Molecular Pathways

Clinical Cancer Research

Molecular Pathways: Resistance to Kinase Inhibitors and Implications for Therapeutic Strategies

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Abstract

The development of targeted therapies has revolutionized the treatment of cancer patients. The identification of "druggable" oncogenic kinases and the creation of small-molecule inhibitors designed to specifically target these mutant kinases have become an important therapeutic paradigm across several different malignancies. Often these inhibitors induce dramatic clinical responses in molecularly defined cohorts. However, resistance to such targeted therapies is an inevitable consequence of this therapeutic approach. Resistance can be either primary (*de novo*) or acquired. Mechanisms leading to primary resistance may be categorized as tumor intrinsic factors or as patient/drug-specific factors. Acquired resistance may be mediated by target gene modification, activation of "bypass tracks" that serve as compensatory signaling loops, or histologic transformation. This brief review is a snapshot of the complex problem of therapeutic resistance, with a focus on resistance to kinase inhibitors in EGF receptor mutant and *ALK* rearranged non–small cell lung cancer, BRAF-mutant melanoma, and BCR-ABL–positive chronic myeloid leukemia. We describe specific mechanisms of primary and acquired resistance and then review emerging strategies to delay or overcome drug resistance. *Clin Cancer Res; 20 (9); 2249–56. ©2014 AACR.*

Disclosure of Potential Conflicts of Interest

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CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the mechanisms of resistance to tyrosine kinase inhibitors and how this knowledge is leading to novel therapeutic strategies to overcome resistance.

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Background

Targeted cancer therapies are drugs designed to interfere with specific mutant signaling proteins. In the setting of "oncogene addiction," certain tumors become dependent on a single oncogenic pathway to promote tumor growth and survival. This "addiction" can serve as an "Achilles' heel" to target cancers with increased precision. Beginning with the marked success of the tyrosine kinase inhibitor (TKI) imatinib in BCR-ABL–positive chronic myelogenous leukemia (CML), the therapeutic targeting of oncogenes has emerged as a preeminent treatment paradigm for multiple other oncogene-driven malignancies. In addition to CML, this approach has been successful in EGF receptor (*EGFR*)-mutant non–small cell lung cancer (NSCLC), *ALK*- and *ROS1*-rearranged NSCLC, *BRAF*-mutant melanoma, *KIT*-mutant melanoma, gastrointestinal stromal tumor (GIST), and *HER2*-amplified breast cancer (1–7). In each case, the identification of the "driver" mutation or rearrangement within the tumor and the administration of genotype-directed antitumor therapy have resulted in improved clinical response rates within the specific molecularly defined cohort.

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Unfortunately, despite these promising results, a common theme among patients with oncogene-driven cancers treated with small-molecule inhibitors is drug resistance. The development of drug resistance remains a major limitation and threat to the successful management of advanced cancer. In this review, we formally define therapeutic resistance, review mechanisms of resistance to kinase inhibitors, and provide examples of therapeutic strategies to attempt to delay or overcome resistance. Given the complexity of the topic, this review focuses on selected tumor types, namely NSCLC, melanoma, and CML. However, many of the concepts discussed are broadly applicable among several tumor types and can serve as paradigms for understanding resistance in other malignancies.

Definition of Therapeutic Resistance

Resistance to targeted therapies can be classified as primary resistance or acquired resistance (Fig. 1). Primary resistance is defined as a *de novo* lack of treatment response. Conversely, acquired resistance refers to disease progression after an initial response to therapy. Importantly, acquired resistance occurs while the patient is still receiving the targeted therapy, implying that the tumor has developed an "escape" mechanism to evade continuous blockade of the target. Specific clinical criteria have been developed for

