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Chemical strategies to overcome resistance against targeted anticancer therapeutics

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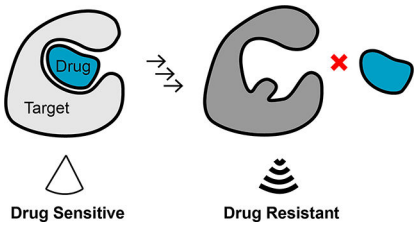
Abstract

Emergence of resistance is a major factor limiting the efficacy of molecularly targeted anticancer drugs. Understanding the specific mutations, or other genetic or cellular changes, that confer drug resistance can help in the development of therapeutic strategies with improved efficacies. Here, we outline recent progress in understanding chemotype-specific mechanisms of resistance and present chemical strategies, such as designing drugs with distinct binding modes or using proteolysis targeting chimeras, to overcome resistance. We also discuss how targeting multiple binding sites with bifunctional inhibitors or identifying collateral sensitivity profiles can be exploited to limit the emergence of resistance. Finally, we highlight how incorporating analyses of resistance early in drug development can help with the design and evaluation of therapeutics that can have long-term benefits for patients.

Graphical Abstract



Chemical inhibitors that selectively block their target's functions can be valuable as probes for dynamic cellular processes, for testing therapeutic hypotheses and as useful starting points for developing drugs. When these inhibitors are active in vivo, they can lead to new molecularly targeted therapeutics, many of which have provided new paradigms for treating diseases such as cancer. For example, aberrant signaling of the BCR-ABL fusion in leukemia or the upregulated activity of epidermal growth factor receptor (EGFR) kinase mutants in lung cancer can be blocked using potent chemical inhibitors and result in

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Competing interests

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improved clinical outcomes[1,2]. However, the long-term efficacy of such targeted therapeutics can be limited as resistance against them inevitably arises[3,4].

The emergence of resistance is driven by evolutionary pressures exerted by drugs on growing cells and can involve multiple mechanisms. Extensive studies of antiviral, antimicrobial and anticancer agents have established paradigms for understanding mechanisms of drug resistance (for reviews see refs. 5,6,7). For example, resistance to antiviral drugs commonly arises due to mutations in the target proteins that can prevent drug binding[8]. Selection of the resistant virus can occur rapidly, as viral populations consist of ensembles of related genotypes (also termed viral quasispecies or swarms[9]) that may arise due to high mutation rates during replication[10]. Emergence of single-point mutations often leads to acquired drug resistance in cells (e.g., bacteria or cancer cells), but unique constraints in different cellular, multicellular and organismal contexts can also lead to a wide range of resistance mechanisms. For example, horizontal gene transfer in bacteria can give rise to acquired resistance by selection of genetic elements that facilitate modifications of drugs and render them ineffective (e.g., hydrolysis of β-lactam antibiotics by β-lactamase)[11]. In cancer cells, mechanisms contributing to resistance can also include reduction of cellular drug abundance by upregulating xenobiotic pathways that promote drug metabolism, as well as increased expression of genes leading to nonspecific multidrug resistance (MDR; for a recent review see ref. 12). Consistent with these studies, drug-resistance mechanisms in patients can be complex, and new chemical strategies are needed to address the emergence of drug resistance and to develop therapeutics with long-term benefits.

Here, we focus on chemotype-specific resistance to chemical inhibitors in cancer, as these mechanisms are now being addressed by innovations in chemistry and chemical biology. In the following sections, we highlight recent examples of drug-resistance analyses and chemical approaches that can help address resistance (Fig. 1).

Designing inhibitors with distinct binding modes

Resistance to small-molecule anticancer agents can result from mutations in genes encoding the target proteins (e.g., BCR-ABL, EGFR or ALK, Table 1) that prevent or reduce drug binding[3,13,14]. An important example of this type of resistance in cancer cells is the mutation of the gatekeeper residue that can prevent binding of drugs targeting the nucleotide-binding site of oncogenic kinases[15]. For instance, the T315I gatekeeper mutation often arises in BCR-ABL-driven leukemias and prevents the binding of different inhibitors targeting the active site of Abl1 kinase such as imatinib or dasatinib[16] (Table 1). Similarly, sustained treatment of anaplastic lymphoma kinase (ALK)-rearranged lung cancers with ATP-competitive inhibitors such as crizotinib invariably leads to emergence of resistance-conferring mutations, including the gatekeeper mutation (ALK-L1196M, Table 1)[14,17]. In these cases, for which the drug resistance mechanisms are known, new drugs and chemical strategies have been designed to address resistance[18,19].

A successful approach to overcome resistance to crizotinib, which is often a result of mutations of the gatekeeper residue L1196M, centers on targeting the active site of ALK with inhibitors that adopt a distinct binding mode compared to that of crizotinib (Fig. 2a and

Table 1). For example, ceritinib binds ALK in the active site and can inhibit a number of crizotinib-resistant alleles including the gatekeeper mutant ALK-L1196M[19]. However, as the binding modes of crizotinib and ceritinib partially overlap (Fig. 2a,b), ceritinib can only block a subset of resistance-conferring mutant alleles in ALK kinase[19]. To overcome resistance to crizotinib and ceritinib (e.g., due to a mutation outside of the active site, C1156Y), a third generation macrocyclic drug called lorlatinib was developed[20] (Fig. 2c,d). Even though all three inhibitors bind in the ALK active site, they can overcome resistance by leveraging distinct inhibitor–residue contacts to achieve selectivity and potency. However, treatment with lorlatinib can also lead to emergence of mutations such as ALK-C1156Y-L1198F that confer resistance to this drug[20]. Interestingly, the ALK-C1156Y-L1198F double mutant can bind crizotinib (Fig. 2e) and re-sensitizes the resistant cancer cells to crizotinib treatment[20]. Taken together, these studies establish a paradigm for how designing drugs with distinct binding modes can help overcome resistance to targeted therapy.

Combining preclinical analyses of resistance and compound testing is becoming a successful approach to anticipate and overcome resistance[21]. Entrectinib and larotrectinib are first-generation, ATP-competitive inhibitors for treating cancers driven by lesions in *NTRK1–3* genes that encode tropomyosin receptor kinases (TrkA, TrkB and TrkC)[22,23]. As is the case with other molecularly targeted therapeutics, acquired resistance to these compounds was found to arise upon treatment with these inhibitors[24,25]. Analyses of resistance in tumor samples from patients and in cell culture models of *NTRK*-driven cancers revealed a number of mutations that confer resistance to larotrectinib and entrectinib[21]. In particular, binding of these drugs was suppressed by mutations in the solvent-exposed loop at the front of the kinase nucleotide-binding pocket (e.g., G595R and G667C in TrkA and G623R in TrkC) and in the activation loop of the DFG motif (e.g., G667C in TrkA). Testing compound libraries against these mutant alleles revealed a series of 13-member macrocyclic scaffolds, based on the larotrectinib core scaffold, that potently block the activity of Trk alleles resistant to larotrectinib and entrectinib[21]. One of the identified compounds, LOXO-195 (Table 1), was rapidly advanced to clinical trials. In these trials, patients that developed resistance to larotrectinib, possibly due to rapid selection of pre-existing resistant clones, were treated with LOXO-195. Remarkably, treatment with LOXO-195 resulted in tumor regression in most patients, likely because of on-target inhibition[21]. These encouraging results illustrate how analyses of resistance can be valuable for designing therapeutics with improved efficacies. Therefore, for the promise of molecularly targeted drugs for long-term cancer treatment to be realized[26], integrating analyses of resistance early in drug development, by approaches such as resistance analysis during design (RADD, Box 1), is needed.

