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Emergence of allosteric drug-resistance mutations: new challenges for allosteric drug discovery

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Allosteric drugs have several significant advantages over traditional orthosteric drugs, encompassing higher selectivity and lower toxicity. Although allosteric drugs have potential advantages as therapeutic agents to treat human diseases, allosteric drug-resistance mutations still occur, rendering these drugs ineffective. Here, we review the emergence of allosteric drug-resistance mutations with an emphasis on examples covering clinically important therapeutic targets, including Breakpoint cluster region-Abelson tyrosine kinase (Bcr-Abl), Akt kinase [also called Protein Kinase B (PKB)], isocitrate dehydrogenase (IDH), MAPK/ERK kinase (MEK), and SRC homology 2 domain-containing phosphatase 2 (SHP2). We also discuss challenges associated with tackling allosteric drug resistance and the possible strategies to overcome this issue.

Introduction

Allostery, or allosteric regulation, refers to the regulation of protein function induced by the binding of an effector to an allosteric site topographically distinct from the orthosteric site [1–3]. Allosteric regulation is common in cells and results from non-covalent binding (e.g., proteins, ions, lipids, small molecules, and DNA/RNA), covalent events (e.g., phosphorylation, point mutations, and reactions with small molecules), environmental disturbances (e.g. temperature, irradiation, pH, and ionic strength), and light absorption [4–7]. Upon allosteric effector binding, the introduced perturbation triggers the reorientation of atoms close to the allosteric site, creating strain energy and forcing the next layer of atoms to move, which then affects the next layer, and so on. Thus, allosteric perturbation passes through the protein structure from allosteric to orthosteric sites via propagation of an allosteric 'wave', leading to fine-tuning of the conformational dynamics of its orthosteric site [4,8,9]. In addition to this structural pathway view of allostery, the concept of dynamics-driven allostery was recently put forward, which suggests that allosteric perturbation spreads throughout the protein, including its orthosteric site, changing its entire vibration mode and conformational population [10,11]. Allostery is implicated in most aspects of cellular life, realizing the exquisite orchestration of myriad cellular processes, including signal transduction, enzymatic catalysis, cellular metabolism, and gene regulation [1,9,12], and, thus, is regarded as 'the second secret of life' [6].

Allosteric drugs have several significant advantages over traditional orthosteric drugs, including quiescence in the absence of endogenous orthosteric activity, greater selectivity based on less homologous sequences at allosteric sites, lower off-target toxicity, and lower dose requirements [13–16]. Owing to these advantages, harnessing allostery has been established as a novel mechanism for drug discovery, leading to a surge in the discovery, optimization, and clinical use of allosteric drugs [5,17–24].

