

REVIEW

A tale of two approaches: complementary mechanisms of cytotoxic and targeted therapy resistance may inform next-generation cancer treatments

Kenta Masui^{1,†}, Beatrice Gini^{1,†}, Jill Wykosky^{1,†},
Ciro Zanca^{1,†}, Paul S.Mischel^{1,2,3}, Frank B.Furnari^{1,2,3}
and Webster K.Cavenee^{1,2,3,*}

¹Ludwig Institute for Cancer Research, ²Moore's Cancer Center and
³University of California San Diego, La Jolla, CA 92093-0660, USA

*To whom correspondence should be addressed. Tel: +858-534-7805;
Fax: +858-534-7750;
Email: wcavenee@ucsd.edu

Chemotherapy and molecularly targeted approaches represent two very different modes of cancer treatment and each is associated with unique benefits and limitations. Both types of therapy share the overarching limitation of the emergence of drug resistance, which prevents these drugs from eliciting lasting clinical benefit. This review will provide an overview of the various mechanisms of resistance to each of these classes of drugs and examples of drug combinations that have been tested clinically. This analysis supports the contention that understanding modes of resistance to both chemotherapy and molecularly targeted therapies may be very useful in selecting those drugs of each class that will have complementing mechanisms of sensitivity and thereby represent reasonable combination therapies.

Introduction

The past decades of research have led to a deep understanding of the intricate mechanisms that regulate tumor growth and development. However, the intrinsic and acquired resistance of tumors to drug treatment remains a fundamental challenge to improving patient outcome. Early efforts to develop effective anticancer drugs resulted in several chemotherapeutics. Although these could potentially induce cell death in rapidly dividing cells, they did not effectively discriminate between tumor and normal cells. This necessitated reliance on a therapeutic window where efficacy was maximized, whereas non-tumor toxicity was minimized. For patients with some types of cancer, such as pediatric acute lymphoblastic leukemia, intensive combinations of cytotoxic chemotherapies resulted in cures (1). However, for most cancer patients, these combination approaches have proven to be too toxic or insufficiently effective to warrant their use.

Advances in genomics have ushered in a new era of 'targeted' cancer therapies that offers hope to the majority of cancer patients for whom intensive cytotoxic chemotherapy is unlikely to produce a cure. Discovery of the distinct molecular characteristics of cancer cells that make them unique from normal cells has led to the emergence of therapies that selectively target these cancer-related genetic lesions, mostly without the major chemotherapy-induced toxicities. However, it has become apparent that drug resistance to this category of therapy also occurs regardless of drug target and mechanism of

Abbreviations: ALK, anaplastic lymphoma kinase; BH3, Bcl-2 homology domain 3; CML, chronic myelogenous leukemia; EGFR, epidermal growth factor receptor; GB, glioblastoma; IGF, insulin-like growth factor; MDR, multidrug resistance; MGMT, O(6)-methylguanine-DNA methyltransferase; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung cancers; PARP, poly (ADP ribose) polymerase; PDGF, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RTKs, receptor tyrosine kinases; TMZ, temozolomide; TKIs, tyrosine kinase inhibitors.

[†]These authors contributed equally to this work.

action. Despite resistance, we remain clinically dependent upon use of both classes of drugs due to their unique respective benefits for treating cancer. As resistance remains the most critical impediment to success of either drug type, the current challenge is to determine how to rationally and effectively utilize these drugs in combination.

A number of significant scientific, logistical and financial issues pose a serious challenge. Understanding the many mechanisms by which cancers adapt to evade drug therapies and leveraging this knowledge into more effective combinations will require a thoughtful and rigorous integration of pre-clinical models and clinical trials. An empiric approach will not suffice: cancers contain too many targetable mutations and activated signaling pathways to develop sufficiently powered clinical trials to test them all. Combining targeted agents, although fully scientifically justified, poses significant challenges including cost, intellectual property considerations and cumulative toxicities. Have studies to identify mechanisms of intrinsic and acquired resistance to cytotoxic and targeted agents provided potential cues that could guide the next generation of combination therapies? Here, we review the existing considerable data supporting the idea that a deep knowledge of mechanisms of sensitivity and resistance may effectively guide rational combinations of cytotoxic and targeted agents, potentially suggesting a testable set of hypotheses that can be accomplished through well-designed clinical trials integrated with pre-clinical studies (Figure 1).

This review will compare and contrast drug resistance mechanisms in response to cytotoxic chemotherapy and molecularly targeted therapy across cancer types. We hope to present a case that distinctive mechanisms of resistance to cytotoxic and targeted agents provide a unique opportunity to develop complementary combinations that are less likely to have combined toxicities and more likely to produce clinical benefit. We will emphasize glioblastoma (GB) because of the breadth of genomic knowledge about the disease; however, several other cancer types will be discussed to illustrate generality. Further, the emphasis will be on pathway-specific, not organ-specific, resistance mechanisms and their implications for combination therapy. The focus will be on similarities and differences in the context of issues that are relevant to future treatment decisions and clinical treatment paradigms (2–4).

Resistance to cytotoxic chemotherapy

Chemotherapy uses highly potent chemicals that kill rapidly dividing cells. As most cancer cells are fast growing, tumors are especially sensitive to these drug treatments. Chemotherapy drugs directly interfere with cell division, often at the level of DNA, and are divided into classes based on their mechanism (Table I) (reviewed in ref. 5). Alkylating agents, such as carmustine and temozolomide (TMZ), and platinum compounds, such as cisplatin, directly damage DNA at any phase of the cell cycle, inducing a DNA-damage response that leads to apoptosis. Mitotic inhibitors, such as the taxanes (paclitaxel and docetaxel) and vinca alkaloids (vinblastine and vincristine), prevent cell division by acting on microtubules. Antitumor antibiotics, the major group being the anthracyclines such as doxorubicin, interfere with DNA synthesis by intercalating between DNA strands and, in some cases, also with DNA replication by inhibiting the activity of topoisomerases. Topoisomerase inhibitors, such as irinotecan and topotecan, also act in this way to inhibit DNA replication. Antimetabolites act by substituting for normal constituents of RNA, DNA or other cellular metabolites during the specific phases of the cell cycle in which these molecules are synthesized. Incorporation of these drugs causes macromolecular damage and cell death. Such antimetabolites include the pyrimidine antagonists 5-fluorouracil and capecitabine as well as the folic acid antagonist methotrexate.

Figure 1- Combination Cytotoxic and Targeted Therapy at a Glance

•**Primary and acquired drug resistance** represent major obstacles to successful cancer therapy.

•**Cytotoxic therapies**, the initial and still common pharmacologic approach, have limitations because their nonselective mode of action causes toxicity for normal proliferative tissues. Compounding this, tumors become resistant to chemotherapy.

•**Targeted therapeutic approaches** offer the opportunity to aim at cancer-specific targets that drive tumor growth. However, similarly to cytotoxic therapies, either pre-existing or acquired pathway and target alterations confer resistance.

•**Rational combinations** of cytotoxic and targeted therapies, based on understanding the mechanisms underlying sensitivity and resistance to each, represents an improved approach for cancer treatment, by simultaneous inhibition of complementing general proliferation mechanisms together with blockade of driver mutations or pathways conferring survival. Indeed, data gained from trials using agents that would be predicted to complement have been promising. This opens the possibility of even more effective and personalized therapies based on advances in understanding the cancer genome and tumor-specific biomarker networks of drug resistance.

Fig. 1. Summary of main points.

Drug-induced toxicity is a serious limitation because non-cancerous fast-growing cells in the body, such as those in the digestive tract, bone marrow, mucous membranes and hair follicles, are also susceptible to these drugs. Each class of drugs has a specific toxicity profile, and clinical use of the drugs is dependent on achieving an ideal therapeutic index, or maximizing the magnitude of the desired therapeutic effect while limiting toxic side effects. Moreover, although they are potent and effective in inducing cell death, resistance often occurs, thus rendering many of these drugs less clinically effective. Examples of mechanisms of resistance to chemotherapy drugs are shown in Figure 2 and Table I.