Designing cysteine-targeting covalent inhibitors

Another chemical strategy to overcome resistance involves the design of covalent inhibitors[27,28]. This approach hinges on the presence of an electrophilic moiety (e.g., acrylamide, epoxide or α-halocarbonyl group) in the inhibitor that can form a covalent bond with a nucleophilic residue in the binding site (e.g., cysteine or lysine)[27]. A number of drug targets contain a native cysteine that can be leveraged for inhibitor design. For example, the EGFR-T790M mutant allele, which is the most common resistance-conferring mutation that arises upon prolonged treatment of EGFR-driven cancers with ATP-competitive inhibitors

such as gefitinib or erlotinib[2,3,29], does not prevent binding of these compounds in the active site. Instead, this mutant allele binds the ATP substrate with increased affinity, which has been proposed to contribute to drug resistance[30]. EGFR contains a cysteine residue at the entrance of the ATP-binding site, and electrophilic compounds that form a covalent bond with this residue (C797) can potently block the activity of EGFR-T790M and substantially increase the inhibitor's occupancy in comparison to that of reversible inhibitors[31,32]. Optimizing interactions between the covalent inhibitors such as osimertinib[33] or WZ4002 (ref. 31) and the methionine side chain of the mutant gatekeeper residue (T790M) can also help reduce the undesired side effects due to the inhibition of wild-type EGFR in noncancerous cells[31]. These studies illustrate how targeting native cysteines in the active sites of proteins can be leveraged for designing compounds that can overcome resistance to targeted anticancer drugs.

Designing inhibitors targeting allosteric binding sites

The efficacy of ATP-competitive or covalent inhibitors can be compromised by active site mutations that can arise upon prolonged drug treatment[16,34,35] (Fig. 3a). A complementary strategy to target the ATP-binding site is designing inhibitors that can bind distinct pockets on the protein, such as regulatory sites or allosteric pockets, that become accessible when the protein adopts alternative conformations (e.g., the inactive state in kinases[36,37]). These pockets can be less conserved than ATP-binding sites, and inhibitors targeting them can potentially achieve high selectivity and have additional favorable properties (e.g., longer on-target residence times)[38,39]. For example, inhibitors targeting the myristate pocket of Abl1 kinase can potently block kinase activity[40,41]. In addition, inhibitors targeting the myristate pocket, such as ABL001 (also termed asciminib, Table 1), can also block the activity of BCR-ABL alleles with mutations in the active site that confer resistance to ATP-competitive inhibitors such as imatinib or dasatinib[40]. Therefore, targeting alternative pockets on proteins can be a useful strategy to overcome resistance.

However, mutations can also arise in the allosteric pockets and confer resistance to these inhibitors (Fig. 3a). Studies of acquired resistance to asciminib reveal that mutations in the myristate pocket of Abl1 kinase, the proposed binding site of the inhibitor, can confer resistance and prevent binding of the drug[40,42] (Fig. 3b). In this case, mutations that block the binding of asciminib are distinct from those that confer resistance to active-site inhibitors[40] (Fig. 3b and Table 1). BCR-ABL alleles with mutations in the allosteric pocket do not substantially alter kinase activity and can be blocked by active site inhibitors such as imatinib or dasatinib (Table 1). Interestingly, combining allosteric and active site inhibitors to block BCR-ABL in cells can help reduce and even prevent the acquisition of on-target resistance[40] (Fig. 3c). Further studies will be needed to determine whether such drug combinations could also limit the development of resistance to BCR-ABL targeting drugs in patients.

Designing compounds targeting multiple binding pockets

mTOR kinase plays critical roles in the PI3K–Akt–mTOR pathway, which is frequently hyperactivated in human cancers[43], and inhibitors that bind mTOR in the rapamycin-binding

site (rapalogs) or the ATP-binding site (TOR kinase inhibitors, TORKi's) have been evaluated in clinical trials[44,45] (Fig. 4a,b). However, mutations in these drug-binding sites can arise and confer resistance[46]. Rapalog binding is prevented by mutations (e.g., A2034V or F2108L) in the FKBP12-rapamycin-binding domain (FRB domain) that have also been reported to confer resistance to everolimus in patients[47]. Interestingly, the M2327I mutation, which confers resistance to the TOR kinase inhibitor AZD8055, maps to the kinase domain ~15 Å away from the ATP-binding site and does not prevent compound binding[46]. This mutation leads to an approximately threefold increase in the basal kinase activity, which likely contributes to resistance against inhibitors targeting the active site of mTOR in cells.

Resistance to the two classes of mTOR inhibitors was recently addressed by designing a bivalent compound that can bind the rapamycin-binding site and the ATP-binding site in the mTOR kinase[46] (Fig. 4a). This inhibitor, termed RapaLink-1 (Fig. 4c), has a linker between the rapalog and the TORKi moieties that allows the compound to simultaneously interact with the rapamycin- and ATP-binding sites. Studies in cells indicate that RapaLink-1 limits proliferation by blocking mTOR signaling and suggest that the compound binds in both pockets of the target[46]. Interestingly, RapaLink-1 also inhibits activity of mTOR alleles with mutations in both FRB and the kinase domains even though this double mutant confers resistance to rapalogs, TORKi's, as well as their combinations[46]. Bivalent compounds such as RapaLink-1 could also help reduce off-target toxicities associated with each drug, as the linker moiety and the topology of the drug conjugate can be tuned to improve selectivity for the chosen target. It will be important to examine whether similar approaches could be applied to other drugs and cancer pathways for which we also have extensive structural and biochemical data.

Overcoming resistance by targeted degradation

PROteolysis Targeting Chimeras (PROTACs[48]) belong to a class of heterobifunctional compounds that can induce targeted degradation of proteins (Fig. 5a). The use of PROTACs has recently emerged as a promising strategy to modulate protein function and also to target drug-resistant alleles in cancer[49]. PROTACs are composed of a ligand that recruits E3 ubiquitin ligase enzymes such as von Hippel-Lindau (VHL) or cereblon (CRBN) ligases[50,51]. This ligand is covalently attached via a linker to a second moiety that interacts with the protein of interest[52]. The concomitant binding of PROTACs to the target protein and the E3 ligase can lead to the formation of a ternary complex that promotes ubiquitination of the target by the E3 ligase and degradation of the ubiquitinated target by the proteasome[53] (Fig. 5a). However, to engage two distinct targets, the PROTAC concentration must be precisely adjusted to avoid formation of 'unproductive' dimers with the target protein or the E3 ligase at high concentrations that can preclude ternary complex formation (so called 'hook effect'[54]). In addition to complex pharmacology, developing PROTACs can be challenging, as designing optimal linkers can also be difficult. Interestingly, PROTACs can act sub-stoichiometrically as degradation of the ubiquitylated proteins releases the PROTAC molecule, which can then bind another molecule of the target protein and repeat the degradation cycle. This mode of action differs from the common 'occupancy-driven' pharmacology of inhibitor–target binding that may require high doses of the inhibitor to effectively block activity of the target. An additional advantage of targeted

degradation compared to reversible inhibition is the longer sustained blockade of the target, as its function and activity can only be restored by protein re-synthesis[55].