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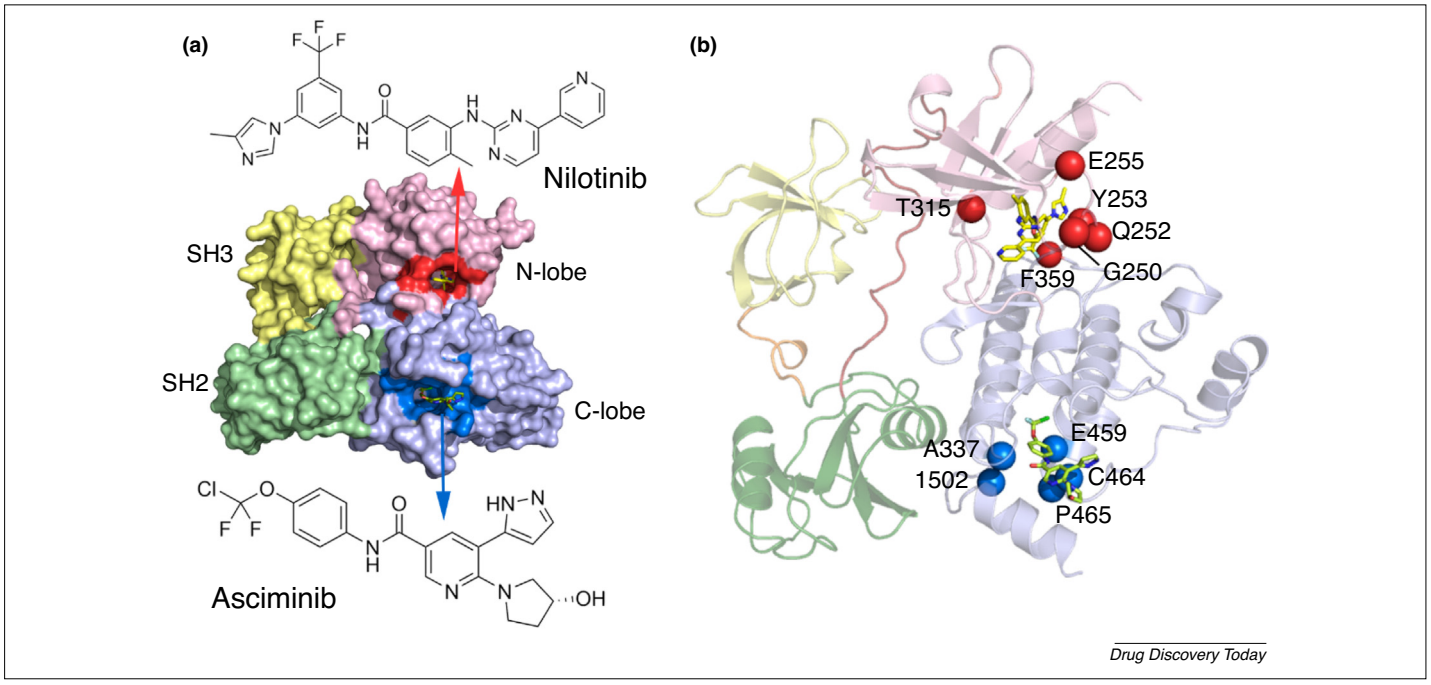

FIGURE 1

Allosteric and orthosteric drug-resistance mutations in Abl1. (a) Surface representation of the X-ray crystal structure of the autoinhibited Abelson tyrosine kinase 1 (Abl1) complexed with the orthosteric drug imatinib and an allosteric inhibitor asciminib (ABL001) [Protein Databank (PDB) ID: 5MO4]. Abl1 is colored by domains, SH3 (yellow), SH2 (green), N-lobe (pink), and C-lobe (light blue), with orthosteric and allosteric sites highlighted in red and blue, respectively. (b) Cartoon representation of Abl1. The sites of drug-resistance mutations depicted by spheres at the orthosteric site (Gly250, Gln252, Tyr253, Glu255, Thr315, and Phe359) and the allosteric site (Ala337, Pro465, Val468, Cys464, and Ile502). Molecular model structures were rendered using PyMOL v1.3 [61].

However, accumulating evidence shows that, because allosteric sites have lower evolutionary pressure than orthosteric sites, there is a increased chance for drug-resistance mutations to occur at the allosteric sites [20,25]. Moreover, drug-resistance mutations can occur not only at the allosteric sites, but also in the allosteric communication pathway, with the latter leading to indirect resistance towards allosteric modulators [26,27]. As a result, drug resistance emerges, hampering the application of allosteric drugs and threatening our ability to target a series of crucial diseases, such as cancers and infectious diseases [4,28,29]. Revealing the molecular mechanisms for drug resistance towards allosteric modulators is important to circumvent such challenges and will provide a promising opportunity for facilitating future drug design and optimization.

In this review, we discuss examples of drug-resistance allosteric mutations, focusing on clinically important targets, such as Bcr-Abl, Akt kinase (also called PKB), IDH, MEK, and SHP2. We provide structural mechanistic insights into their drug resistance and highlight potential strategies to tackle allosteric drug resistance and the possible approaches to predicting and analyzing them.