Pharmacokinetics and drug metabolism

Resistance to chemotherapy drugs falls into two categories with respect to the tumor cell: extrinsic and intrinsic. Extrinsic resistance

encompasses the failure of drugs to reach their site of action in an active form due to pharmacokinetic/pharmacodynamic reasons, such as a short serum half-life or rapid clearance by the kidneys and/or liver (Figure 2, I (19)). Alterations in endogenous tumor cell gene products involved in drug metabolism also affect their response to chemotherapy. As avenues toward overcoming some of these problems, chemotherapeutics have been conjugated to tumor-targeted antibodies or incorporated within carriers such as liposomes or nanoparticles. These approaches have demonstrated improved drug uptake and decreased drug-induced toxicity to normal tissue through enhancement of specific and efficient delivery to the tumor. Liposomal doxorubicin, for example, was the first encapsulated anticancer drug to gain Food and Drug Administration approval (20) and is now used routinely for the treatment of several tumor types such as multiple myeloma and

Table I. Cytotoxic chemotherapies

Cancer type	Standard therapy	Mechanism of action	Combinatorial treatment in clinical trials	Additional cell death inducing action
NSCLC (6)	Cisplatin (II, V)	DNA damage	Erlotinib	EGFR-targeted therapy
Cervical cancer (7,8)	Cisplatin (II, V)	DNA damage	Topotecan/paclitaxel (III)	Topoisomerase inhibitor/ microtubules stabilizer
Pancreatic cancer (9,10)	Gemcitabine (V)	Nucleoside analog	Capecitabine (V)	Nucleoside analog
Biliary tract cancer (11)	Gemcitabine (V)	Nucleoside analog	Cisplatin (II, V)	DNA damage
Pediatric childhood acute lymphoblastic leukemia, acute myelogenous leukemia, CML (12)	Methotrexate (II)	Folate antimetabolites	Rapalogs	MDR reversal
Breast cancer (13)	Anthracycline/taxane/ trastuzumab	Mixed	Lapatinib/capecitabine (V)	HER-2/EGFR inhibitor/ nucleoside analog
Bladder cancer (14)	Cisplatin (II, V)	DNA damage	Gemcitabine (V)	Nucleoside analog
Colorectal cancer (15)	Oxaliplatin (II, V)	DNA damage	Capecitabine (V)	Nucleoside analog
Cancer type	Standard therapy	Mechanism of action	Overcoming resistance treatment in clinical trials	Mechanism for overcoming resistance
GB (16)	TMZ (V)	DNA damage	O6-benzylguanine (V)	O6-alkylguanine-DNA alkyltransferase inhibitor
NSCLC (17)	Docetaxel (II, VI)	Microtubules stabilizer	AT-101	Bcl-2 family members inhibition
Chronic lymphocytic leukemia (18)	Standard or monoclonal therapy (II, VI)	Mixed	Navitoclax	Bcl-2 family members inhibition

Roman numerals (I–VI) indicate groups corresponding to Figure 2. I, pharmacokinetics/drug metabolism and tumor microenvironment; II, efflux pumps; III, drug-inactivating enzymes; IV, acidic vesicle compartments; V, DNA repair; VI, Bcl-2 family member-mediated resistance to apoptosis.

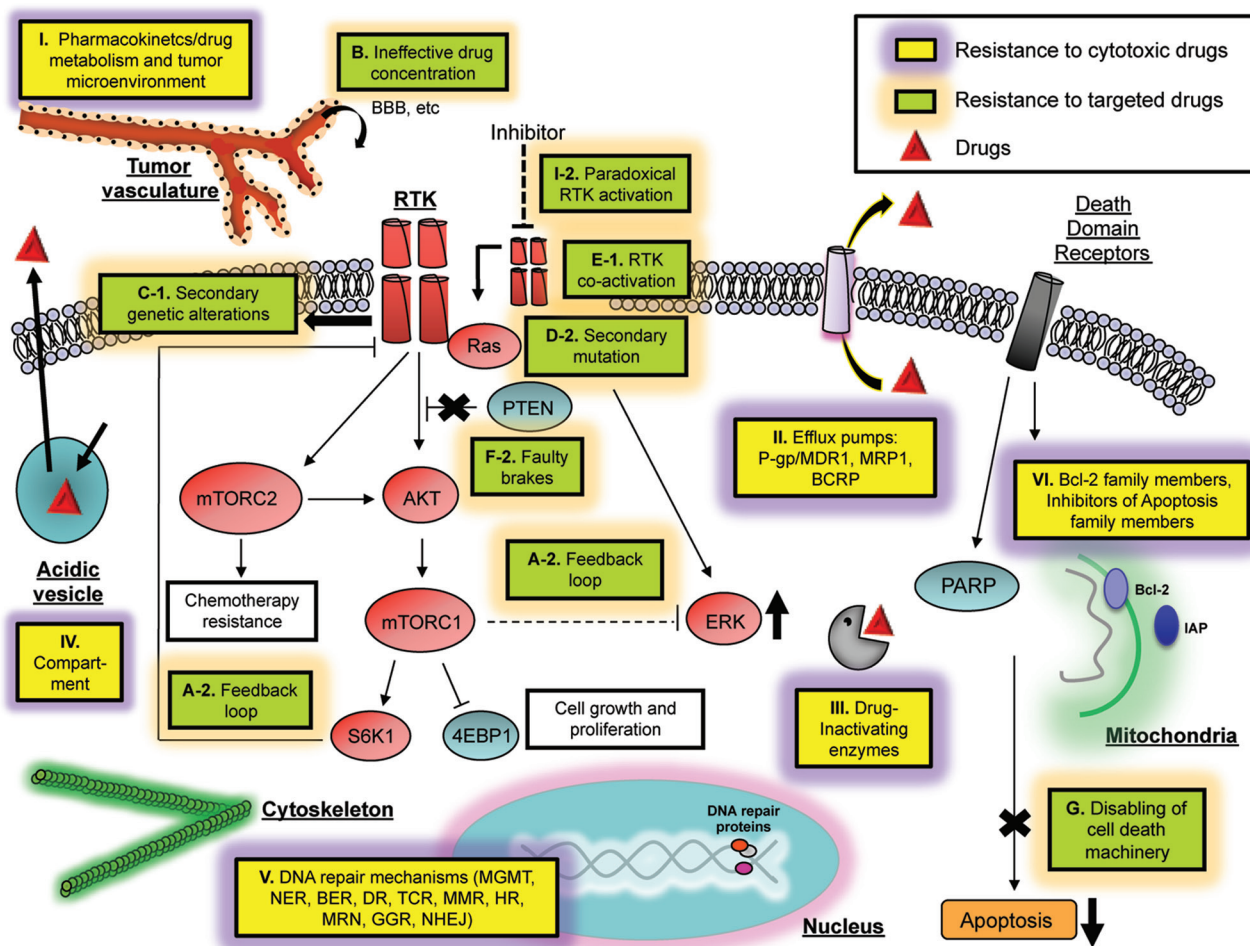


Fig. 2. Cellular mechanisms of resistance to cytotoxic chemotherapies and molecular targeted therapies. Resistance to cytotoxic therapy is commonly a result of failure of the drug to reach its intended target. This is mediated by enhanced activity of drug transporters, induction of emergency response genes to increase the repair of damaged DNA or dampening of mitochondrial-dependent apoptotic cell death. The main mechanisms for resistance to targeted therapies are genetic alteration/mutation of the target itself, persistent activation of downstream signaling pathways, bypass mechanisms such as activation of alternative driver-oncogenes or other downstream signaling pathways or pathway-independent mechanisms such as epigenetic alterations. Yellow boxes represent the major mechanisms of resistance to cytotoxic therapies, and green boxes denote resistance mechanisms to target therapies. Letters and numbers in each box correspond with those in Tables I, II, and III detailing resistance mechanisms.

metastatic breast and ovarian cancer (reviewed in ref. (21)). Substantive clinical evidence shows that liposomal encapsulation does increase tolerability when chemotherapy drugs are used in combination (22). Currently, there are a number of liposomal chemotherapeutic drugs in clinical development, including cisplatin (22) and irinotecan (23), and major efforts are focused on the development of novel, more efficient liposomal formulations. Similarly, antibody–drug conjugates are also under clinical development and exploit tumor-selective antigens to target chemotherapy. Two examples include brentuximab vedotin, which delivers the antimicrotubule agent monomethyl auristatin E to CD-30–positive lymphoma cells (24), and trastuzumab-DM1, which delivers a different antimicrotubule agent to HER-2-overexpressing breast cancer cells (25). In addition, some drugs are dependent upon intracellular activation by metabolic enzymes, such as cytochrome P450. Expression of and genetic polymorphisms in this and other drug-metabolizing enzymes significantly influences plasma levels of active drug and, by extension, drug sensitivity (26) (Figure 2, I).