Targeting proteins for proteasomal degradation using the PROTAC approach has now been demonstrated for a number of proteins in cancer (e.g., BCR-ABL[56], EGFR[55] or Bruton's kinase (BTK)[57]). Recent advances also suggest that PROTACs could help overcome resistance associated with mutations in these targets. For example, resistance-conferring alleles in Bruton's kinase often arise by a substitution of the active-site cysteine residue to a serine (C481S), which reduces binding of the covalent inhibitor ibrutinib[58]. Interestingly, PROTACs based on the ibrutinib scaffold (e.g, MT-802, Table 1), which can still bind to the mutant protein, albeit with lower potency, can induce robust degradation of the BTK alleles that confer resistance to the parental compound[57,59]. This indicates that alleles conferring resistance to anticancer drugs may be targeted using the PROTAC approach.

In cases in which resistant mutations completely preclude binding, PROTACs targeting distinct binding pockets on the protein may be needed to provide an alternative mode for degradation (e.g., the myristate pocket of BCR-ABL[60]). Combining PROTACs targeting different sites on a protein (e.g., an active site and allosteric sites) could also be a strategy to limit resistance similar to what has been observed for 'occupancy-driven' drug combinations (see below).

Recent data show that resistance to PROTACs can arise via mutations not only in the target protein but also within the ubiquitination machinery[61,62]. Interestingly, these studies indicate a limited overlap of mutations that confer resistance to cereblon- and VHL-targeted degraders. This difference could be leveraged for designing robust treatment regimens that could potentially limit the emergence of resistance against PROTAC-based therapeutics.

Analogous 'molecular glue' mechanism of actions have been demonstrated for thalidomide-related compounds (also referred to as immunomodulatory drugs, or IMiDs) that promote formation of a tertiary complex with E3 ligase cereblon (CRBN) and an endogenous target protein (e.g., IKAROS family zinc finger proteins 1 and 3 (IKFZ1/3))[51,63,64,65,66]. Recent studies have also identified aryl sulfonamide-based inhibitors[67], such as indisulam and tasisulam, as 'molecular glues' that induce protein–protein interactions. Studies applying an approach similar to DrugTargetSeqR (see below) in HCT116 cells revealed mutations that map to the *RBM39* gene (RNA binding motif splicing factor 39) and confer resistance to a number of aryl sulfonamide compounds[67,68]. Biochemical and in vivo studies indicate that these compounds facilitate formation of a ternary complex between the RBM39 protein and DCAF15-CUL4-RBX1-DDB1 E3 ubiquitin ligase that can promote RBM39 ubiquitination and its degradation by the proteasome. Resistance-conferring mutations in RBM39 block aryl sulfonamide binding and RBM39 association with the E3 ubiquitin ligase. Disrupting this complex protects the RBM39 splicing factor from degradation and can promote cancer growth. Recent structural characterizations of E3 ligases in complex with aryl sulfonamides match the predicted binding sites of these compounds and suggest the molecular basis of their selectivity[69,70]. It is possible that these different molecular glues can be modified to induce the degradation of additional cellular targets, which could help address resistance

mechanisms. These studies also raise the possibility that endogenous molecular glues that regulate protein–protein interactions may exist and could be discovered.

Overcoming resistance by using combinations of drugs

Combination therapy is a well-established clinical approach for cancer treatment and can also help address emerging resistance. Using cocktails of multiple chemotherapeutics has been a successful approach to cancer therapy, as drug combinations can have synergistic effects and provide more potent anticancer outcomes than single drugs alone (for reviews see refs. 5,71,72,73). Here, we highlight recent advances of drug combination strategies that leverage principles of non-overlapping resistance profiles and synthetic lethality to help overcome drug resistance.

Resistance to drugs often arises due to genetic changes in the drug's target (e.g., point mutations, deletions or misregulation of expression), and multiple changes would be needed to confer resistance to drug combinations that target distinct proteins or binding sites. However, multiple genetic changes (e.g., single-point mutations) are less likely to occur simultaneously in a single cell. Therefore, using combinations of drugs with non-overlapping profiles of resistance-conferring mutations (e.g., due to targeting distinct sites on a protein or by targeting distinct protein targets) could be a powerful strategy to limit and prevent the emergence of resistance.

A nice example of drug combinations that have distinct patterns of resistance include drugs targeting BCR-ABL kinase. For instance, the active site inhibitor dasatinib in combination with an allosteric site-targeting inhibitor asciminib can substantially limit the emergence of resistant cells in preclinical models of the disease and may even lead to tumor eradication40. Additional studies will be needed to identify combinations of drugs with distinct resistance profiles.

Identifying combinations of drugs can not only enhance efficacy of targeted therapies but also uncover synthetic lethal strategies to suppress the evolution of drug resistance. For example, targeting mutant alleles of EGFR in lung cancers can elicit beneficial therapeutic responses, but is often followed by a relapse associated with acquired resistance[29]. Although the precise evolutionary trajectories leading to acquisition of the resistant phenotype in these cancers are not known, recent evidence suggests that the activity of Aurora A kinase contributes to the development of resistance in response to treatment with anti-EGFR agents[74]. Preclinical studies indicate that suppression of Aurora A kinase activity can improve outcomes of EGFR inhibition and can even limit the emergence of resistance to EGFR inhibitors in xenograft tumor models[74]. These studies suggest that drug combination regimens could be designed to help prevent developing resistance to targeted therapeutics. Further studies will be needed to determine whether this approach could also limit emergence of resistance to other classes of targeted therapeutics.

Synergistic drug combinations (i.e., when drugs induce larger effects in combination than predicted by their individual activities) often lead to increased potency of the two drugs in cancer cells. Interestingly, though, in the case of antimicrobial agents, synergistic drug

combinations were shown to promote acquisition of resistance[75]. Interestingly, some antagonistic combinations (i.e., when the effect of drugs in combination is less than the predicted effect of each drug alone) of antimicrobial drugs can reduce and may even invert the selection pressure for resistance[75]. However, these experiments are currently limited mainly to bacterial systems, and additional studies will be needed to establish whether using antagonistic combinations of inhibitors could also limit the emergence of resistance in cancer cells.

Exploiting collateral sensitivity to limit emerging resistance

A potential strategy to reduce acquisition of a drug-resistant phenotype, as well as to treat disease after relapse, can be to target distinct evolutionary trajectories that promote emergence of resistance[76]. In principle, cells can acquire genetic changes during evolution of resistance to one drug that may sensitize these cells toward another drug (Fig. 5b).