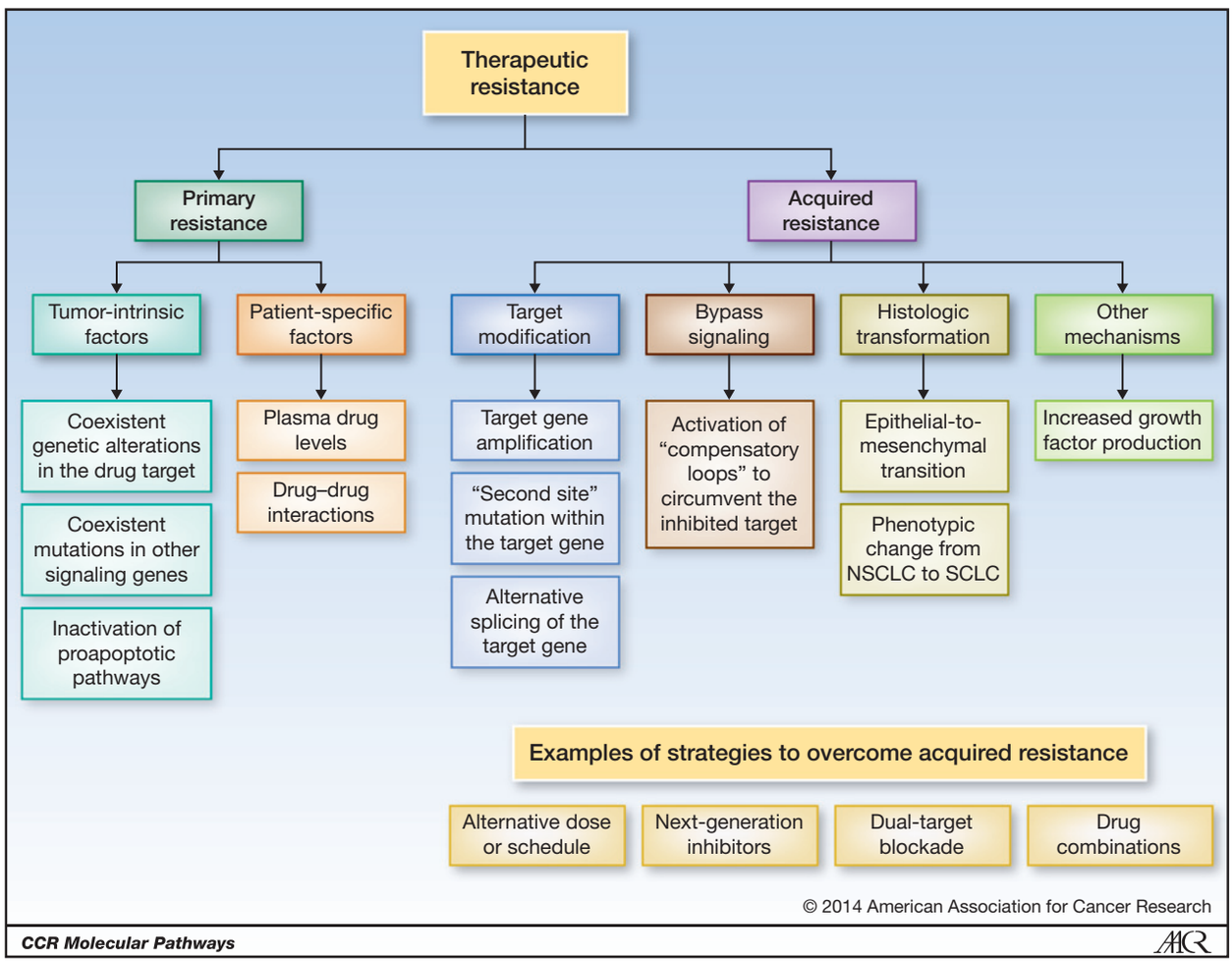

Figure 1. Mechanisms of therapeutic resistance to kinase inhibitors. Resistance to targeted therapies can be classified as primary resistance or acquired resistance. Primary resistance is defined as a *de novo* lack of treatment response and can be mediated by tumor intrinsic factors, such as concurrent genetic alterations within the drug target or within other signaling molecules, and by patient-specific factors, such as drug–drug interactions. Conversely, acquired resistance refers to disease progression after an initial response to the targeted therapy. Acquired resistance develops while the patient is still receiving the targeted therapy, implying that the tumor has developed an "escape" mechanism to evade continuous blockade of the target. These "escape" mechanisms include target modification (gene amplification, second-site mutations, splice variants), the emergence of bypass signaling tracks, histologic transformation, as well as other less well-characterized mechanisms such as increased growth factor production. Examples of strategies to overcome acquired resistance, which are discussed in more detail within the text, include alternative doses or schedules of the targeted inhibitor, development of more potent "next-generation" inhibitors, dual blockade of the initial target with two or more target-specific agents, and combination drug strategies designed to suppress compensatory signaling loops.

formally defining acquired resistance to EGFR TKIs in *EGFR*-mutant NSCLC (8) and ABL TKIs in CML (9).