The tumor microenvironment is another major component that is emerging as a significant mediator of extrinsic drug resistance (Figure 2, I). The abnormal vasculature of tumors often hinders their accessibility to chemotherapeutic drugs, but agents that increase tumor vessel density and perfusion can improve drug distribution and delivery. For example, inhibition of cellular signaling through the Hedgehog pathway leads to depletion of the stromal tissue and more efficient delivery of

gemcitabine in a model of pancreatic ductal adenocarcinoma (27). The hypoxic environment of tumors is also known to induce chemoresistance because it inhibits cell proliferation, and most chemotherapy drugs rely on rapid cell division for efficacy. In addition, the transcriptional changes induced by a hypoxic response include increased levels of molecules within tumor cells that contribute to resistance (28). Targeting of hypoxia-inducible factor-1, the major mediator of a hypoxic response, is one approach for blocking tumor hypoxia in an effort to increase drug sensitivity and response (reviewed in ref. 29). Importantly, modulation of the tumor microenvironment at the level of vascularization can also greatly impact hypoxia, which has a dual benefit in increasing both delivery and sensitivity of tumor cells to chemotherapeutic agents.

Drug efflux and decreased cellular uptake

Intrinsic cellular mechanisms of resistance involve processes such as removal of the drug from its site of action by increased efflux or decreased uptake, enzymatic modification/inactivation of the drug and alteration of drug target(s) within the cell. All of these processes represent avenues the cell can exploit to mount multidrug resistance (MDR): the ability of tumor cells to exhibit cross-resistance to several cytotoxic drugs at once (Figure 2, II). MDR was first discovered in the context of the p-glycoprotein family of proteins (30,31). These proteins are components of the adenosine triphosphate-binding

cassette transporter efflux pumps that, when expressed in tumor cells, effectively remove drugs from the cell. As a result, chemoresistance occurs due to insufficient intracellular drug concentrations. The specificity of efflux pumps for drugs has a wide spectrum and includes the anthracyclines (e.g. doxorubicin), the vinca alkaloids (e.g. vincristine and vinblastine) and microtubule stabilizing drugs such as paclitaxel (reviewed in ref. 4). Overexpression of p-glycoprotein/MDR1 and other adenosine triphosphate-binding cassette drug transporter genes, such as *MRP1* and *BCRP*, have been shown to be associated with resistance *in vitro* (32) and clinically with poor response to therapy and shorter overall patient survival (33,34).

Alternatively, some water-soluble drugs depend on cellular energy/nutrient transporters for entrance into cells. Altered expression of these transporters can reduce uptake and resulting intracellular concentration of drugs such as carboplatin (35) and the antifolate methotrexate (36). Moreover, expression and activity of glutathione-S-transferase detoxification enzymes is typically high in MDR cells, further contributing to resistance (37). Expression of these and other related drug-inactivating enzymes represent another cell-intrinsic mechanism of resistance (Figure 2, III).

Several approaches have been taken to re-sensitize cells that have become chemoresistant through efflux-pump-mediated MDR. For example, MDR reversal agents such as verapamil, quinidine and cyclosporine analogs act as direct or indirect/allosteric inhibitors against the efflux pump to prevent drug binding to the pump and, consequently, they block transport of the drug out of the cell (reviewed in ref. 38). Many of these have been tested clinically, but unfortunately have produced marginal benefits due to problems with high toxicity, low efficacy at non-toxic doses and metabolic inactivation (39–45) (reviewed in ref. 46).

Intracellular drug sequestration

Another tactic that tumor cells employ to avoid the cytotoxic effects of some chemotherapeutics is to sequester the drug away from its intracellular site of action. Chemotherapeutic drugs are localized predominantly in specific organelles within the cytoplasm of drug-resistant cells, whereas sensitive cells exhibit more nuclear drug localization and a more general distribution throughout the cytoplasm (47). It is thought that the weak basic charge of many chemotherapy drugs results in increased trapping within acidic vesicles followed by secretion from the cells by normal vesicular transport. Consistent with this model, drug-sensitive cells have been shown to harbor reduced pH gradients across vesicular membranes compared with resistant cells. Furthermore, reversal of drug resistance occurs upon disruption of pH gradients in resistant cells (48) (Figure 2, IV).

DNA repair enzyme-mediated resistance

As cancer cells rely on DNA synthesis to meet their proliferative needs, a large number of chemotherapeutic drugs are DNA damaging agents. DNA damage normally prompts either repair of the damaged DNA or induction of signals for the cell to undergo apoptosis as a means for preventing expansion of cells harboring damaged DNA. The DNA repair machinery is a complex of enzymes whose function is to assure the integrity of DNA strands and ensure repair in the event of damage. DNA damage induces specific damage-type classes of repair enzymes, including direct reversal, transcription-coupled repair, mismatch repair, homologous recombination, Mre11–Rad50–Nbs1 complex, base excision repair, nucleotide excision repair, global genome repair and non-homologous end joining (reviewed in ref. 49).

Tumors utilize various strategies to overcome the DNA damage induced by chemotherapy, many of which rely upon the enzymes that mediate the various forms of DNA repair (Figure 2, V). TMZ, an alkylating agent used for the treatment of GBM, is known to induce cytotoxic cell death through the formation of O(6)-methylguanine adducts in the DNA. The primary mechanism associated with TMZ resistance in patients is the expression of the DNA repair enzyme O(6)-methylguanine-DNA methyltransferase (MGMT) in malignant cells where it repairs specific damage caused by TMZ, thus allowing cancer cell survival. The regulation of MGMT expression has

been widely investigated and of particular interest is the methylation status of its promoter, which is often altered in GB patients. MGMT promoter methylation is predictive of improved response to TMZ treatment in glioma patients as compared with patients with unmethylated MGMT promoters (50). However, MGMT is not the only enzyme responsible for TMZ failure in glioma patients. Recently, a base excision repair-associated enzyme called alkylpurine-DNA-N-glycosylase has also been shown to contribute to TMZ resistance (51). Indeed, multiple mechanisms likely collaborate to neutralize cytotoxic therapies making the circumvention of drug resistance particularly challenging.

Defects in apoptosis

All chemotherapeutic drugs, regardless of their specific target or mechanism of action, produce the same cytotoxic end effect in sensitive cells: apoptotic cell death. In fact, the success of chemotherapy is almost completely dependent upon the ability of the cell to undergo apoptosis. This provides the tumor cell with another avenue to chemoresistance mediated through the cellular reprogramming of pathways that are responsible for inducing apoptosis. Inhibition or loss of pro-apoptotic proteins and pathways is often associated with the clinical onset of resistance to chemotherapy, with an important role played by the ATM–Chk2–p53 axis, as exemplified in the case of breast cancer (52). Another important role is played by the Bcl-2 family of proteins. For example, the expression of pro-apoptotic Bim, which inhibits the pro-survival proteins Bcl-2 and Bcl-xL and acts directly at the mitochondria to engage apoptosis-inducing Bax and Bak, appears central for the ability of cells to undergo apoptosis in response to chemotherapeutic drugs (reviewed in ref. 53). In addition, the p53-regulated pro-apoptotic members Puma and Noxa are required for apoptotic cell death in response to DNA damage-inducing agents in lymphoma (54). Many tumors inherently or in response to therapy express high levels of pro-survival proteins such as Bcl-2 and Bcl-xL (53) as well as inhibitor of apoptosis family members such as cIAP, XIAP and ML-IAP (55) (Figure 2, VI). As a result, there is significant effort in overcoming chemotherapy-induced resistance to apoptosis by using agents that are specifically targeted to components of the apoptotic machinery within the cell. A promising therapeutic approach is the use of Bcl-2 homology domain 3 (BH3) mimetic compounds, such as ABT-737, which mimic the function of Bim and inhibit Bcl-xL, Bcl-2 and BclW (56). Pre-clinical studies have clearly demonstrated the ability of these drugs to chemosensitize resistant cells (57) and their clinical efficacy in combination with chemotherapeutics is encouraging (17,18). Resistance to apoptosis is also a predominant theme in tumor cells that have failed to respond to molecularly targeted therapy and represents an area where the distinctions between resistance to chemo- and targeted therapy begin to overlap (Figure 2, VI and G).