This approach, first described in bacteria[77] and termed collateral sensitivity, exploits drug-specific vulnerabilities and trade-offs that can emerge during the evolution of resistant phenotypes[78,79,80]. For example, BCR-ABL leukemia cells treated with the active-site-targeting drugs dasatinib or bosutinib often acquire multiple resistance-conferring mutations in the active site (e.g., V299L-E255K or V299L-F317L double mutants[78]). In these cases, the intermediate state of clonal evolution (i.e., cells that acquired the BCR-ABL-V299L mutant allele) can represent a particularly stable and robust state that is susceptible to treatment with other drugs. This ‘sensitization window’, also named ‘temporal collateral sensitivity’, is lost when cells acquire the double mutant genotype[78]. In particular, chemical screens revealed that proliferation of cell subpopulations expressing the single-point mutant allele BCR-ABL-V299L were sensitized to inhibitors such as vandetanib or foretinib that are chemically dissimilar to known BCR-ABL inhibitors (e.g., dasatinib or bosutinib)[78]. Interestingly, although vandetanib and foretinib can bind a number of distinct kinases, studies of isogenic cell lines suggest that the collateral sensitivity in these cells likely arises due to on-target inhibition of the BCR-ABL-V299L allele[78]. Molecular modeling and biochemical studies also indicate that these drugs can exploit different binding modes in the active site of BCR-ABL and potently inhibit the ‘sensitized’ mutant allele (V299L). Most notably, treating animals with BCR-ABL-V299L-driven tumor models with vandetanib or foretinib can lead to significant increase in survival in comparison to animals with tumors expressing wild-type BCR-ABL.

Together, these studies suggest that identifying and predicting evolutionary trajectories of cancer cells in response to drug treatment could be exploited for chemotherapy, although further studies will be needed to establish whether targeting temporal collateral sensitivity could be achieved in the clinic. Further studies will also be required to establish rational approaches and methods to systematically identify evolutionary trajectories and collaterally sensitive stages of cancer cells that could be targeted to overcome resistance.

‘Crash-testing drugs’

It is becoming clear that understanding resistance mechanisms can help design therapeutic strategies. Therefore, unbiased analyses of resistance-conferring mechanisms in different cell lines (e.g., established cancer cell lines or patient-derived cell lines) need to be developed and should be incorporated at early stages of drug development.

Chemical biology approaches, such as the DrugTargetSeqR[81,82,83] that can be used for drug target identification in cells, can also help uncover high-frequency mechanisms of resistance. The first step of DrugTargetSeqR is to isolate cells resistant to the inhibitor. In the case of toxic compounds, such resistant clones can be selected from compound-treated genetically diverse cell populations. Genomic analyses, such as RNA-seq or exome sequencing, of the resistant clones can then be used to identify lesions that likely confer resistance to the compound. As passenger mutations will likely be unique to each clone, the lesions mapping in multiple independent clones to the same gene or a set of genes in a common pathway (e.g., kinase signaling cascade) likely indicate the targets of the inhibitor. In many cases, these high-frequency mutations are sufficient to confer resistance[67,81,82,83]. Importantly, these mutations can also match those that confer drug resistance in the clinic. For example, single-point substitutions in the *PSMB5* gene (e.g., M104V) often arise and confer resistance in cultured cell lines upon prolonged treatment with the proteasome inhibitor bortezomib[82] (Velcade). Analogous mutations have been identified in patient samples following relapse following bortezomib therapy[84]. Therefore, ‘crash testing’ drugs in different cancer cell lines can help reveal mechanisms of chemotype-specific resistance.

Variations in cancer cell genomes and karyotypes impose distinct constraints on the emergence of drug resistance. For example, applying DrugTargetSeqR in near-haploid and diploid cancer cell lines revealed distinct mechanisms of resistance to the mitotic kinesin CENP-E inhibitor GSK923925. This compound was evaluated in clinical trials for cancer, as blocking CENP-E activity can be toxic to cancer cells. However, similar to other targeted therapeutics, resistance can emerge in cells upon prolonged treatment with the inhibitor. In diploid cells, resistance to GSK923295 was found to arise via single-point mutations in the N-terminal motor domain of CENP-E, which can prevent inhibitor binding[83]. In contrast, analysis of GSK923295-resistant near-haploid cells revealed a deletion of the C-terminal domain of CENP-E. In contrast to chemical inhibition of CENP-E protein, which leads to cell death in diploid and haploid cells, genetic knockout of the *CENPE* gene does not block cell growth in haploid cells, and thus disrupting the gene in these cells can confer resistance. Though additional studies are needed to examine the emergence of drug resistance in cells with different ploidies, this recent study suggests that different karyotypes in cancer cells can constrain the trajectories of the genetic changes underlying drug resistance.

Cancer cell lines with impaired DNA mismatch repair (MMR) pathways exhibit increased mutation rates[85], and identifying resistance-conferring alleles in these cells can be efficient[86]. However, most cells and cancer cell lines with intact MMR harbor low mutation frequencies, which can hinder selection of resistant clones. This can pose a barrier for incorporating analyses of resistance to established drug development pipelines. New approaches have recently been developed to address this limitation. For example, genetic

ablation of MSH2, a gene involved in MMR pathways, in a number of cancer cell lines can induce higher mutation rates and facilitate selection of drug resistance-conferring alleles[87]. Incorporating these techniques in drug development projects could be particularly valuable for identifying mechanisms of resistance in cancer models with low mutational rates (e.g., childhood retinoblastomas or Ewing sarcomas[88,89]).

Conclusion and outlook

We have outlined a number of recent innovations in chemical biology for analyzing and addressing chemotype-specific resistance to anticancer therapeutics. As resistance to targeted drugs inevitably arises, new approaches are needed to develop drugs with improved efficacies and to devise strategies to address drug resistance. In particular, we highlighted how analyses of resistance can help the design of new drugs with distinct binding poses. In addition, simultaneous targeting of multiple sites on a protein (e.g., the active site and the allosteric site) can reduce the emergence of resistance, as multiple mutations blocking compound binding are less likely to occur. Identifying compounds with non-overlapping resistance signatures should therefore be prioritized early in the design process to help develop drugs that could reduce and even prevent the emergence of drug resistance. Innovative chemical biology strategies (e.g., the design of bivalent compounds that bind their target simultaneously in two proximal binding sites[46]) can also help in this process and can provide new leads for the development of therapeutics. Predicting trajectories of resistance and sensitivity to drugs in cancer cells using computational modeling will also help improve efficacies of new therapeutics[90,91]. Finally, advances in computational drug design and machine learning will likely facilitate the discovery of drugs that have binding poses distinct from those of other drugs and could help overcome resistance. Moving forward, integrating preclinical resistance analyses and new chemical approaches to tackle resistance should help realize the promise of targeted therapeutics for cancer treatment.