Drug-resistance allosteric mutations: some quintessential examples

Bcr-Abl

The Bcr-Abl fusion oncoprotein is an aberrantly active tyrosine kinase that causes chronic myeloid leukemia (CML) and 30–50% of adult acute lymphoblastic leukemia (ALL) cases [30]. The Abl regulatory module (RM), containing the SH3, SH2, SH3–SH2 linker, SH2–KD linker, and Cap domains, modulates the activation of the kinase domain (KD) by shifting between two distinct docking poses. Abl is autoinhibited when the RM docks at the back of the KD (Fig. 1a) via the interactions of its SH2 and SH3 domains with the C- and N-lobes, respectively. Upon activation, the SH2 domain disengages from the C-lobe and docks on the top of the N-lobe, yielding an extended state. The open conformation of Abl exposes the T421 residue located at the activation loop and enables substrate binding and phosphorylation, strongly enhancing its kinase activity [31]. Therefore, the structural shift towards the extended state has a vital role in the leukemogenic activity of Bcr-Abl [32]. Traditional ATP-competitive orthosteric drugs (e.g., imatinib and nilotinib) bind to the ATP site in the KD (Fig. 1a) and directly block the catalytic function of Abl, representing front-line therapy against CML [33]. However, despite initially satisfying responses, a fraction of patients treated with orthosteric inhibitors inevitably develop drug resistance, which is mainly driven by spontaneous point mutations adjacent to the ATP site, including G250H, Q252H, Y253H, E255K, T315I, and F359V (Fig. 1b) [34]. The most common mechanism, detected in 4–15% of patients with imatinib resistance, is the multidrug-resistant 'gatekeeper' mutation T315I, which is located at the hydrophobic pocket of the catalytic site and abolishes the crucial hydrogen bond formed by orthosteric inhibitors and the T315 residue [35].

One solution to overcome orthosteric drug resistance is the development of allosteric drugs by attaching to the myristoyl-binding pocket in the C-lobe (Fig. 1a). Asciminib (Fig. 1a), a potent and specific allosteric inhibitor, occupies the allosteric myristoyl-binding pocket and stabilizes the autoinhibited conformation. More importantly, asciminib is effective against all orthosteric