To summarize, the dominant intrinsic mechanisms of resistance to cytotoxic chemotherapies include efflux-pump-mediated MDR to remove the agent from cells, activation of DNA repair pathways and failure of the cellular apoptotic machinery, both of which prevent DNA damage from killing tumor cells. Of note, these mechanisms can be at the cell surface, mitochondria or nucleus and none of them are known to be dependent upon activated cancer signaling pathways downstream of oncogene and tumor suppressor mutations, although such altered signaling could regulate their function.

Resistance to molecularly targeted cancer therapy

The cancer treatment field has evolved considerably over the past years with the introduction of targeted therapies that are characterized by higher specificities of action than chemotherapy and which promise the potential for tumor eradication with decreased toxicity. Cancers typically contain multiple mutations, but may develop dependence on a single mutation for growth and survival rendering them preferentially susceptible to targeted inhibition (58,59). Experimental models, and some clinical examples, support the ‘oncogene addiction’ hypothesis, providing compelling rationale for

targeted cancer therapy (60,61). The National Cancer Institute defines targeted therapies as drugs that interfere with specific key molecules involved in tumor growth and progression. These compounds comprise small molecules or monoclonal antibodies that block tumor proliferation and may indirectly or directly induce apoptosis. The small molecules penetrate cells to reach their intracellular targets, whereas the monoclonal antibodies are typically directed against cell surface or extracellular antigens. Targeted therapies affecting cell growth signaling include inhibitors of receptor tyrosine kinases (RTKs) and drugs affecting downstream kinases. Aberrant RTK activation has a pivotal role in cancer progression and is due to gene amplification, receptor overexpression, autocrine activation or gain of function mutations (62). Many RTKs and downstream kinases are being targeted by clinically approved agents and include epidermal growth factor receptor (EGFR) (63), HER-2 (64), MET (65), phosphatidylinositol 3-kinase (PI3K) (66), mammalian target of rapamycin (mTOR) (67) and BRAF (68), among others (Tables II and III). As a representative example, hyperactivation of the EGFR/PI3K/Akt/mTOR cascade occurs in up to 50% of GB tumors, which generally respond poorly to standard non-targeted therapies. Clinical trials with small molecule EGFR-selective tyrosine kinase inhibitors (TKIs) have been conducted with improved outcome for a subgroup of patients (63). Specifically, inhibition of EGFR with erlotinib is preferentially effective in GBM patients co-expressing the common constitutively active mutant EGFR (EGFRvIII) and the tumor suppressor phosphatase and tensin homolog (PTEN) (63), demonstrating the necessity to molecularly characterize each tumor for prediction of therapeutic efficacy (69). Further, erlotinib is an inhibitor of EGFR in its active conformation and poorly blocks the EGFR extracellular domain mutants expressed in GB as compared with lapatinib, which inhibits EGFR in its inactive conformation (70). In contrast, non-small cell lung cancers (NSCLCs) primarily express EGFR that is mutated within the kinase, rather than the extracellular, domain and inhibitors such as erlotinib demonstrate remarkable efficacy for NSCLC (70). This highlights the need to carefully pair specific drugs with specific targets, as it is clear that the drug effect can be drastically different based on the specific mutations of the target.

As shown in Tables II and III, targeted therapy can also involve inhibition of downstream effectors of oncogenic mutations. EGFRvIII-expressing GB cells rely preferentially on the activity of mTORC2, which confers tumorigenicity through activation of the nuclear factor- κ B pathway (135). This implies the possibility of suppressing GB growth by targeting mTORC2 and clinical trials are now testing combinatorial therapies designed to achieve more complete inhibition of EGFR/PI3K/Akt/mTOR signaling. The strategy is to inhibit both upstream oncogenic signaling and downstream effectors by administering allosteric mTOR inhibitors in combination with erlotinib, or using dual PI3K/mTOR kinase inhibitors (100,136). However, just as for chemotherapies, the molecularly targeted therapies have thus far achieved clinically relevant efficacy in only a limited number of cases because of the emergence of drug resistance and because the pairing of patients who are genotypically subject to the agent is in its infancy.

Examples of targeted therapies and corresponding mechanisms that cancers invoke to evade treatment are shown in Tables II and III and Figure 2. Resistance to targeted drugs can be classified as intrinsic (primary) or acquired (secondary) and often relies on cellular responses that maintain the signal flux despite effective RTK targeting and maintain signaling through alteration of downstream effectors and/or disable the cell death machinery through compensatory cell survival pathways. Other mechanisms of targeted drug resistance have also been described in the literature and are described.

Maintaining signal flux while targeting RTKs

One of the most common mechanisms of resistance to targeted therapies involves the maintenance of oncogenic signaling through downstream pathways despite efficient target inhibition

by a drug (Tables II and III, Figure 2). These include co-activation of other RTKs (Figure 2, E-1), signaling by physiologically regulated RTKs while mutant RTKs are inhibited (Figure 2, I-2), and secondary activating resistance mutations (Figure 2, C-1). EGFR TKIs and the allosteric mTOR inhibitor rapamycin (and its derivatives) have failed to display durable efficacy in gliomas even though the rationale for targeting these molecules has been clearly demonstrated (63,67,137–139). These failures may be explained by the relative ease with which cancers develop compensatory resistance mechanisms to maintain signal flux. In this regard, it was reported that when treated with EGFR inhibitors, other RTKs such as c-MET and/or platelet-derived growth factor receptor (PDGFR) become co-activated, engaging PI3K to maintain downstream pathway activation (140,141). Thus, effective targeted therapy may require combined regimens targeting multiple RTKs. This is consistent with the demonstration that inhibition of AKT induces the expression and phosphorylation of multiple RTKs, primarily due to mTORC1 inhibition and secondary to a FOXO-dependent transcriptional activation of receptor expression (142). Furthermore, pre-clinical studies in GB have shown that upon EGFR inhibition, compensatory signaling is mediated by fibroblast growth factor receptor and SRC family kinases and involves the inactivating phosphorylation of PTEN at Y240 (104).

EGFR kinase domain mutations have been identified in NSCLC patients who are responsive to the reversible EGFR TKIs gefitinib and erlotinib. Tumor responses to these agents are dictated by the presence of EGFR somatic mutations, increased gene copy number and certain clinical and pathological features (72). Despite dramatic responses to such inhibitors in some cases, most patients eventually become resistant and progression of the disease follows. In those tumors with acquired resistance, a second point mutation occurs in exons encoding the kinase domain of EGFR (T790M) in about half of these cases (72,143). Structural and functional analyses revealed that this second mutation confers gefitinib and erlotinib resistance by increasing the affinity of the kinase domain for adenosine triphosphate, thereby decreasing affinity for the TKIs. In addition, a second-site D761Y EGFR mutation reduced the sensitivity of the drug-sensitive L858R EGFR mutant to TKIs in a brain metastasis of NSCLC (144). Irreversible second-generation EGFR inhibitors and those designed to specifically bind the T790M mutant receptor are under clinical development and may overcome second-site mutation-mediated resistance (145,146).