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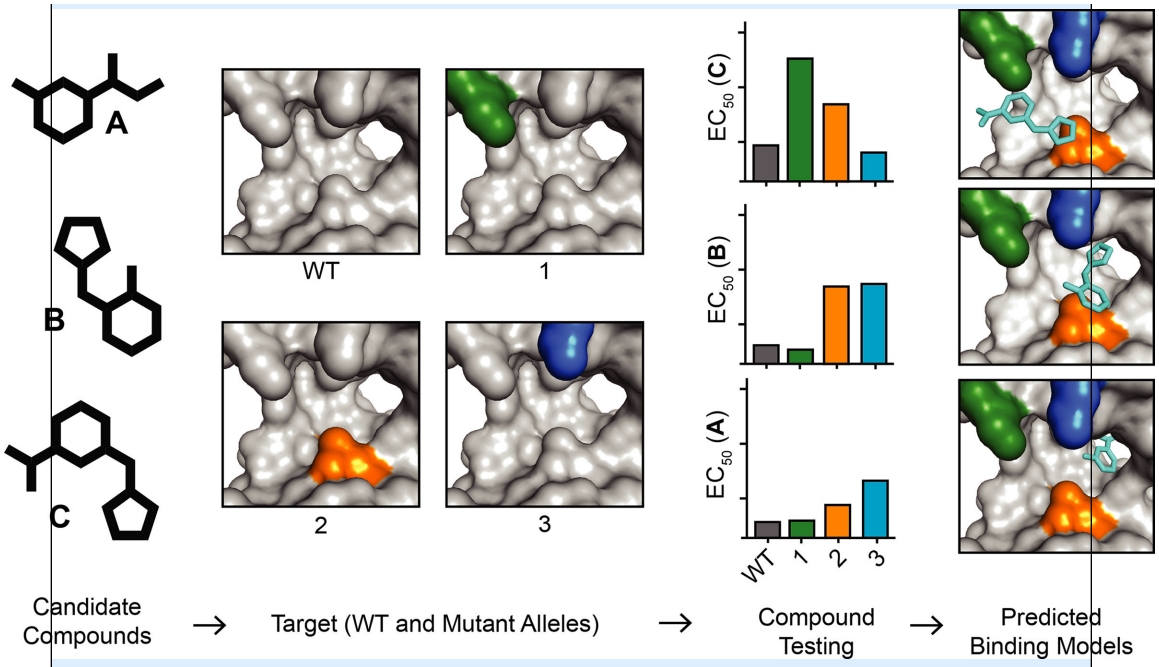
Box 1

Resistance analysis during design (RADD)

Binding of inhibitors to their targets is driven by the their complementary steric and electrostatic interactions[92]. Mutations that are functionally silent and lead to changes in the shape and electrostatics of the drug-binding sites can reveal such protein–inhibitor interactions. Therefore, identifying key residues and inhibitor–protein contacts can be useful not only for anticipating resistance and validating targets of inhibitors in cells[93], but also for optimizing inhibitor potency and specificity. An approach termed resistance analysis during design (RADD)[94] has recently been used for the design of spastazoline, a cell-permeable, potent and selective chemical probe of spastin[95]. In particular, RADD involves engineering biochemically silent mutations in the target protein and testing compounds against them. Mutations altering compound potency identify residues that form key inhibitor–target interactions and predict inhibitor–target binding poses. Briefly, to identify target–inhibitor interactions needed for the design of the pyrazolylpyrrolopyrimidine-based spastazoline, selected inhibitor scaffolds were tested against spastin mutant alleles that retain enzymatic activity (Box 1 figure). These analyses helped develop a compound-binding model that guided optimization of the inhibitor. Furthermore, testing diaminotriazole-based scaffolds, which are chemically unrelated to spastazoline, against spastin mutant alleles revealed how even minor modifications of the inhibitors can lead to distinct binding modes in the active site[94]. Together, these studies indicate that analyses of resistance can be valuable for the inhibitor design process[94,95].

RADD can be especially useful for evaluating unoptimized compounds (e.g., screening hits) as models of inhibitor–target interactions for these compounds are often not readily available. In addition, identifying starting scaffolds with distinct binding modes could facilitate the design of new inhibitors to overcome resistance. Recent advances in genome editing (e.g., CRISPR–Cas9 technology) can help introduce resistance-conferring mutations in cells, such as those identified by RADD or using unbiased target-focused approaches, and analyze those that alter compound activity[96,97,98,99,100]. Finally, analyzing compound–target interactions using RADD could help identify combinations of drugs with non-overlapping resistance signatures that may delay and even prevent emerging drug resistance (see text).



Box1 figure:

RADD approach for developing inhibitor-target binding models. Schematic shows the RADD approach. Selected candidate compounds are tested against wild-type (WT) and mutant alleles of a chosen target to identify key inhibitor–target interactions that can guide the development of robust binding models.

