanoma), suggesting that nilotinib may provide benefit in this subgroup (Lee et al., 2015b). As in GIST, *KIT* status seems to be a biomarker of response to imatinib in melanoma, but resistance inevitably develops. Current strategies to combat resistance are the same as GIST, with stronger inhibition of KIT, but response rates are low and not durable. Further understanding of the mechanisms of resistance and means to effectively suppress drivers of drug resistance are crucial to achieve durable responses in GIST and melanoma patients.

Acquired resistance to BRAF inhibition

BRAF, a serine/threonine kinase, plays a key role in the MAPK signaling pathway, which affects cell division, differentiation, and growth. Mutations in *BRAF* have been associated with numerous cancers, including CRC, melanoma, thyroid carcinoma, NSCLC, and non-Hodgkin's lymphoma. Most commonly observed are somatic mutations causing activation of BRAF, specifically a valine to glutamine or lysine substation at position 600 (V600E/K), and constitutive signaling through the MAPK pathway. The discovery of *BRAF* mutations in cancer led to development of drugs aimed at inhibiting this oncogenic driver. In 2011, the first selective BRAF inhibitor, vemurafenib, was FDA-approved for the first-line treatment of *BRAF V600E*-positive melanoma after interim review of a phase 3 randomized controlled trial that demonstrated improved OS (84% vs. 64%) and PFS (5.3 months vs. 1.6 months) (Chapman et al., 2011). Debrafenib, a second BRAF inhibitor, demonstrated similar efficacy (PFS 5.1 vs. 2.7 months, HR 0.30, $p<0.0001$) and, was approved for the treatment of *BRAF*-positive melanoma in 2013 (Hauschild et al., 2012). It was realized early that, despite high initial levels of response, efficacy of BRAF inhibitors alone is not durable, with most patients developing resistance within 6–8 months (McArthur et al., 2014).

Most mechanisms of acquired resistance to BRAF inhibitors involve reactivation of the MAPK pathway; unlike EGFR and ALK, no secondary or gatekeeper-type mutations have been identified as resistance drivers (Nazarian et al., 2010). Secondary resistance may be driven upstream (e.g., upregulation and activation of the other receptor tyrosine kinases), downstream (e.g., activating *MEK1/2* mutations), at the level of *BRAF* (e.g., alternative splicing, *BRAF V600E* amplification), or elevated *CRAF* levels. Observed genetic alterations in the setting of acquired resistance include mutations that activate *NRAS*, *MEK1*, and *MEK2* (Emery et al., 2009; Nazarian et al., 2010; Van Allen et al., 2014). Amplification of mitogen-activated protein kinase kinase kinase 8 (*MAP3K8* or *COT*) is another mechanism of resistance, which results in RAF-independent activation of MEK and ERK signaling (Johannessen et al., 2010).

One potential strategy to circumvent or delay BRAF inhibitor resistance is dual inhibition of components of the MAPK-pathway. In a phase 3 clinical trial of BRAF and MEK inhibition vs. BRAF inhibition alone in melanoma, the combination of dabrafenib plus trametinib improved PFS when compared to debrafenib alone (9.3 vs. 8.8 months, HR 0.75, p= 0.03); significant improvements in response rates and OS were also observed (Long et al., 2014). Trametinib is now FDA approved as monotherapy or in combination with dabrafenib. Interestingly, the benefit of MEK inhibitors was not observed when administered as monotherapy in patients who had progressed after initial benefit from a BRAF inhibitor, suggesting that MEK inhibition alone is not sufficient to overcome BRAF resistance (Kim et al., 2013). Preclinical data suggests another potential strategy to delay or overcome resistance caused by increased MAPK signaling is concurrent or sequential inhibition of BRAF and ERK (Hatzivassiliou et al., 2012; Herrero et al., 2015; Wong et al., 2014). However, while dual inhibition of the MAPK pathway has shown benefit, acquired resistance occurs within 12 months. Known mechanisms of acquired resistance to BRAF plus MEK inhibition are similar to those observed with BRAF inhibitor monotherapy, and include amplification of *BRAF V600*, which activates CRAF and subsequently MAPK signaling, and acquired *MEK1/2* mutations (Moriceau et al., 2015; Villanueva et al., 2013; Wagle et al., 2014). Preclinical studies investigating even broader combination therapy consisting of concurrent BRAF, MEK, and PI3K/mTOR inhibition have demonstrated potential efficacy in BRAF/MEK-induced resistance (Carlino et al., 2014; Moriceau et al., 2015; Villanueva et al., 2013). In addition, like *ALK*, *BRAF V600E* is a client of HSP90, and preclinical studies suggest that treatment with an HSP inhibitor may be another successful strategy to overcome BRAF and MEK inhibitor resistance (Smyth et al., 2014).