Maintaining the signal through downstream effectors

Another type of acquired drug resistance is achieved by tumor cells through rewiring of downstream signaling pathways thereby conferring faulty brakes on growth checkpoints (Figure 2, F-2), secondary mutations in downstream signaling effectors (Figure 2, D-2) and/or feedback loop activation (Figure 2, A-2). The previously cited demonstration that expression of the constitutively active mutant EGFRvIII sensitized GBs to EGFR inhibitors only in the context of intact PTEN expression and function underscores the combinatorial nature of drug sensitivity. As loss of PTEN function is a common event in GB, many patients expressing EGFRvIII, but without functional PTEN, were resistant to erlotinib (63) due to uncoupled inhibition of EGFR from PTEN-mediated inhibition of downstream PI3K signaling flux (63). Consistent with this, patients whose tumors had high levels of EGFR coupled with low levels of activated AKT were more likely to respond to the TKIs (138). These studies indicate that intact negative regulation of downstream PI3K signaling appears to be critical for effective response to EGFR inhibitors and that efficacy of targeted agents, or such agents with chemotherapy, is likely to require consideration of genetic synthetic lethality or complementation for optimal effect.

The PI3Ks are a family of lipid kinases grouped into three classes with class IA PI3Ks being activated by the binding of a growth factor to its associated cognate RTK. The *PIK3CA* gene

Table II. Targeted therapies with small molecules

Cancer type	Small molecule and target	Determinant of sensitivity	Determinant of resistance	Clinical trials to overcome resistance
NSCLC (71)	1) <i>Gefitinib</i> -EGFR (72); 1) <i>Crizotinib</i> -ALK (73)	<i>EGFR</i> mutations (74)	C) T790M <i>EGFR</i> mutation (72); E) <i>MET</i> amplification (72,75); G) <i>BIM</i> mutations (76)	<i>EGFR</i> and <i>MET</i> inhibitors (65); Combination with retinoid (71); Combination with chemotherapy (77) <i>ND</i>
GIST (78)	1) <i>Imatinib</i> -KIT, PDGFR α (78)	C) Types of KIT (78)	CPDGFR α (79) mutations	<i>ND</i>
Colorectal cancer (80)	2) <i>Everolimus</i> -mTOR (80); 2) <i>Vemurafenib</i> -BRAF (81), <i>olaparib</i> -PARP (82), <i>regorafenib</i> -TKI, <i>cediranib</i> -VEGFR;	PIK3CA mutation, PTEN loss of function (80), V600E BRAF mutation (81)	D) <i>KRAS</i> mutation (80) E) Increased <i>EGFR</i> expression (81)	Combination with conventional chemotherapy partially successful (83)
mCRC (83)	<i>Aflibercept</i> -VEGFA/B, PIGF (83)			
CML (84)	2) <i>Imatinib</i> , <i>nilotinib</i> , <i>dasatinib</i> -BCR-ABL1 (84–86)	BCR-ABL1 mutation (84)	C) Target mutations or amplification (87,88); I) extracellular signal-regulated kinase activation (84); I) PI3K, Src, Janus kinases (89,90); G) <i>BIM</i> mutations (76); B) pharmacokinetic failure (91) D) <i>KRAS</i> mutations (94)	Pan-ABL inhibitors (92), treatment with mycophenolic acid (93)
Various (66,94)	2) <i>Sunitinib</i> , <i>everolimus</i> , <i>temsirolimus</i> , PI3K inhibitors, PI3K and mTOR or MEK inhibitors, Akt inhibitors-PI3K/AKT/mTOR pathway (94); 2) <i>Everolimus</i> -mTOR (80);	H1047R PIK3CA mutation (94); <i>BIM</i> mutation (preclinical test (95))		Target therapies are in progress
Pancreatic cancer, HNSCC (80)	3) <i>Olaparib</i> -PARP (96)	PIK3CA mutation, PTEN loss of function (80); <i>BRCA1/2</i> mutations (96)	D) <i>KRAS</i> mutation (80)	<i>ND</i>
TNBC (96)	1) <i>Lapatinib</i> , <i>neratinib</i> , <i>afatinib</i> -HER-2 (64); tamoxifen-ER (97); <i>olaparib</i> -PARP (82); <i>MET</i> inhibitors (65)	PIK3CA mutation (98); PTEN loss of function (80)	<i>ND</i> D) PI3K mutations (97); E) Alternative pathway and HER signaling (99); F) Constitutive activation of downstream effectors (64); D) <i>BRCA1</i> and <i>BRCA2</i> mutations (82); B) drug efflux (102); micro RNA (103); PTEN Y240 (104); pharmacokinetic failure (70); A) Redundant signaling through alternate pathways (67); Not understood (105)	PI3K and PARP inhibitors (96) TKI plus chemotherapy (64)
Breast cancer (64)				
GB (100)	1) <i>Erlotinib</i> -EGFR (101); 1) <i>Lapatinib</i> -EGFR (70); 2) <i>Rapamycin</i> -mTOR (67)	PTEN expression (101); PTEN loss (67)		Dual inhibitor PI3K and mTOR (100)
Renal cancer (105)	1) <i>MET</i> antagonists, <i>MET</i> kinase inhibitors- <i>MET</i> (65,105); 2) <i>Temsirolimus</i> -mTOR(106); 2) <i>Everolimus</i> -mTOR(107)	Hypoxia-inducible factor-1 α (106)		New immunotherapeutic strategies are emerging (105); Combination with antibody (107) <i>ND</i>
Ovarian cancer (66)	2) <i>Temsirolimus</i> -PI3K/AKT/mTOR pathway (98); 3) <i>olaparib</i> -PARP (82); 1) A6-uPAR (108)	PIK3CA and <i>KRAS</i> or <i>BRAF</i> mutations (98)	D) <i>BRCA1</i> , <i>BRCA2</i> mutations (82) D) <i>KRAS</i> mutation(98,109)	
Cervical cancer, Endometrial cancer (98,109)	2) <i>Temsirolimus</i> -PI3K/AKT/mTOR pathway(98)	PIK3CA and <i>KRAS</i> or <i>BRAF</i> mutations(98)		Combined inhibition of the RAS/RAF/MEK and PI3K/AKT/mTOR pathways suggested (110)
Melanoma (68)	2) <i>Vemurafenib</i> -mitogen-activated protein kinase dependent and independent pathways (68,111); 2) <i>Everolimus</i> -mTOR (80); <i>Olaparib</i> -PARP (82)	V600E BRAF mutation, KIT mutation (68); PIK3CA mutation, PTEN loss of function (80)	D) NRAS-mutant clones (112); H) amplification of MAP3K8 (113); E) increased IGF-1R signaling (114); F) loss of PTEN and G) <i>Bim</i> (115); D) MEK mutations (116); D) BRAF alterations (117,118); E) PDGF overexpression (112); D) <i>BRCA1</i> , <i>BRCA2</i> mutations (82)	Combination of BRAF and MEK inhibitors (119,120)
DLBCL (121)	<i>Fostamatinib</i> -SYK, <i>enzastaurin</i> -PKC β (122,123); <i>ruxolitinib</i> -JAK/STAT5B1518, <i>alisertib</i> -Aurora A (121); <i>panobinostat</i> -histone deacetylase, <i>bortezomib</i> -proteasome (121,124)	BCR signaling (122) PKC β over-expression (123); Aurora A over-expression (121)	<i>ND</i>	Therapies still in clinical trials (124)

Numbers refer, respectively, to 1), RTK targets; 2), downstream kinase; 3) cell death targets. Letters refer to resistance mechanisms in Figure 2. GIST, gastrointestinal stromal tumor; HNSCC, head and neck squamous cell carcinoma; TNBC, triple negative breast cancer; DLBCL, diffuse large B-cell lymphoma; ND, not determined.

Italicized text in 'small molecule and target' column indicates drug names.