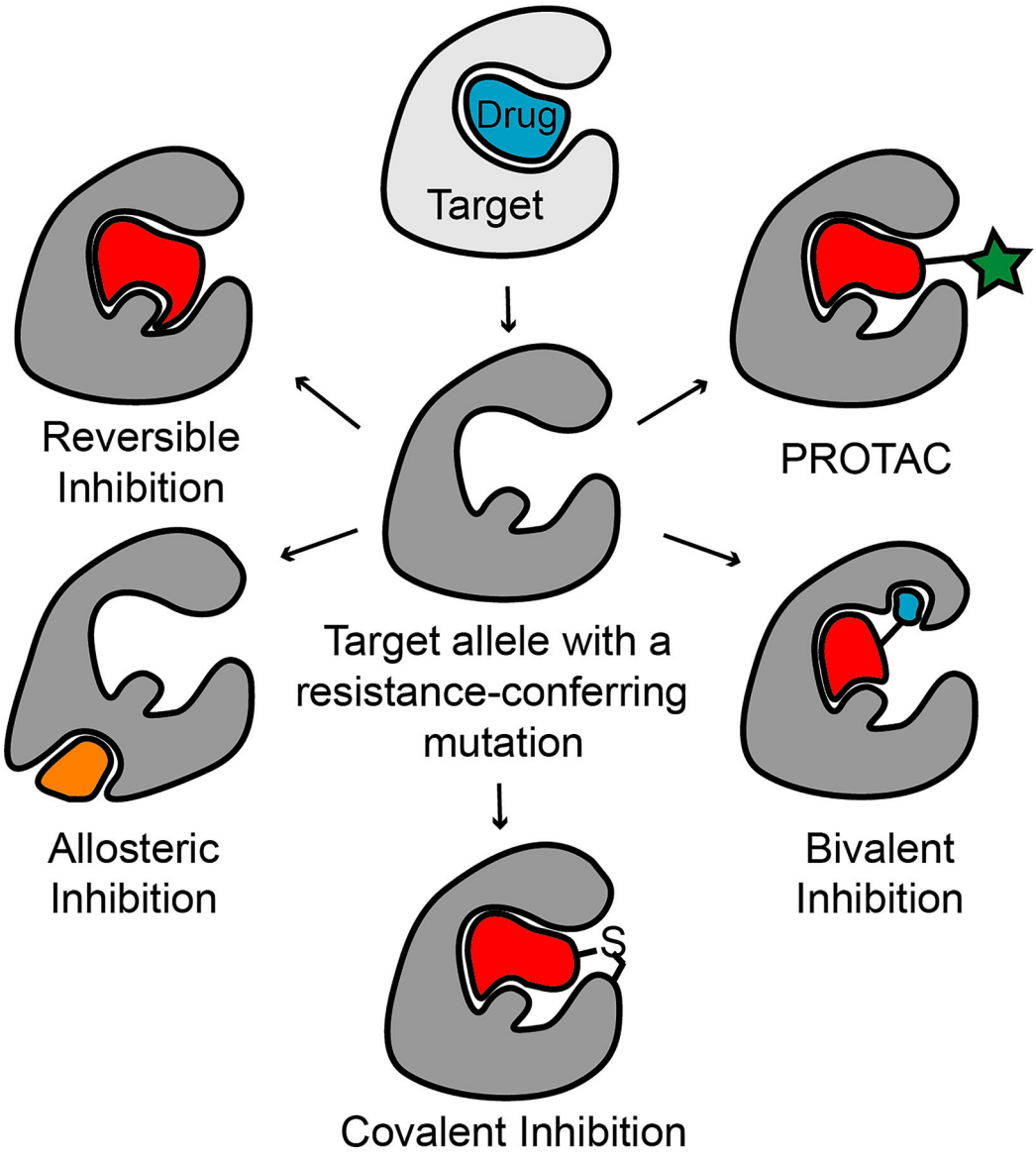


Fig. 1 |. Strategies to overcome resistance against molecularly targeted therapeutics.
Schematic shows strategies, which are highlighted in this Review, to overcome chemotype-specific resistance to inhibitors. The activity of resistance-conferring alleles (dark gray, center) can be blocked by inhibitors with distinct binding modes, allosteric inhibitors, covalent inhibitors, or bivalent compounds. Resistance-conferring alleles can also be targeted for degradation by the proteasome using PROTACs (red ligand with a green star, see text for details).

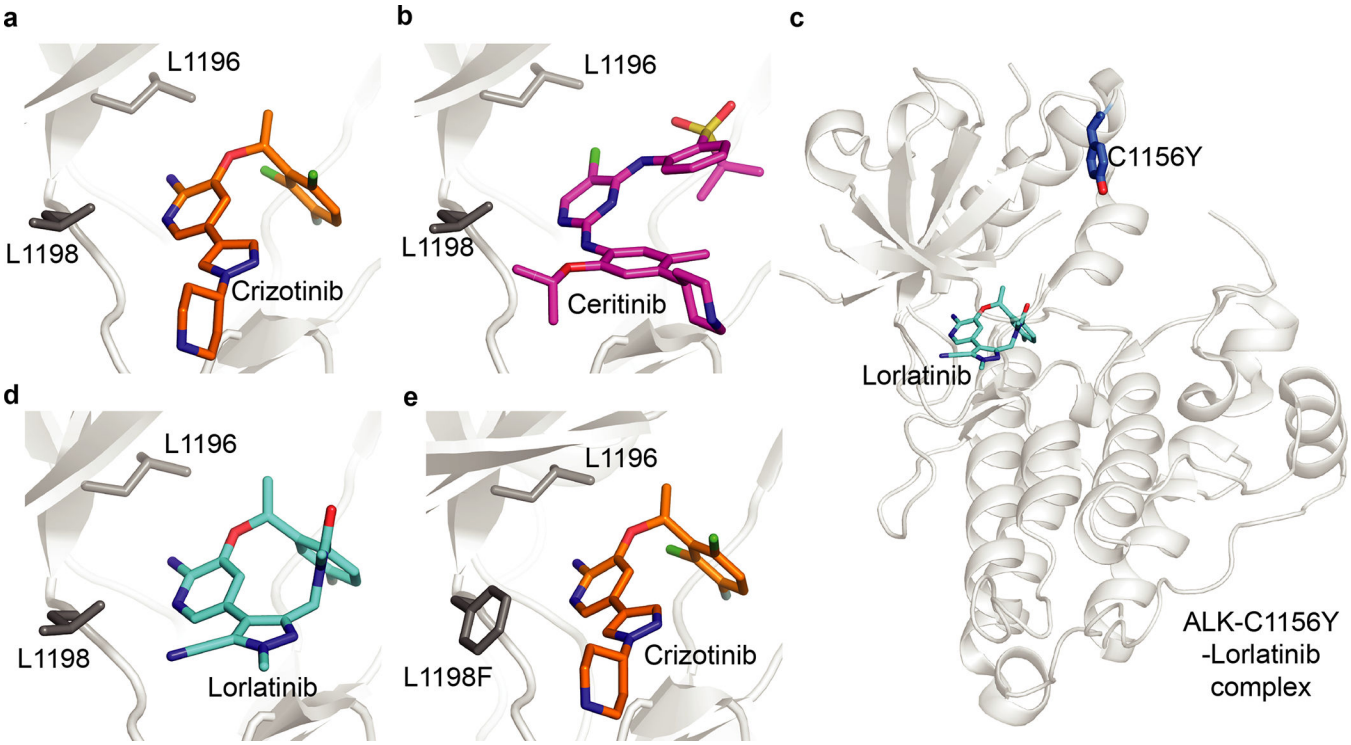


Fig. 2 |. overcoming resistance by designing inhibitors with distinct binding modes.

a–**e**, Structural models of ALK kinase alleles in complex with crizotinib (**a**, ALK-WT, PDB: 2XP2; **e**, ALK-C116Y-L1198F, PDB: 5AAB), ceritinib (**b**, ALK-WT, PDB: 4MKC) and lorlatinib (**c**,**d**, ALK-C1156Y, PDB: 5A9U). Selected residues are indicated (stick representation).