MAPK-independent mechanisms of acquired resistance to BRAF inhibition have also been identified, and dual inhibition has been proposed as a strategy to overcome this resistance. The most well studied bypass track of BRAF inhibitor resistance is activation through the PI3K/Akt signaling pathway. For example, increased expression of *PDGFRB* or *IGF-1R* has been observed in cell culture and patient xenograft models of secondary resistance. Over-activation of these receptors results in activation of alternate signaling pathways (e.g., PI3K/Akt), which can reduce the cancer cells' dependency on MAPK signaling, rendering the cells resistant to BRAF-mediated inhibition (Nazarian et al., 2010; Villanueva et al., 2010). Secondary mutations in PI3K pathway regulatory genes, such as *AKT1/3, PIK3CA, PIK3CG, PIK3R2*, and *PTEN* have also been observed, further supporting combination

therapy with PI3K inhibitors (Shi et al., 2014a). Preclinical data demonstrated efficacy of concurrent PI3K and MEK inhibition in BRAF resistant cell lines, and clinical trials of this combination are currently underway (NCT01363232, NCT01337765). Recently, expression of yes-associated protein 1 (*YAP1*), a member of the Hippo signaling pathway, was associated with resistance to BRAF and MEK inhibition, and preclinical studies demonstrated that triple therapy with a BRAF, MEK, and YAP inhibitor may be a promising strategy to increase response in the setting of resistance (Lin et al., 2015).

Conclusions

Targeted therapies are increasingly common and recommended first-line in some cancer types due to their impressive increases in response rates and survival benefits when compared to standard cytotoxic chemotherapy. However, even in the presence of a genetic biomarker predictive of response, not all patients will benefit from such therapies (intrinsic resistance), and for those who do, durable response rates are low (acquired resistance). Therefore, equally important as identifying targetable oncogenic alterations is the identification of biomarkers of intrinsic resistance and the ability to anticipate potential mechanisms of acquired resistance that may develop.

As discussed in this article, the most common mechanisms of acquired resistance to targeted therapies induce upregulation of the drug target or bypass signaling through the same or similar pathways, resulting in cancer progression. These patterns imply an evolutionary-like process in which the cells most fit to regulate cellular proliferation and survival are selected. The question of whether or not resistant cells are present at undetectable concentrations at diagnosis or whether they develop post-treatment remains unanswered. Specifically, does resistance arise from pre-existing clones or is there drug-induced selection pressure that drives acquisition of mutations? It is likely that both may be the case.

An important concept in the context of resistance to anticancer therapies is that a biomarker conferring response or resistance in one tumor type may or may not be predictive in all tumor types. For example, approximately 10% of CRCs carry *BRAF V600* mutations, but are resistant to BRAF inhibition (Corcoran et al., 2015; Kopetz et al., 2015). However, in a recent “basket” study of vemurafenib in *BRAF V600*-mutated non-melanoma cancers (n = 7 tumor classifications, including CRC), patients with NSCLC and Erdheim-Chester disease or Langerhans’-cell histiocytosis met the predetermined overall response rate of >35%, suggesting that *BRAF* status may be important in those tumor types (Hyman et al., 2015). Recently, vemurafenib also demonstrated efficacy in *BRAF*-positive hairy cell leukemia (Tiacci et al., 2015). Another example is *RAS*-status as a predictor of intrinsic resistance to EGFR monoclonal antibodies in CRC, but not predictive of response to EGFR inhibition in NSCLC. Interestingly, secondary mutations in *EGFR* have been reported as mechanisms of acquired resistance in both tumor types.