Table III. Targeted therapies with antibodies (125,126)

Cancer type	Small molecule and target	Determinant of sensitivity	Determinant of resistance	Clinical trials to overcome resistance
mCRC (83)	1) <i>Bevacizumab</i> -VEGF, <i>ramucirumab</i> -VEGFR2 (83)	ND	ND	Combination with conventional chemotherapy partially successful (83)
Breast cancer (64)	1) <i>Trastuzumab</i> , <i>pertuzumab</i> , <i>ertumaxomab</i> -HER-2 (64)	<i>PIK3CA</i> mutation (98)	C) Altered target, E) alternative pathway and HER signaling (99); F) constitutive activation of downstream effectors (64)	<i>Trastuzumab plus pertuzumab</i> and docetaxel (127), <i>Trastuzumab-DM1</i> (128); double HER-2 inhibition with antibodies plus small TKI molecules (64)
Glioma (125)	1) <i>Cetuximab</i> -EGFR (125,129)	ND	ND	ND
Renal cancer (105)	1) <i>Bevacizumab</i> -VEGF (107)	ND	ND	New immunotherapeutic strategies are emerging (105); combination with TKI (107)
Ovarian cancer (66)		<i>PIK3CA</i> and <i>KRAS</i> or <i>BRAF</i> mutations (98)	D) <i>KRAS</i> mutation (98,109)	ND
Cervical cancer, endometrial cancer (98,109)	1) <i>Bevacizumab</i> - 2) PI3K/AKT/mTOR pathway (98)	<i>PIK3CA</i> mutation (98,109)	D) <i>KRAS</i> mutation (98,109)	Combined inhibition of the RAS/RAF/MEK and PI3K/AKT/mTOR pathways suggested (110)
Melanoma (68)	1) <i>Bevacizumab</i> -VEGF inhibitor (130); <i>Ipilimumab</i> -CTLA-4 (131)	ND	ND	Combination with cancer vaccine and chemotherapy (68,132); combination of <i>ipilimumab</i> and <i>vemurafenib</i> -V600E <i>BRAF</i> mutation inhibitor- in progress (128)
DLBCL (121)	<i>Rituximab</i> -CD20 (121)	ND	ND	Aurora A inhibitor, chemotherapy and <i>rituximab</i> (121); Combination with chemotherapy (124,133)

Numbers refer, respectively, to 1), RTK targets; 2), downstream kinase target. Letters refer to resistance mechanisms represented in Figure 2. DLBCL, diffuse large B-cell lymphoma; ND, not determined.

Detailed review on immunotherapy and targeted therapies combination is available in Vanneman *et al.* (134).

Italicized text in 'small molecule and target' column indicates drug names.

encodes the p85 regulatory and the p110 catalytic subunits of PI3K. Activating mutations in *PIK3CA* have been found in approximately 25% of primary breast cancers, and these occur almost exclusively in PTEN-positive tumors (147). *PIK3CA* mutations contribute to increased risk for progression in trastuzumab-treated HER-2-positive breast cancer (148) and *PIK3CA* exon 20 mutations are also associated with a lack of cetuximab sensitivity in colorectal tumors (149). This suggests that the relationship between mutant *PIK3CA* and other aberrations affects response to PI3K/AKT/mTOR axis inhibitors and the necessity for further characterization and understanding of which mutation combinations cause sensitization or resistance.

mTOR acts within the canonical PI3K signaling pathway to mediate cell growth and proliferation, and mTOR is an important integrator of several targetable signaling cascades (69). However, studies in patients with recurrent malignant gliomas have failed to demonstrate consistent responses to the mTOR inhibitor rapamycin or its analogs (139,150–152). Paradoxically, rapamycin treatment leads to AKT activation by disrupting the negative feedback loop that normally attenuates PI3K signaling and is associated with significantly shorter time-to-progression (67). This finding suggests that a more effective way to target mTOR signaling and to prevent PI3K pathway reactivation might be to use a dual PI3K/mTOR inhibitor (153). Dual PI3K/mTOR inhibitors may also suppress the ability of mTORC1 to activate extracellular signal-regulated kinase signaling by a PI3K-dependent mechanism, an example of the remarkable plasticity of tumor cells and their capacity for rewiring (154). Additionally, it has been increasingly recognized that rapamycin only partially inhibits

the phosphorylation of 4E-BP1, which may further contribute to the resistance to rapamycin treatment in cancer (155).

mTOR inhibition has been explored in pre-clinical studies with breast cancer cells where inhibiting mTORC1/C2 activity with mTOR kinase inhibitors had a biphasic effect on Akt phosphorylation and function (156). Specifically, the mTOR inhibitor induced a temporary inhibition of Akt-Thr308 phosphorylation followed by reactivation through hyperactivation of PI3K signaling, which is mediated by insulin-like growth factor (IGF)-1 receptor/insulin receptor signaling. By preventing the relief of this RTK survival feedback loop with the combinatorial treatment of mTOR and HER-kinase inhibitors, T308 phosphorylation has been shown to be stably inhibited in breast cancer xenograft models, providing a rationale to consider this approach in cancer therapy.

Failure to induce cell death due to compensatory cell survival pathways and other mechanisms of resistance to targeted therapies

The failure to cause cell death due to disabling of the cellular death machinery (Figure 2, G) is a mechanism that has been characterized in chronic myelogenous leukemia (CML) and NSCLC. In CML and EGFR-mutant NSCLC, a germ-line polymorphism results in expression of a novel isoform of the pro-apoptotic Bcl-2 family protein Bim (76). High levels of Bim expression are required to induce apoptosis upon TKI exposure (76). However, because this novel Bim isoform lacks the pro-apoptotic BH3, it is deficient in inducing apoptosis and thus contributes to poor intrinsic clinical response to TKI treatment. For these patients, sensitivity to targeted therapy might be generated using a combination of TKI and BH3

mimetics (76). This highlights the existence of inherited resistance determinants that are shared between different cancer types and are fundamental in guiding new synergistic treatment combinations for specific molecularly defined patients.

Two other examples of mechanisms of resistance to targeted therapies are resistance dependent on differential cell-type sensitivity or on DNA repair pathways. The presence of a small population of cancer cells, defined as cancer stem cells, that have the ability to self-renew and are refractory to conventional drug treatment because of MDR activity (157) or enhanced DNA damage response, has been described ref. 158.

With respect to EGFR inhibition, a selected population of NSCLC and prostate cancer cells develop a chromatin-mediated state of tolerance upon EGFR inhibitor treatment that is dependent on epigenetic regulation of chromatin and IGF-1 signaling. By inhibiting IGF-1 receptor or using chromatin-modifying compounds, this secondary resistance-triggering action can be prevented (159).

More than 80% of endometrioid endometrial cancers are characterized by loss of PTEN function. This deficiency, similarly as found in GB, is generally correlated with resistance to TKI inhibition (101), and, in endometrioid endometrial cancer, with sensitization to poly (ADP ribose) polymerase (PARP) inhibitors (160). This lethal effect in PTEN-mutant endometrioid endometrial cancer has been correlated with genomic instability elicited by defects in homologous recombination DNA repair (160).

All of the examples above present compelling evidence that the best approach to achieving a clinical response to molecularly targeted therapy will result from the utilization of molecular tools to stratify patients and clinical trials to assess the most effective strategy to inhibit both the triggering mutations and the mechanisms that evade drug efficacy. Importantly, several studies using massively parallel sequencing technologies have reported intratumoral genetic heterogeneity in solid cancers (161,162) and revealed that specific mutations, including PIK3CA or PTEN mutations, may only be predominant in a subset of tumor cells in a given cancer (163,164). This observation implies the necessity to develop robust and accurate diagnostic biomarkers to collect representative tumor specimens pre- and post-drug treatment and personalize therapies throughout the disease course.