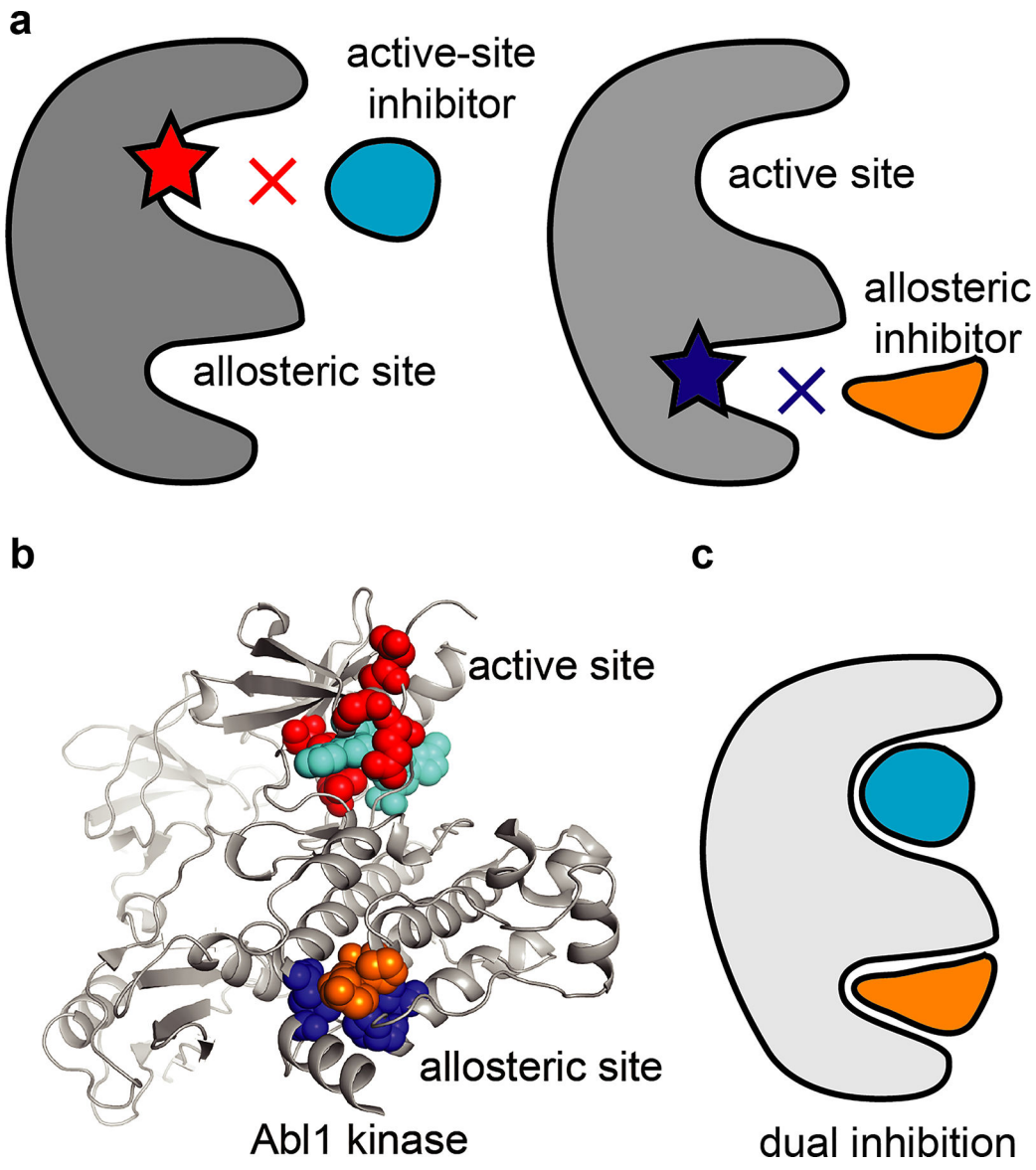


Fig. 3 |. inhibitors with non-overlapping resistance profiles to overcome drug resistance.
a, Schematics show mutant alleles of a target protein with resistance-conferring mutations in the active site (red star, left) and the allosteric site (blue star, right). Schematics of active-site and allosteric-site inhibitors are also shown (cyan and orange, respectively). **b**, Structural model of Abl1 kinase with inhibitors (sphere representation) bound in the active site (inhibitor in cyan) and the allosteric site (inhibitor in orange). Residues (sphere representation) that can be mutated without loss of protein activity and that confer resistance to inhibitors in the active site (residues in red) or the allosteric site (residues in blue) are shown. PDB: 5MO4. **c**, Schematic of a target protein with two inhibitors concomitantly bound in the active and allosteric sites.

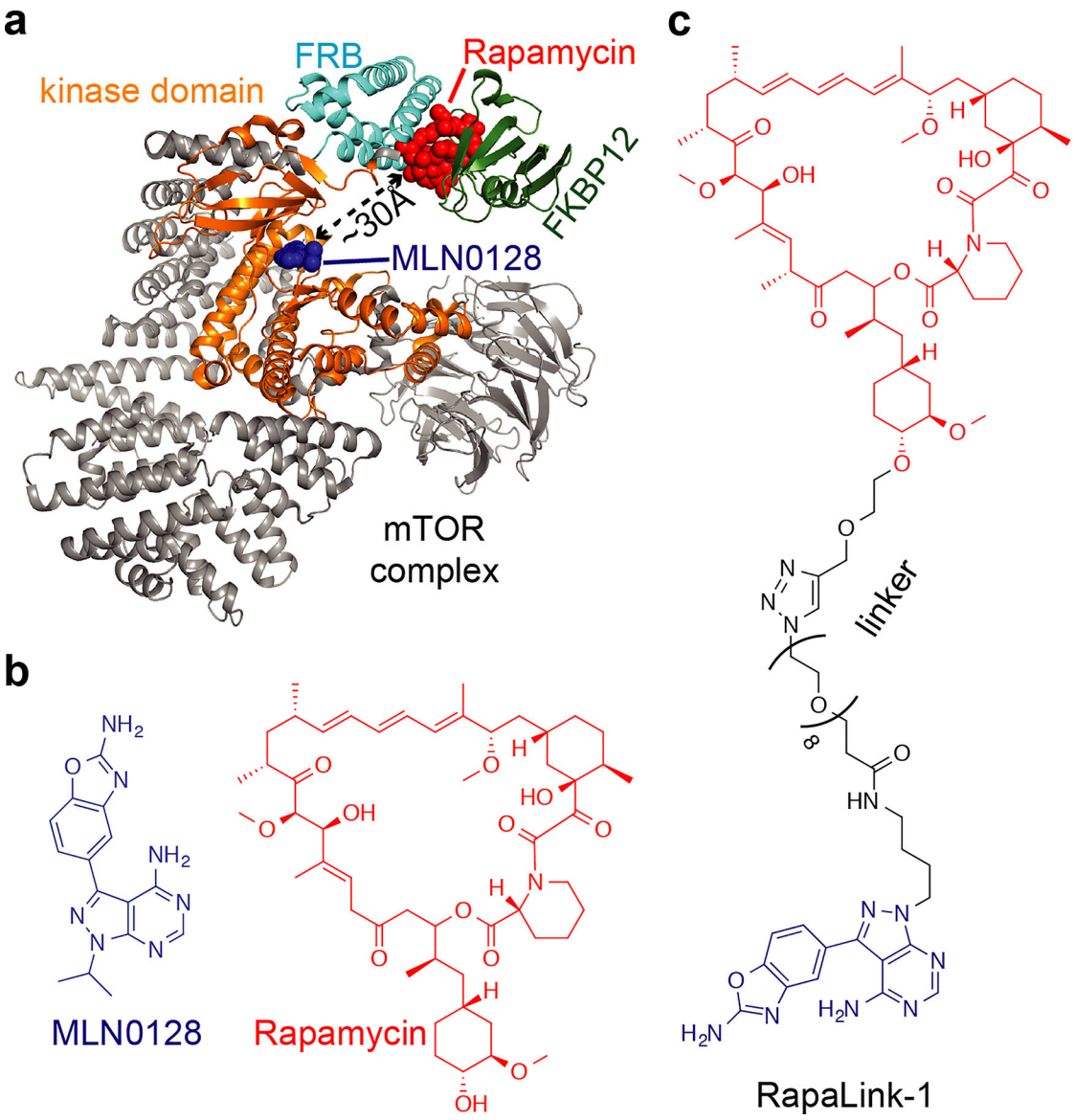


Fig. 4 |. Bivalent inhibitors to overcome drug resistance.

a, Model of mTOR kinase (gray, constructed from structural models of PDB IDs 4JT5 and 1FAP). FKBP12 (green), FRB (cyan) and kinase (orange) domains are indicated. Inhibitors bound in the kinase active site and the FRB site are also shown (blue and red spheres, respectively). Shortest distance between rapamycin and MLN0128 inhibitors is indicated. **b**, Chemical structures of mTOR inhibitors binding in the active site (MLN0128, blue) and the FRB-site (rapamycin, red). **c**, Chemical structure of the bivalent mTOR inhibitor RapaLink-1.