Lack of efficacy across tumor types may be reflective of differential oncogenic drivers or compensatory resistance mechanisms. In CRC, data suggests that BRAF inhibition is overcome through increased EGFR-mediated signaling; this bypass track is not clinically relevant in melanoma due to low basal levels of EGFR in this cancer type (Corcoran et al.,

2012). Therefore, dual BRAF and EGFR inhibition may be required to increase response rates in *BRAF*-mutant CRC (Atreya et al., 2015; Napolitano et al., 2015). The benefit of imatinib in hematologic malignancies with *BCR-ABL* translocations and solid tumors with *KIT* or *PDGFRA* mutations is a unique example of how different oncogenic drivers may respond to the same therapy, but result in different genetic mechanisms of acquired resistance.

Future directions

Treatment modalities to prevent and overcome drug resistance are critical to increase the rate of durable responses to cancer therapies (Fig. 4). To date, most strategies used in clinical practice involve sequential dosing once resistance develops (Fig. 4A). An alternative strategy is to predict and target known resistance pathways from the outset using combination therapy (Fig. 4B). The strength of this approach is supported by the increased benefit of dabrafenib plus trametinib vs. dabrafenib alone in *BRAF*-mutated melanoma (Long et al., 2015). However, while this prolongs duration of response, resistance inevitably develops. Studies indicate development of BRAF inhibitor dependence in melanoma cells, which may also be combatted with intermittent or continuous dosing (Das Thakur et al., 2013; Dooley et al., 2014) (Fig. 4C). Another possible strategy is pulse dosing, in which the targeted therapy is administered for a short time (maybe until progression), stopped temporarily, and then restarted (Fig. 4D). This method is supported by serial biopsies of *EGFR*-positive NSCLC resistance which demonstrated that patients may re-develop the T790M mutation after withdrawal and, therefore, respond to reinitiation of an EGFR inhibitor (Sequist et al., 2011). Finally, some patients with advanced disease may benefit from immunotherapy, but identification of this subset is much less understood.

Despite overall response rates of 50% to 80% in clinical trials of targeted anticancer therapies, mechanisms of intrinsic resistance have yet to be fully elucidated. Intrinsic resistance may be attributed to germline genetics (e.g., *BIM* or *TPMT*) or somatic alterations (e.g., *KRAS* in CRC). Drug metabolism and germline variants that may affect exposure to cancer therapies are also an important consideration in the context of intrinsic resistance [reviewed in (Hertz and Rae, 2015; Kathawala et al., 2015)]. However, robust examples of intrinsic resistance to targeted anti-cancer therapies are lacking. Furthermore, mechanisms of resistance to PKIs that inhibit multiple targets, such as sorafenib and sunitinib, are even more challenging to elucidate due to the heterogeneity of their effects, and are even less studied. Another interesting gap in the area of resistance to anticancer therapies involves potential racial disparities. Certain alterations are known to be more common in some races (e.g., *EFGR* mutations in Asian populations), suggesting potential differences in racial trends of oncogenic drivers. Because clinical and genetic studies enroll a vast majority of Caucasian patients, these potential disparities in genetic oncogenesis between races, which can greatly affect response rates, have yet to be elucidated.