Primary Resistance

Clinical and molecular mechanisms leading to primary (*de novo*) resistance may be broadly categorized as tumor intrinsic factors or patient/drug-specific factors. It is worth noting that true primary resistance to a targeted inhibitor in a molecularly defined patient population is relatively uncommon. Technical issues in the identification of the molecular marker as well as compliance of the patient to the prescribed therapy are also important considerations when evaluating for primary resistance.

Tumor intrinsic factors

Factors leading to tumor intrinsic primary resistance include the specific target mutation as well as coexistent genetic alterations in either the target gene itself or other signaling genes. For example, lung tumors with *EGFR* exon 20 insertions, which account for approximately 4% of *EGFR* mutations, are associated with a lack of response to clinically achievable doses of the EGFR TKIs, erlotinib, and gefitinib (10). In addition, a small percentage of patients with *EGFR*-mutant lung cancer harbor both a somatic EGFR activating mutation as well as a germline T790M mutation. Typically associated with acquired resistance to EGFR TKIs, the T790M alteration is found as a heterozygous germline variant in 0.5% of never smokers with lung adenocarcinoma and has been associated with primary resistance to EGFR TKI therapy (11). Analogously, patients with GIST harboring *KIT* exon 9 mutations typically display reduced responses to standard doses of imatinib compared with patients with GIST harboring *KIT* exon 11 mutations (6).

Primary resistance may also result from coexistent alterations within other signaling genes. For example, *de novo MET* amplification is associated with primary resistance to EGFR TKIs in *EGFR*-mutant NSCLC (12). In addition, drug resistance through inactivation of proapoptotic pathways has also been described. Polymorphisms in the proapoptotic *BIM* gene have been shown to result in intrinsic resistance to EGFR TKIs in *EGFR*-mutant NSCLC as well as in imatinib resistance in CML (13).

Patient/drug-specific factors

Drug levels and kinetics of drug exposure are affected by several patient-specific pharmacokinetic factors, including absorption, distribution, metabolism, and excretion (ADME). ADME properties may influence the efficacy of targeted therapies in clinical use and result in primary drug resistance. For example, imatinib drug levels correlate with response to therapy (14); however, several studies have shown significant variability of imatinib plasma levels among patients with CML receiving this inhibitor (15). In addition, drug–drug interactions may influence drug levels. For example, it has been reported that coadministration of erlotinib with fenofibrate results in lower plasma levels of erlotinib because fenofibrate induces cytochrome P450 3A4 (CYP3A4), which is involved in the metabolism of erlotinib (16). Finally, drug levels may be affected by interindividual differences in drug absorption and metabolism.

Acquired Resistance

Acquired resistance to a kinase inhibitor after an initial response develops in most patients. Acquired resistance is a complex and diverse phenomenon, but the end result for each potential mechanism is continued signaling through downstream pathways, despite the continued presence of the inhibitor. Here, we focus on clinically relevant mechanisms of acquired resistance.

Target modification

Alterations in the target oncogene, including gene amplification and second-site mutations, have been described as mechanisms of acquired resistance to many different kinase inhibitors. For example, amplification of *EGFR*, *BCR-ABL*, and *EML4-ALK* have been described in cases of acquired resistance to erlotinib/gefitinib, imatinib, and crizotinib, respectively (17–19). Amplification of the target may mediate drug resistance by shifting the equilibrium in favor of the kinase, resulting in an "out-competition" of the drug. Second-site mutations within the target oncogene have also been described for *BCR-ABL* in CML (20) and *EML4-ALK* (19, 21), *EGFR* (22, 23), and *ROS1* (24) in NSCLC. In the case of EGFR, the T790M "gatekeeper" mutation is the most common target-specific alteration identified in approximately 50% of patients with acquired resistance to the EGFR TKIs, erlotinib, and gefitinib (22, 23). Mutation of the EGFR T790 residue, which is located in the ATP-binding cleft of the kinase domain, has been shown to confer drug resistance by increasing the kinase's ATP affinity (25). In contrast, multiple different "second-site" mutations that confer reduced drug sensitivity *in vitro* and *in vivo* have been described for both BCR-ABL and EML4-ALK (18, 19, 21). These mutations seem to span the entire kinase domain of the respective targets and confer variable levels of drug resistance. More specifically, for EML4-ALK, the mutations seem to cluster around the ATP-binding pocket, and molecular modeling studies have demonstrated that the presence of the various ALK kinase domain mutations found at the time of resistance (L1196M, G1269A, G1202R, S1206Y, 1151Tins, and C1156Y) results in diminished crizotinib binding due to steric interference (19, 21). Mutations analogous to the EGFR gatekeeper T790M mutation have also been detected in patient samples at the time of resistance to imatinib and crizotinib (ABL T315I and ALK L1196M, respectively).