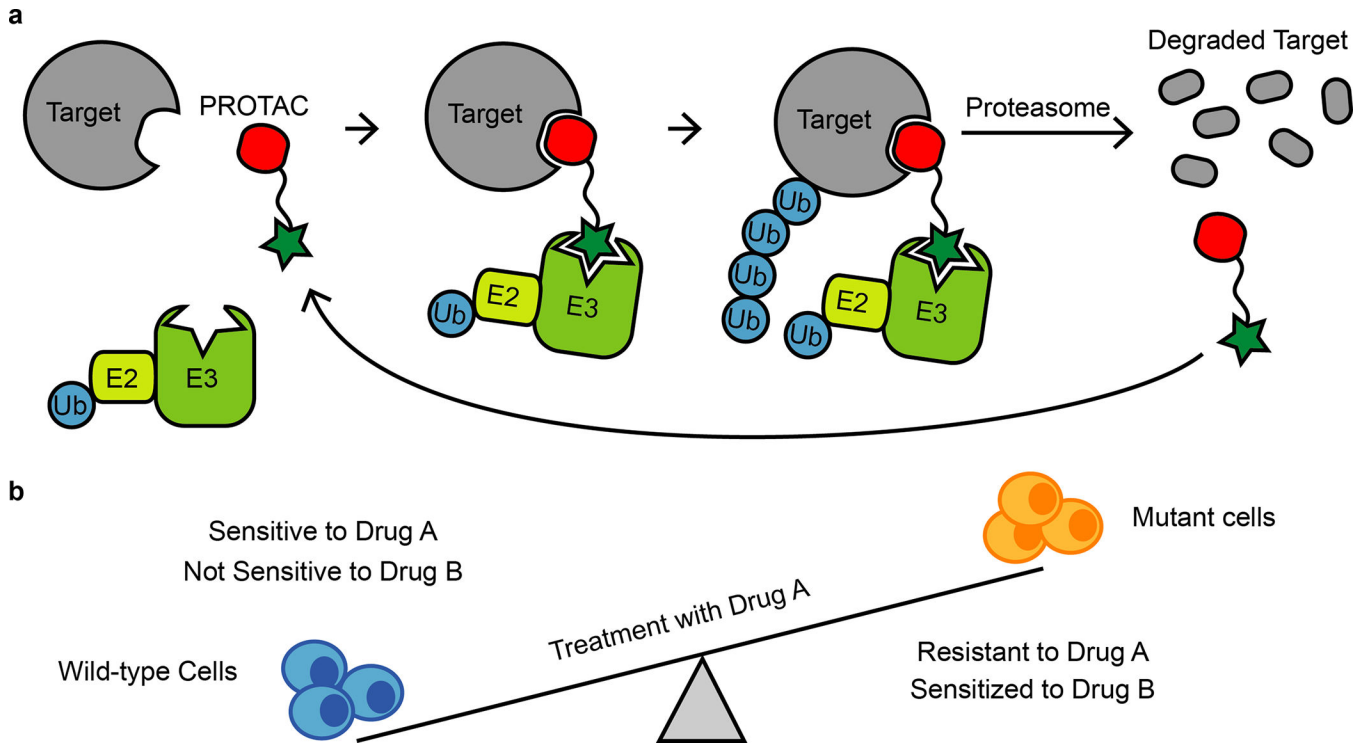
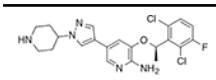
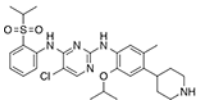
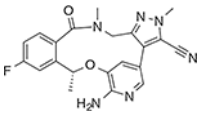
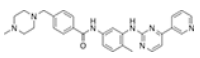
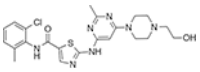
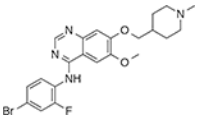
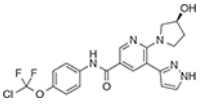
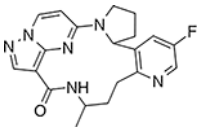
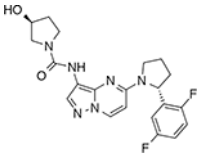
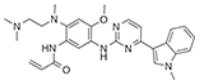
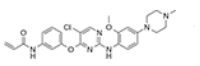


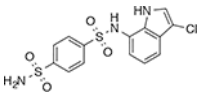
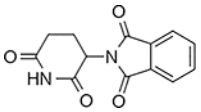
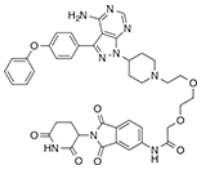
Fig. 5 |. Schematic of PROTAC and collateral sensitivity strategies.

a, Schematic of the PROTAC strategy. A ternary complex formed upon binding of PROTAC, a target protein (e.g., drug-resistant mutant allele of a selected protein) and an E3 ligase complex can promote target protein ubiquitination (Ub) and degradation by the proteasome. **b**, Schematic showing wild-type and drug A-resistant cells (blue and orange, respectively). Wild-type cells are sensitive to inhibition with drug A but not drug B. Cells that acquire resistance to drug A can become collaterally sensitive to drug B.

Table 1

Selected drugs discussed in the manuscript

Chemical structure	Name(s) (Brand name)	Target(s)	Examples of resistance- conferring mutations[Ref]	Mode of Action
	Crizotinib (Xalkori™)	ALK	ALK-L1196M, C1156Y[14,17,19]	ATP-competitive
	Ceritinib (Zykadia™)	ALK	ALK-L1198F[19,20]	ATP-competitive
	Lorlatinib (Lorbrena™)	ALK	ALK-C1156-L1198F[19,20]	ATP-competitive
	Imatinib (Gleevec™)	BCR-ABL	BCR-ABL-T315I[3,16]	ATP-competitive
	Dasatinib (Sprycel™)	BCR-ABL	BCR-ABL-T315I[3,16]	ATP-competitive
	Vandetanib (Caprelsa™)	BCR-ABL-V299L EGFR	BCR-ABL-V299L-E255K, V299L-F317L[78]	ATP-competitive
	ABL001 Asciminib	BCR-ABL-WT BCR-ABL-T315I	BCR-ABL-A337C, P465S, V468F[40]	allosteric
	LOXO-195	TrkA, B, C	N/A	ATP-competitive
	Larotrectinib LOXO-101 (Vitrakvi™)	TrkA, B, C	TrkA-F589L, G595R, G667C[24,25] TrkC-G623R, G696A[24,25]	ATP-competitive
	Osimertinib (Tagrisso™)	EGFR-T790M	EGFR-C797S, L718Q[35]	covalent
	WZ4020	EGFR-T790M	EGFR-C797S[35]	covalent

Chemical structure	Name(s) (Brand name)	Target(s)	Examples of resistance- conferring mutations[Ref]	Mode of Action
	Indisulam	RBM39 DCAF15	RBM39-G268V,W,R,E[67]	“molecular glue”
	Thalidomide	CRBN IKFZ1 IKFZ3	IKFZ1-Q146H[64,65] IKFZ3-Q147H[64,65] CRBN-W386A[64,65] CRBN-W400A[64,65]	“molecular glue”
	MT-802	CRBN BTK(C481S)	N/A	PROTAC