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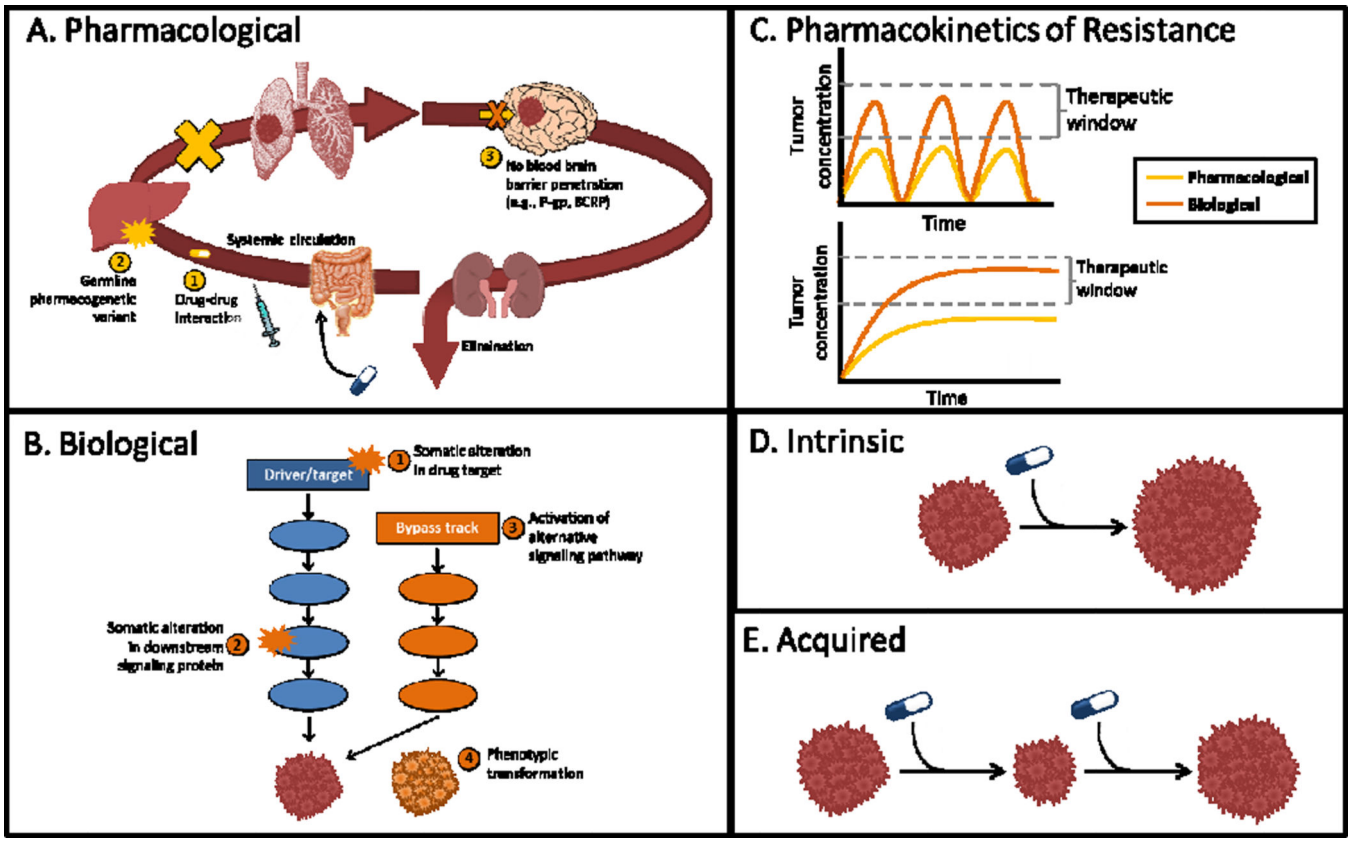

Figure 1. Mechanisms of oncology drug resistance

(A) Pharmacological resistance. Drug-drug interactions and germline pharmacogenetic variants can affect drug exposure at the tumor site. Pharmacological properties can affect drug penetration into the central nervous system. (B) Biological resistance. Somatic (acquired) mutations in the drug target can affect the drug's ability to effectively inhibit oncogenesis. Somatic alterations downstream of the drug target can result in constitutive upregulation of oncogenic pathways. Genetic alterations may also activate alternative oncogenic signaling pathways. Some tumor types have been shown to transform into other tumor types (e.g., non-small cell lung cancer to small cell lung cancer). (C) Pharmacological drug resistance results from inadequate drug levels at the site of action, whereas biological drug resistance results despite adequate drug levels at the site of action. (D) Intrinsic resistance is the lack of even transitory clinical benefit – the tumor continues to progress despite treatment. (E) Acquired resistance is the lack of tumor response to medication despite initial benefit.