A more recent addition to the realm of targeted therapy is the drug crizotinib, an inhibitor of the RTKs anaplastic lymphoma kinase (ALK) (165) and c-Met (165,166). It has been approved and shown to be efficacious for the treatment of NSCLC patients carrying the specific fusion gene involving EML4 and ALK, namely EML4-ALK (73,167), and is entered in clinical trials for anaplastic large-cell lymphoma (NCT00585195). It was also shown to be effective in treating a patient with a myofibroblastic tumor (168). Despite initial enthusiasm, resistance mechanisms have been described, such as ligand overexpression to overcome receptor signaling blockade (166), poor blood-brain barrier penetration (169), mutations in the ALK protein that inhibit crizotinib action (170–172) or paracrine signaling activating additional survival pathways (173). A novel ALK inhibitor, LDK378, capable of overcoming resistance to crizotinib is now in phase II clinical trials (NCT01685138 and NCT01685060). A recent mouse neuroblastoma model expressing an ALK mutation highlighted mTOR as an alternate target to block in overcoming crizotinib resistance (174). All of this suggests that, even for the most contemporary targeted agents, monotherapy is unlikely to produce long-term benefit.

To summarize, the dominant mechanisms of intrinsic resistance to targeted therapies involve maintained signal flux to downstream signaling effectors as a consequence of: second-site resistance mutations or amplification of drug targets, loss of downstream pathway suppressors, secondary genetic alterations and feedback loops or co-activation of other upstream effectors such as other altered RTKs. Moreover, the mechanisms of resistance are largely cytoplasmic; they engage the signaling cascades that are not the core components of, but may actually impact the drug efflux-MDR system, the intrinsic apoptotic circuitry and DNA repair pathways. In conclusion, a rigorous

analysis of resistance mechanisms may suggest a set of complementary combination cytotoxic plus targeted therapy approaches.

Therapeutic combination of cytotoxic and molecular targeted agents

Drug resistance is perhaps the predominant factor limiting the clinical success of cytotoxic chemotherapy as well as molecular targeted therapy. The complexity of cancer cells and tumors prevents the success of either type of agent alone or combinations of the same types of drug. Each therapeutic approach hinges upon an essential dependency: on DNA synthesis and/or cell division in the case of chemotherapy and on the targeted pathway or oncogene in the case of molecular targeted therapy. Like their dependencies and mechanisms of action, resistance is reflective of the type of drug. Resistance to cytotoxic therapy is commonly a result of failure of the drug to reach its intended target in an active form within a rapidly dividing cell. In contrast, the major mechanisms for resistance to targeted therapy are genetic alteration/mutation of the target itself, persistent activation of downstream signaling pathways, bypass mechanisms such as activation of alternative oncogenes or other downstream signaling pathways, or pathway-independent mechanisms such as epigenetic alterations or epithelial-to-mesenchymal transition (175,176). Analogous to the concept of genetic complementation or synthetic lethality, the combination of these two classes of drugs clinically would appear to be promising. However, in order to glean the most information, combinations should be approached rationally and not in the opportunistic and less-informed way in which combinations have been tested and failed to demonstrate clinical benefit. In illustrating this point, it is useful to consider examples of combinations that have demonstrated clinical efficacy (or that have failed) as a way to inform the development of future treatment paradigms.

The treatment of advanced HER-2-positive breast cancer offers a good example of recently introduced protocols that have markedly improved the progression-free survival of patients (Figure 3A). Conventional clinical practice for HER-2-positive breast cancer until recently was based on sequential chemotherapies and/or antihormonal treatments (64). Targeted therapies in breast cancer are mainly directed against HER-2 that is overexpressed or amplified in 15–20% of cases (64) and involves HER-2 inhibition by monoclonal antibodies, immunochemotherapy and small molecules (Tables II and III). However, the acquisition of acquired resistance to HER-2-targeted therapies after initial response remains a daunting clinical problem (Tables II and III). The introduction of simultaneous multitargeted therapies also presents logistic limitations for cancer treatment suggesting that a more achievable approach might entail the combination of single targeted therapy together with chemotherapy or immunochemotherapy (Tables II and III, Figure 3A (64)). Indeed, single agent trastuzumab in combination with chemotherapy demonstrated increased patient response rates compared with single agents alone (Figure 2A (177)). In addition, immunochemotherapy with trastuzumab-DM1 demonstrated higher progression-free survival than chemotherapy combined with TKI in a randomized phase III clinical trial (128). Phase III trials in which several HER family blockers were used simultaneously with chemotherapy also have proven to be more effective than trastuzumab alone (127). New TKIs are also being tested and could potentially enhance the outcome for breast cancer patients (64).

The breast cancer example is one of several demonstrating that complementing chemo- and targeted therapy can be an effective approach that minimizes the toxicity limitations of chemotherapy. Another case in point is mTOR inhibition using rapalogs in combination with methotrexate for hematological malignancies such as acute myelogenous leukemia and acute lymphocytic leukemia (Figure 3B) where improvements in survival have been achieved (12,178). For acute promyelocytic leukemia, degradation of the oncogenic fusion gene PML-RAR α is mediated by treatment with retinoic acid and arsenic and produces disease remission (179,180) and upon relapse, combination with chemotherapy induced complete remission of the disease (181,182).