It is worth noting that although second-site mutations have been described as important mechanisms of acquired resistance to inhibitors of tyrosine kinases, such as EGFR, ALK, and ABL, no such second-site mutations have been described in *BRAF*-mutant melanoma tumors with acquired resistance to the BRAF inhibitor, vemurafenib. Interestingly, however, *BRAF* splice variants, which lack the RAS-binding domain, were found in 6 of 19 vemurafenib-resistant tumor biopsy samples (26). This *BRAF* splice variant leads to

enhanced dimerization of RAF and therefore increased downstream signaling.

Bypass of drug inhibition

Oncogenic kinases share many common downstream signaling mediators, thereby potentially providing a mechanism whereby tumors can bypass dependency on a given "driver" alteration. The presence of a targeted kinase inhibitor provides a selective pressure to circumvent the inhibited kinase and thereby continue signaling through critical downstream pathways to promote sustained tumor proliferation even in the continued presence of the inhibitor. To date, such "bypass tracks" have been most extensively characterized in the context of *EGFR* mutant and *ALK*$^+$ NSCLC. In the case of *EGFR*-mutant NSCLC, amplification of the *MET* receptor tyrosine kinase is detected in approximately 5% of tumors with acquired resistance to EGFR TKI therapy (17). *MET* amplification has been demonstrated to confer resistance by driving ERBB3-mediated activation of downstream PI3K-AKT signaling (27). In addition, *HER2* amplification has been detected in 12% of tumors with acquired resistance to EGFR TKI therapy (28), resulting in sustained downstream signaling, even in the continued presence of the EGFR TKI.

Analogously, in patients with crizotinib-resistant *ALK*$^+$ NSCLC, EGFR activation has been detected in four of nine (44%) tumor biopsy samples at the time of resistance (19). In this case, EGFR activation was evidenced not by mutation or gene amplification, but rather by increased EGFR phosphorylation in the post-crizotinib compared with the pre-crizotinib tumor biopsy samples. In addition, *KIT* amplification was identified in 2 of 13 (15%) patients with crizotinib resistance. These data suggest that either EGFR or KIT may serve as clinically relevant bypass tracks to compensate for ALK inhibition.

Bypass signaling has also been documented as a potential mechanism of resistance to the BRAF inhibitor vemurafenib in BRAF-mutant melanoma. Increased expression and phosphorylation of platelet-derived growth factor receptor (PDGFR)-β were found in 4 of 11 post-vemurafenib biopsy samples (29). In addition, increased insulin-like growth factor-I receptor (IGF-IR) phosphorylation has also been documented in post-vemurafenib tumor biopsy samples (30). Similar to bypass tracks activated in *EGFR*-mutant and *ALK*-rearranged NSCLC, RAF inhibitor resistance mediated by altered receptor tyrosine kinase expression or activity, such as PDGFR-β and IGF-IR, is thought to be conferred by parallel activation of downstream signaling pathways that bypass the inhibited target. In addition, resistance to BRAF inhibitors may also be mediated through activation of other intracellular signaling molecules in the mitogen-activated protein kinase pathway. For example, alterations in MAP–ERK kinase (MEK)-1 and *NRAS* have been found in tumor rebiopsy samples at the time of acquired resistance to vemurafenib (29, 31).

Histologic transformation

Changes in tumor histology have been documented at the time of acquired resistance to EGFR TKIs. These histologic changes include epithelial-to-mesenchymal transformation as well as transformation to small cell (neuroendocrine) histology (17). In particular, transformation to small cell histology was documented in 5 of 37 (14%) patients with *EGFR*-mutant lung cancer who developed acquired resistance to EGFR TKI therapy. Notably, all of the patients examined had adenocarcinoma histology in their pre-EGFR TKI tumor biopsy samples, all retained the original EGFR activating mutation, and some patients also had concurrent *PIK3CA* mutations in the context of the histologic transformation.