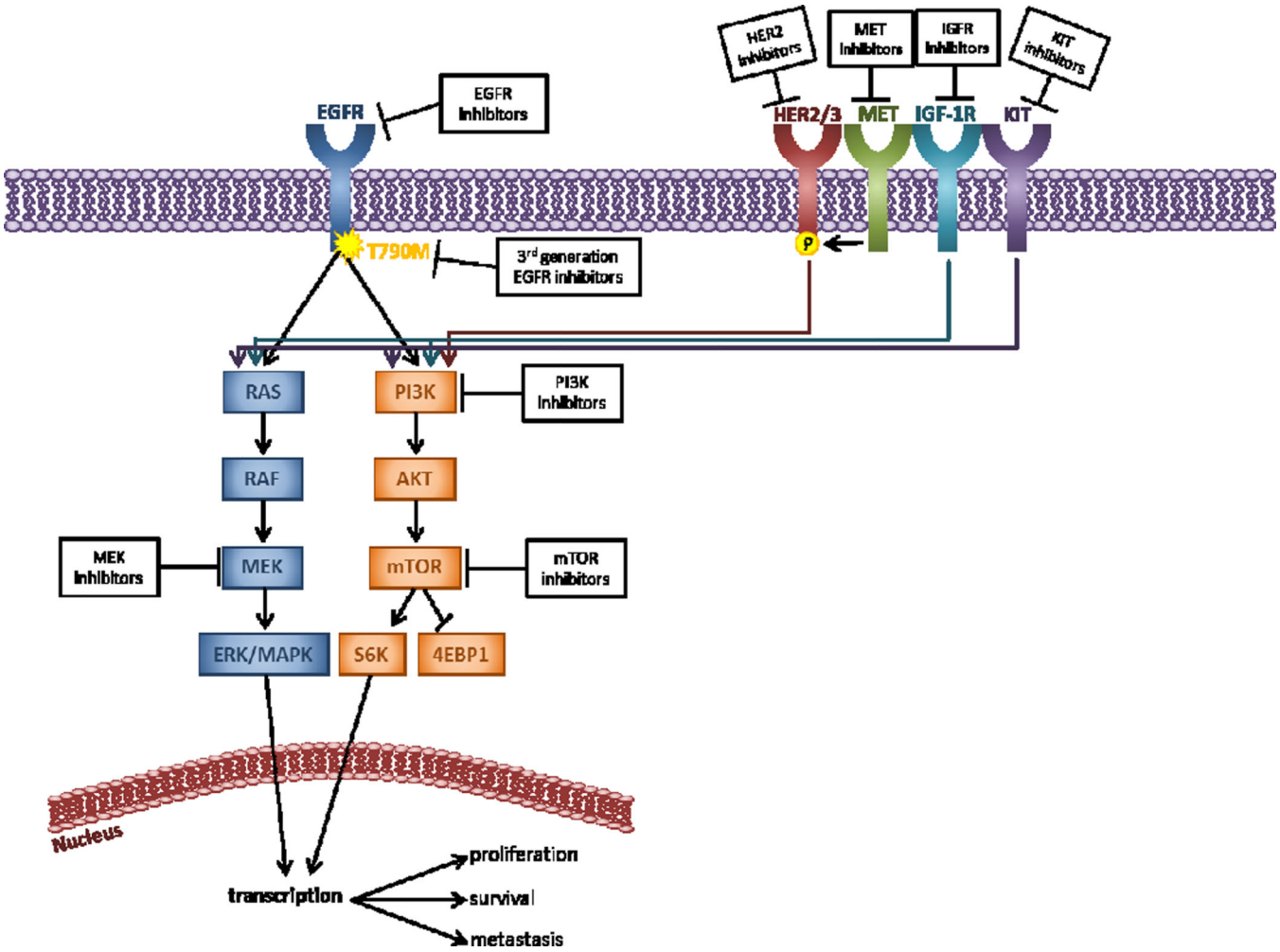

Figure 2. Example mechanisms of resistance to EGFR inhibitors and potential treatment strategies: The MAPK and PI3K/AKT pathways

EGFR-mutated non-small cell lung cancers show initial response to EGFR inhibitors (e.g., erlotinib, gefetinib). Mechanisms of acquired resistance include secondary *EGFR* mutations, downstream mutations that result in EGFR-independent activation of MAPK or PI3K/AKT signaling pathways, or mutations in alternative protein kinases that bypass EGFR-mediated signaling through MAPK and/or PI3K/AKT pathways. Potential treatment strategies to combat acquired resistance to EGFR inhibitors include stronger inhibiton of EGFR (e.g., afatinib), combination therapy with a MEK (e.g., trametinib) or mTOR (e.g., sirolimus) inhibitor, or inhibition of bypass tracks.