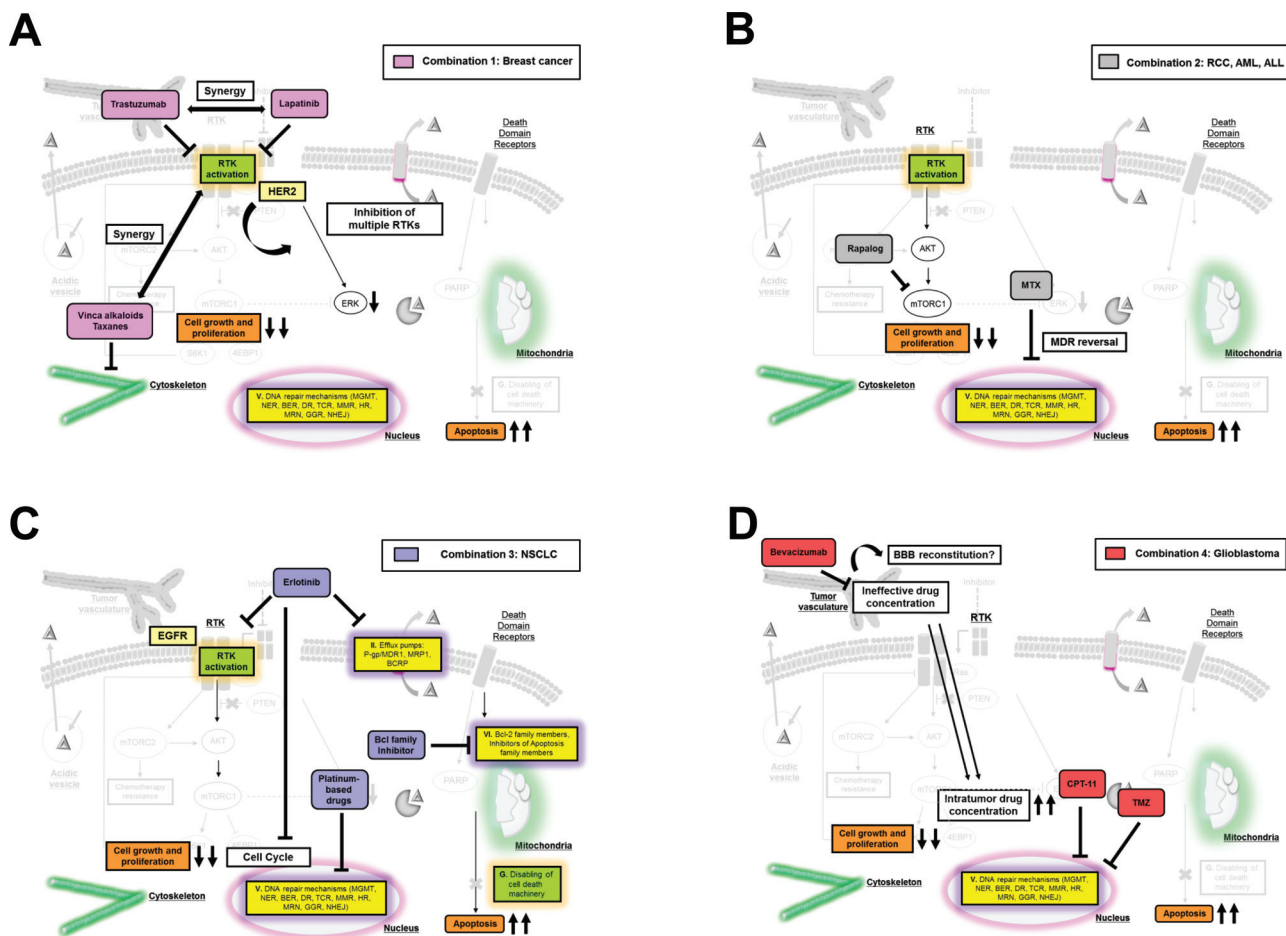


Fig. 3. Examples of rational combinations of chemotherapies and targeted therapies. Chemo- and targeted therapies are interrelated in that the limitations of one approach can be overcome by corresponding benefits of the other. Several combinations of chemotherapy and targeted therapy for different types of cancers are depicted by corresponding color boxes. (A) Combinations of HER-2 targeted therapies and chemotherapies for breast cancers demonstrate synergistic effects as well as inhibition of multiple RTKs. (B) Rapalog and methotrexate combinations for renal cancers and leukemias improve their effects through MDR reversal. (C) Erlotinib induces G₁-arrest and inhibition of efflux pumps, which can synergize with chemotherapeutics, and sequential administration of EGFR TKIs following chemotherapy provides a greater effect than concurrent administration. Also, resistance to chemo- and targeted therapies converges on an inability to undergo apoptosis, and pharmacological inhibition of pro-apoptotic members including the Bcl-2 family could overcome chemotherapy drug resistance. (D) Addition of bevacizumab can promote the efficacy of combined cytotoxic agents through the improved delivery of cytotoxic agents *via* normalization of the tumor vasculature. On the contrary, it could reconstitute the blood–brain barrier in GBM, preventing the diffusion of anticancer drugs.

Although chemotherapy drugs are extremely potent in their ability to induce apoptosis of rapidly dividing cells, it has been shown that cell types exist within the tumor that are not susceptible. Cancer stem cells tend to be slow-growing components of a tumor and are therefore less likely to be affected by these drugs (183) resulting in tumor re-growth following chemotherapy. Combining chemotherapy with a targeted agent directed against the specific pathways responsible for cancer stem cell survival represents a potential remedy to this problem.

Importantly, resistance to both chemo- and targeted therapy converges on an inability to successfully undergo apoptosis. As discussed previously, pharmacological inhibition of pro-apoptotic members of the Bcl-2 family of proteins as a strategy for overcoming chemotherapy drug resistance has been well characterized at the pre-clinical level. The Bcl-2/Bcl-xL dual inhibitor ABT-263 (Navitoclax; Abbott Laboratories/Genentech) has been used in phase I clinical trials for lymphoid malignancies (18,184) and in a phase II trial for small cell lung cancer (185) (Figure 3C). Although this is a promising approach, efficacy still needs to be established for each of the cancer types because another Bcl-2 family inhibitor, AT-101, failed in clinical trials for small cell lung cancer (186), in NSCLC in combination with docetaxel (17) and in metastatic castration-resistant prostate cancer (187).

Another issue that must be taken into careful consideration when contemplating combinations for clinical use is that chemotherapy and

molecularly targeted therapy can interact with each other in both positive and detrimental manners. For example, bevacizumab (Avastin; Genentech), a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), can improve the efficacy of combined cytotoxic agents (188,189) through following several mechanisms: (i) normalization of the tumor vasculature culminating in the efficient delivery of cytotoxic agents to the tumor, (ii) prevention of rapid tumor cell re-population after cytotoxic therapies and (iii) augmentation of the antivascular effects of chemotherapeutic agents (190). However, to the contrary, it also has the ability to hinder the effect of the combination therapy by normalizing blood vessels and reconstituting the blood–brain barrier in brain tumors, thereby preventing the diffusion of anticancer drugs (Figure 3D (191)).

For advanced NSCLC, the standard treatment has long been a platinum-based two-drug chemotherapy regimen. However, disease progression following acute response is common. Because a large proportion of NSCLC patients have tumors that overexpress or harbor mutated EGFR, there has been a clinical effort to incorporate the use of EGFR TKIs with chemotherapy for treatment of this disease (Figure 3C). The EGFR TKI erlotinib (Tarceva®) was approved for patients as second-line treatment following failure of chemotherapy or as a maintenance therapy following completion of chemotherapy based on successful clinical trials (192,193). This combination of

EGFR TKIs with concurrent cytotoxic chemotherapy was then tested in four randomized phase III clinical trials (194–197), all of which failed to demonstrate a benefit of the combination compared with chemotherapy alone. Pre-clinical data suggest that pharmacodynamic reasons are to blame for the failure of this combination approach. Studies have shown that the G₁-arrest induced by erlotinib antagonizes chemotherapy drugs that depend on a progressing cell cycle for efficacy (198). Thus, the prediction would be that sequential administration of EGFR TKIs following chemotherapy provides a greater effect than concurrent administration (199–201). In fact, a phase II trial demonstrated that treatment-naïve NSCLC patients receiving erlotinib on days 15–28 of a 4 week cycle following chemotherapy had significantly better response rates and longer progression-free survival than patients receiving a placebo (202). Interestingly, the Tarceva OR CHemotherapy (TORCH) randomized phase III trial, which was designed to test first-line erlotinib followed by cisplatin-gemcitabine at progression versus the inverse sequence, demonstrated conclusively that erlotinib first followed by chemo was inferior compared with chemo followed by erlotinib (203). These clinical data suggest a rational combination treatment paradigm in which patients are treated with first-line sequential chemotherapy followed by erlotinib. This illustrates the point that clinical success of combining EGFR-targeted agents with chemotherapy is likely to be dependent on a mechanistic understanding of how the drugs are likely to influence one another as well as selection of a patient population that would be predicted to respond to the targeted therapy component of the combination. Given the similarity of action on different targets achieved by the various targeted agents, it can be anticipated that this point is a generalizable one.

Future perspectives

There are many still unanswered questions that confound the combined chemotherapy-molecularly targeted agent approach. For example, it is clear that dose scheduling is a critical component of the success of certain combinations. Should combinations be administered up-front or sequentially and if so, in what order? Does resistance develop more, or less, quickly in response to combinations compared with single agents? Pre-clinical and clinical data would suggest that for toxicity and pharmacodynamic reasons, agents should be administered sequentially and chemotherapy should precede molecularly targeted therapy, but will this be tumor-type and even patient-specific? Ultimately, the approach may even depend on the goal of combination therapy: to prevent resistance from occurring or to be able to successfully treat and clinically manage resistant tumors.

Rapidly evolving technology will provide a platform for even more sophisticated methods of rationally combining chemotherapy and targeted therapy. Molecular network analysis, both computationally and in cells and tumor tissue, could provide a way to identify synergistic strategies for combining drugs and identify new vulnerabilities created upon the emergence of drug resistance. The ever-expanding data of cancer genomes will likely play a role in the design and rationalization of combination therapies by providing insight into the best sequence and combinations of therapies for which specific patients will benefit the most. For example, molecular subgroups of GB based on The Cancer Genome Atlas data provide information not only on the targetable mutations they possess but also on their sensitivity to the chemotherapeutic treatment (204). Moreover, it is becoming increasingly clear that the metabolic pathways that are of critical importance in influencing tumor biology represent novel targets for therapy. Combining chemotherapy and targeted therapy does not negate, but rather underscores, the requirement for patient stratification according to biomarkers for response to the targeted therapy. As a consequence, a personalized medicine approach is of critical importance and becoming increasingly more feasible than ever before.

Overall, there are many layers of complexity underlying successful clinical implementation of what appears to be a very rational and simple biological concept. Toxicity, dose scheduling and molecular heterogeneity are issues that surround drug efficacy and resistance.

Combining traditional cytotoxic chemotherapy drugs with molecularly targeted agents represents a formidable challenge but also a distinct opportunity. The future of the approach requires a conjoint consideration of what is learned by clinical failures as well as successes, driven by mechanistic knowledge of therapeutic sensitivity and resistance to guide the design of rational future clinical trials with the goal of establishing novel and more effective treatment paradigms.

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