Other mechanisms of acquired resistance

Although alterations in the target oncogene, bypass signaling, and histologic transformation remain the most well-characterized resistance mechanisms, other potential means whereby tumor cells can evade the antiproliferative effects of targeted inhibitors have been described. For example, MET activation through increased production of hepatocyte growth factor (HGF), the ligand for MET, has been described as a mechanism of resistance to EGFR TKIs (32). The frequency of increased HGF production in patient tumor samples remains uncertain. Increased stromal levels of HGF have also been described in vemurafenib-resistant *BRAF*-mutant tumors (33). Additional mechanisms of acquired resistance certainly exist and remain to be characterized on a molecular level. To emphasize this point, consider the case of "next-generation" ALK inhibitors. These more potent inhibitors, described below, salvage almost all patients with acquired resistance to crizotinib, yet, only a minority of patients have target gene alteration as the defined mechanism of resistance. These data underscore the critical need to obtain rebiopsies at the time of disease progression to further understand the complexities of therapeutic resistance.

Clinical–Translational Advances

Preclinical and clinical studies that have uncovered mechanisms of therapeutic resistance, as described above, have provided crucial information necessary to inform physicians about the potential therapeutic strategies to attempt to delay or overcome drug resistance.

Strategies to overcome resistance mediated by target modification

Several clinical approaches to overcome resistance are directed at the initial drug target itself. The rationale for these approaches is based on the fact that some oncogene-driven malignancies retain "addiction" to the initial therapeutic target even at the time of resistance. In these cases, resistance is driven by alteration of the target oncogene by either amplification or second-site mutation, as described above.

Alternative doses and schedules. Dose escalation of imatinib has been demonstrated to be an efficacious strategy in patients with both CML and GIST (34, 35). In *EGFR*-mutant NSCLC, mathematical modeling experiments have

suggested that high-dose pulses combined with low-dose continuous EGFR TKI therapy may delay the development of resistance (36). In addition, retrospective reports have demonstrated that pulsatile erlotinib may control central nervous system (CNS) metastases from *EGFR*-mutant lung cancer after failure of standard daily dosing (37). In this case series, 6 of 9 patients (67%) with CNS metastases (brain and/or leptomeningeal), which occurred despite conventional dose EGFR TKI, had a partial response (PR) to pulsatile high-dose erlotinib. In the same series, best response outside the CNS was only evaluable in 5 of 9 patients (56%); 3 had stable disease (including 2 of 3 patients who had a PR in the CNS) and 2 had progressive disease There is also one case report of a patient with ALK^+ NSCLC whose intracranial disease responded to high-dose crizotinib (38). However, this approach needs to be validated in prospective trials.

Development of new, more potent inhibitors. In the case of resistance mediated by target modification, one potential strategy to overcome resistance is through the development of novel inhibitors with increased potency. These so-called "next-generation" inhibitors have already proved to be an efficacious strategy in CML. The second-generation TKIs, dasatinib, nilotinib, and bosutinib, are more potent than imatinib and have demonstrated efficacy in patients with imatinib-resistant CML (39–41). Of particular note, ponatinib was specifically developed to overcome the BCR-ABL T315I gatekeeper mutation (analogous to EGFR T790M and ALK L1196M mutations) and has already demonstrated efficacy in this patient population (42).

Analogous strategies are being explored in *EGFR*-mutant and ALK^+ lung cancer. Preclinical data suggested that "second-generation" irreversible EGFR TKIs, such as dacomitinib, neratinib, and afatinib, may be able to overcome target intrinsic resistance, including inhibiting the EGFR T790M mutation (43–45). Unfortunately, clinical trials with these "second-generation" EGFR TKIs as single agents have been disappointing (46, 47). However, recent phase I trials of the "third-generation" EGFR TKIs, AZD9291 and CO-1686, have demonstrated promising clinical results in patients with advanced *EGFR*-mutant NSCLC and resistance to erlotinib or gefitinib (48, 49). Both drugs are irreversible inhibitors with higher specificity for mutant EGFR (including T790M) compared with wild-type EGFR. Clinical trials with these agents are ongoing. Finally, next-generation ALK inhibitors are being explored in advanced ALK^+ NSCLC. These inhibitors are more potent against ALK than is crizotinib, and they overcome many of the known secondary resistance mutations within ALK. Initial results from phase I trials of the "second-generation" ALK TKIs, ceritinib (LDK378), alectinib (CH5424802), and AP26113, have demonstrated that all of these agents have efficacy in the setting of crizotinib resistance (50–52), including the CNS.

Dual target blockade. Analogous to the use of trastuzumab plus lapatinib (53) or trastuzumab plus pertuzumab (7) in $HER2^+$ breast cancer, dual target inhibition has been attempted as a strategy in *EGFR*-mutant NSCLC. The combination of the EGFR antibody, cetuximab, plus afatinib has been studied in patients with acquired resistance to erlotinib (54). In the initial phase I study of this combination, the confirmed overall response rate was 40%, similar in both T790M-positive and T790M-negative tumors. Adverse events including rash and diarrhea, predominantly grade 1/2, were seen in the majority of patients. Further studies are ongoing with this combination.