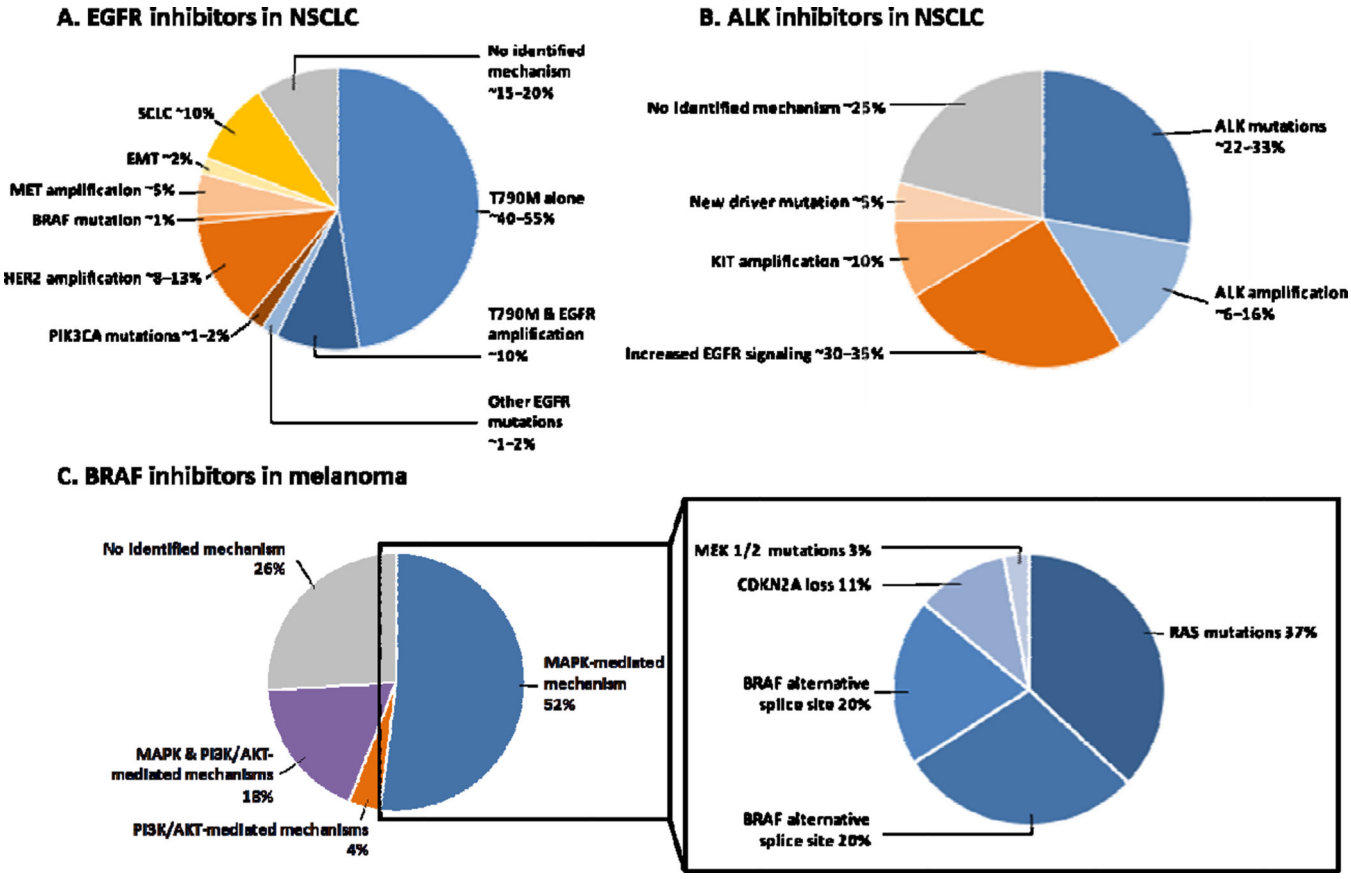

Figure 3. Mechanisms of acquired resistance to protein kinase inhibitors

(A) and (B) data from Camdige et al., 2014. (C) Data from Shi et al., 2013. The all-blue pie chart represents MAPK-mediated mechanisms of resistance. PI3K/AKT-mediated mechanisms include *AKT1/3* mutations (3%), mutations in positive-regulatory genes, *PIK3CA* and *PIK3CG*, and mutations in negative-regulatory genes *PIK3RS, PTEN*, and *PHLPP1*.

Blue shades throughout correspond to alterations within the targeted oncogenic track; orange shades correspond to alterations regulating alternative or bypass tracks; yellow shades correspond to phenotypic transformations. NSCLC: non-small cell lung cancer, SCLC: small cell lung cancer, EMT: epithelial to mesenchymal subtype.

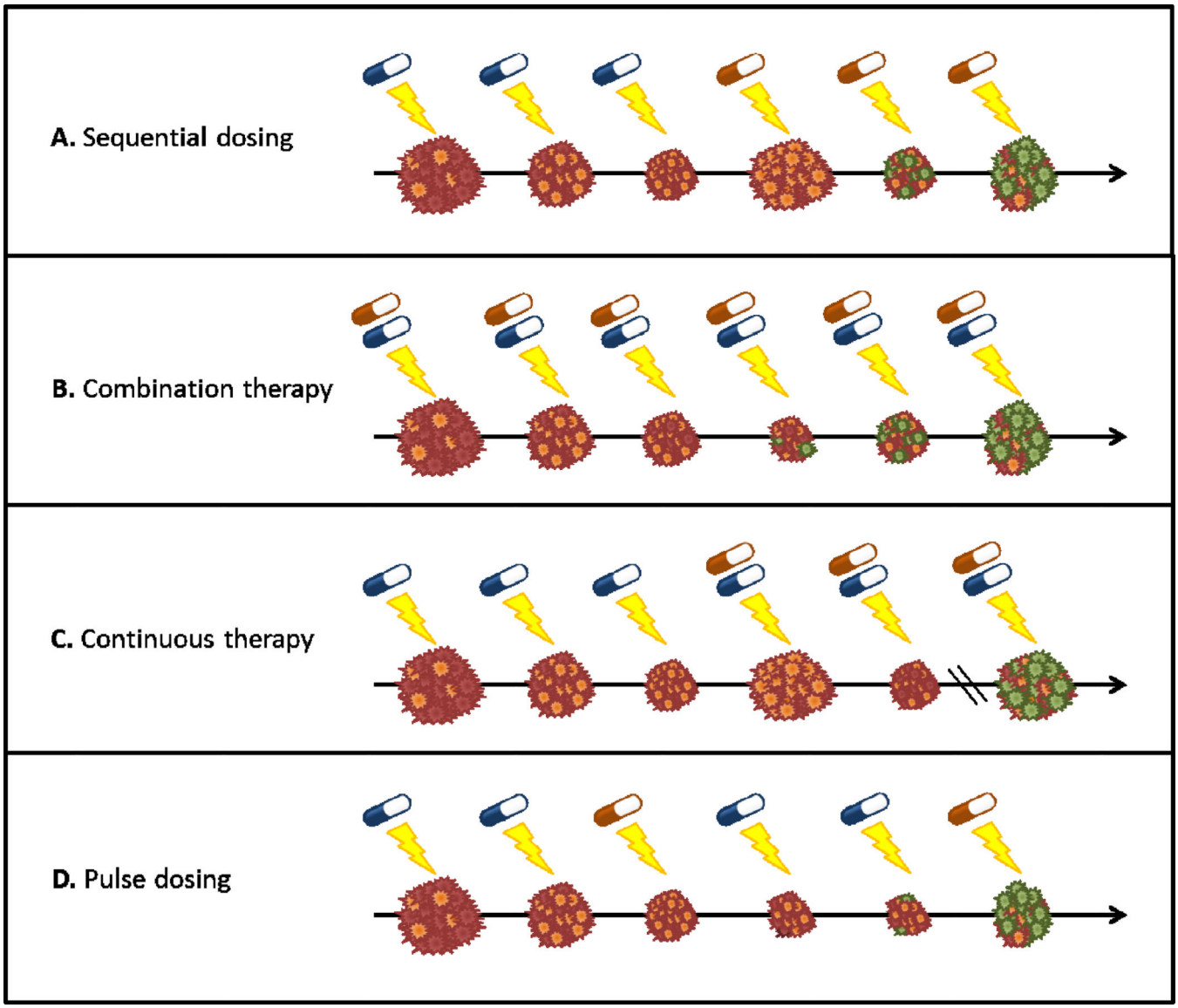

Figure 4. Potential treatment strategies to combat anticancer drug resistance

(A) Sequential dosing. Begin treatment with single targeted therapy, once resistance develops, switch to drug that targets resistant cells. (B) Combination therapy. Begin therapy with a medication that targets the identified oncogenic marker in combination with a drug targeting predicted resistance mechanisms. (C) Continuous therapy. Begin treatment with a single targeted therapy, once resistance develops, add medication that targets resistance mechanism. (D) Pulse dosing. Begin treatment with single targeted therapy and periodically administer medication that targets predicted resistance mechanism. (A) represents the most commonly used strategy to date. Some data suggest that (B), (C), and/or (D) may delay development of acquired resistance.

Table 1

Summary of FDA-approved protein kinase inhibitors and known mechanisms of resistance.

Class	Drug	Generation	Indication	Indication subgroup	Innate (primary) resistance	Acquired (secondary) resistance
EGFR inhibitors	erlotinib	1st	NSCLC	EGFR exon 19 deletions or exon 21 (L858R) substitution mutations	EGFR wt, BIM deletion	secondary EGFR mutations (e.g., T790M), MET amplification, HER2 amplification (*see Fig. 3*)
	gefetinib	1st	NSCLC			
	afatinib	2nd	NSCLC			
	osimertinib	3rd	NSCLC	EGFR T790M–positive	EGFR wt	loss of T790M, secondary EGFR mutations (e.g., C797S)
ALK inhibitors	crizotinib	1st	NSCLC	ALK+, recommended for pts with ROS1 translocation	ALK wt	secondary ALK mutations, ALK fusion amplification, bypass signaling (e.g., EGFR, KIT, IGF1R) (*see Fig. 3*)