Drug Combination Strategies

Drug combination therapies are being implemented clinically to both delay the emergence of resistance and to improve therapeutic efficacy. Drug combinations have been successful in overcoming resistance and improving treatment outcomes in other areas of medicine, particularly in antibiotic resistance and HIV infection, and analogous strategies are currently being investigated in cancer medicine. Here, we review strategies for rational anticancer drug combinations.

Targeting of bypass tracks

Strategies aimed at cotargeting bypass tracks are being actively pursued in a number of malignancies. Such strategies are typically devised to provide continuous inhibition of the primary oncogene (the driver) while also coinhibiting compensatory signaling loops, with the rational that therapeutic sensitivity can be restored when the two agents are given in combination. For example, combined inhibition of both EGFR and MET has been used in *EGFR*-mutant NSCLC with acquired resistance mediated by *MET* amplification (55). There are several MET pathway inhibitors in clinical development, including TKIs (crizotinib, cabozantinib, tivantinib, and foretinib) as well as monoclonal antibodies directed against both MET (onartuzumab) and the HGF ligand (rilotumumab and ficlatuzumab). In addition, the combination of RAF inhibitors with MEK inhibitors has already proved to be an efficacious treatment strategy for patients with *BRAF*-mutant melanoma (56). In a phase I/II study of the combination of the BRAF inhibitor, dabrafenib, with the MEK inhibitor, trametinib, in patients with advanced *BRAF*-mutant melanoma, median progression-free survival was 9.4 months in the combination group versus 5.8 months in the monotherapy group (HR for progression or death, 0.39; 95% confidence interval, 0.25–0.62; $P < 0.001$).

Other rational drug combination strategies

In addition to cotargeting of potential bypass tracks, several other potential therapeutic strategies have been proposed to delay or overcome acquired resistance. One approach involves cotargeting the molecular chaperone, HSP90, together with the primary oncogene. Many oncogenic kinases are HSP90 clients, and this chaperone is necessary for protein folding and stabilization. Single-agent activity of HSP90 inhibitors has been documented in patients with both TKI-resistant *EGFR*-mutant (57) and TKI-resistant ALK^+ NSCLC (58). Numerous ongoing

clinical trials are evaluating the efficacy of HSP90 inhibitors alone or in combination with kinase inhibitors.

Another potential strategy that has garnered much attention recently is the combination of targeted inhibitors plus immune therapy. Particularly in melanoma, the cancer subtype in which immune-based therapies have been most commonly used, there are growing data to suggest that treatment with a BRAF inhibitor results in both increased melanoma antigen expression and tumor recognition by antigen-specific T lymphocytes (59, 60). These data provide the rationale for combining BRAF inhibitors with immune-based therapies. Clinical trials of vemurafenib plus immune checkpoint inhibitors are ongoing in melanoma.

Conclusions

The development and clinical application of targeted inhibitors have led to a paradigm shift in the treatment of patients with cancer. However, the emergence of resistance is inevitable and represents a critical issue. Despite the wealth of information that has already been obtained about resistance to targeted therapies, several questions remain to be answered, including the following (i): How do we address heterogeneity of resistance mechanisms at different sites (or even the same site) within an individual patient? (ii) How do we develop and implement clinical trials to take into consideration the rapid pace of scientific discovery and the need to quickly and efficiently bring more efficacious treatments to the forefront of care while limiting the number of patients who receive potentially less effective treatments? (iii) How do we gain increased access to tumor biopsy samples at the time of resistance to each line of therapy to be able to more fully understand the dynamics of resistance mechanisms? Moving forward, it is incumbent on physicians and translational scientists to identify gaps in knowledge of therapeutic resistance and to implement strategies to address these gaps. We will need better/more extensive systems for studying resistance, such as the development of noninvasive ways to identify resistant tumor subclones. Overall, a better understanding of resistance mechanisms to targeted therapies will allow us to develop more efficacious therapeutic strategies and improve the care of patients with cancer.

Authors' Contributions

Conception and design: C.M. Lovly, A.T. Shaw
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.M. Lovly
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.M. Lovly
Writing, review, and/or revision of the manuscript: C.M. Lovly, A.T. Shaw
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.M. Lovly

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