	ceritinib	2nd	NSCLC	ALK+, resistant to crizotinib, ALK I1171N-positive	ALK wt	secondary ALK mutations (e.g., G1202R)
	alectinib	2nd	NSCLC	ALK+, resistant to crizotinib		secondary ALK mutations (e.g., I1171N)
BCR-ABL inhibitors	imatinib	1st	CML, ALL, MDS, GIST	Ph+	BIM deletion; KIT and PDGFRA wt (GIST)	BCR-ABL1 T315I and others in heme; KIT and PDGFR secondary mutations in GIST
	bosutinib	2nd	CML	Ph+		BCR-ABL1 T315I and others
	dastinib	2nd	CML, ALL			
	nilotinib	2nd	CML			
	ponatinib	3rd	CML,ALL	Ph+ for whom no other TKI is indicated, or **T315I positive**		BCR-ABL1 compound mutations
BTK inhibitor	ibrutinib		CLL, MCL, WM		C481S, point mutations in phospholipase Cy2 (PLCg2)	Mutations in BTK and downstream
HER2 inhibitor	lapatinib		breast	HER2+		deregulation of PIK3CA pathway, AXL over-espression?
BRAF inibitiors	dabrafenib		melanoma	BRAF V600E or V600K	RAC1 mutations, loss of PTEN or NF1, CCND1 over-expression, abundance of HGF	re-activation of the MAPK pathway, activating mutations in NRAS, activating MEK 1/2 mutations, evelated CRAF (*see*
	vemurafenib		melanoma	BRAF V600E		

Drug Resist Updat. Author manuscript; available in PMC 2017 September 01.

Class	Drug	Generation	Indication	Indication subgroup	Innate (primary) resistance	Acquired (secondary) resistance
						Fig. 3)
MEK inhibitors	trametinib		melanoma	BRAF V600E or V600K		
mTOR inhibitor	everolimus		breast, pNET, RCC, angiolyolipoma, SEGA	breast HER2-	TSC1/TSC2 mutations predict *response*	
EGFR monoclonal antibodies	cetuximab panitumumab				RAS mutations	
ER inhibitors	tamoxifen, fulvustrant					ESR1 mutations
HER2 inhibitors	trastuzumab		breast, gastric	HER2+	PIK3CA mutations possibly predictive of response in neoadjuvant setting (not predictive in adjuvant)	
SMO inhibitor	vismodegib		basal cell carcinoma			SMO mutations

Drug Resist Updat. Author manuscript; available in PMC 2017 September 01.