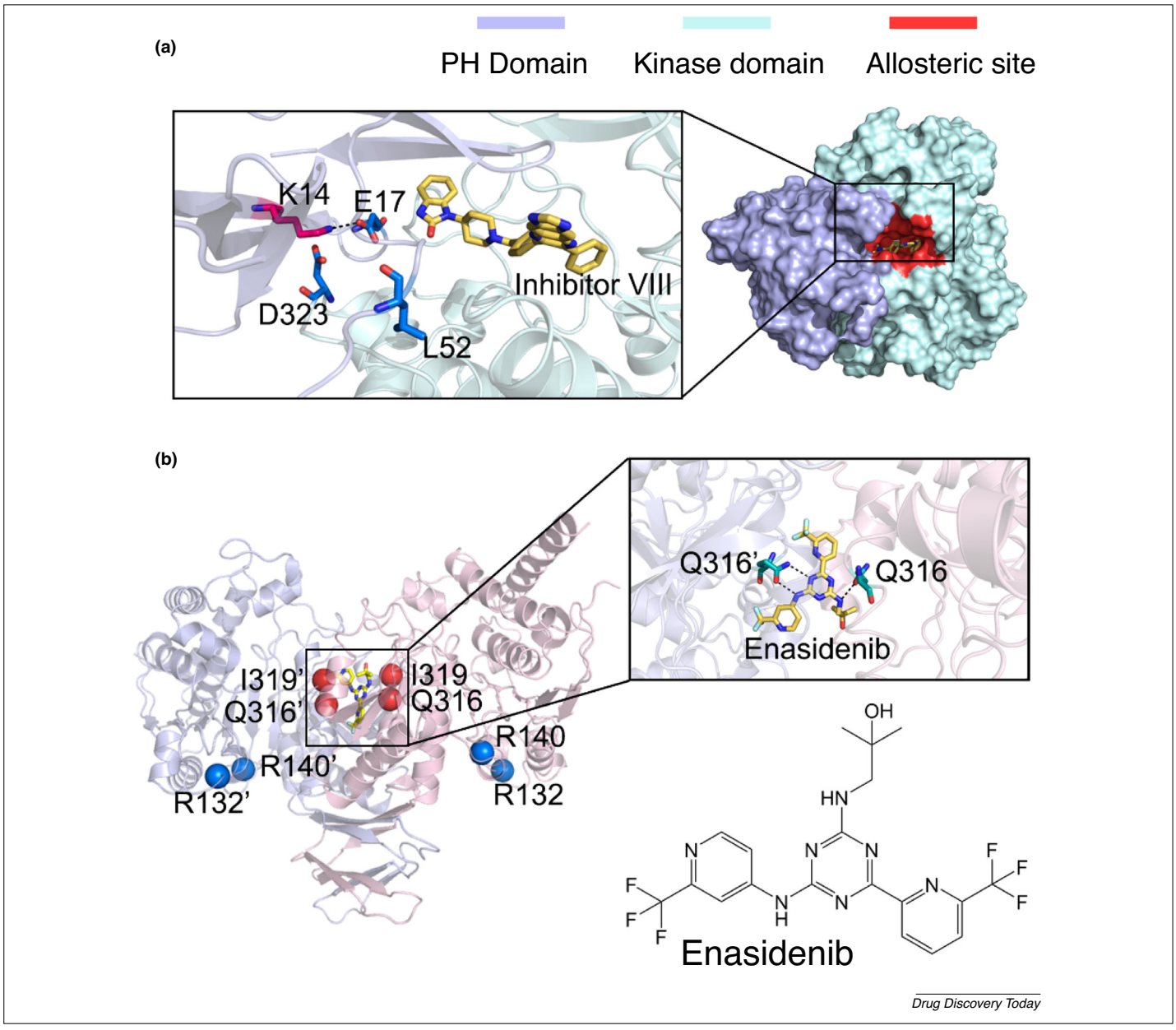

FIGURE 2

Allosteric drug-resistance mutations in AKT1. (a) Surface representation of the X-ray crystal structure of Akt kinase 1 (AKT1) in complex with an allosteric inhibitor (Inhibitor VIII; yellow sticks) [Protein Databank (PDB) ID: 3O96]. The kinase (KD) and pleckstrin homology (PH) domains are colored cyan and light blue, respectively. The allosteric pocket is highlighted in red. The enlarged image depicts the sites of allosteric drug-resistance mutations (blue sticks) at the PH–KD interface (Glu17, Leu52, and Asp323) and the ionic interaction between Glu17 and Lys14 (pink sticks). (b) Cartoon representation of the IDH2 dimer with enasidenib (AG-221; yellow sticks) bound to the dimer interface (PDB ID: 5I96). The wild-type residues affected by orthosteric neomorphic mutations (Arg132, Arg132′, Arg140, and Arg140′; blue) and allosteric drug-resistance mutations (Gln316, Gln316′, Ile319, and Ile319′; red) are highlighted by spheres. Enlarged image shows the crucial hydrogen bonds formed between Gln316, Gln316′ (dark-teal sticks) and enasidenib.

mutations, including T315I [34]. Currently, this inhibitor is undergoing a Phase III clinical trial in patients with CML (ClinicalTrials.gov: NCT03106779). However, recent studies demonstrated that allosteric drug resistance to asciminib is now occurring in Bcr-Abl. Qiang *et al.* illustrated in vitro that allosteric mutations within or near the myristoyl-binding pocket (e.g. A337V, P465S, V468F, C464W, and I502L) led to asciminib resistance (Fig. 1b). For instance, the C464W mutation introduces a bulky sidechain at the myristoyl-binding pocket, causing a steric clash to hinder asciminib binding. Cell proliferation experiments of theses mutants revealed a decreased response to asciminib, whereas their sensitivity to orthosteric drugs remains undiminished [34,36].

A recent in vitro study revealed a novel combination of allosteric (asciminib) and orthosteric (nilotinib) drugs to overcome kinase drug resistance. The dual-targeting treatment not only prevented the emergence of both allosteric and orthosteric drug-resistance mutations, but also provided additive effects to complete tumor regression [34]. This approach is currently in a Phase II clinical trial in patients with CML (ClinicalTrials.gov: NCT03578367).

Akt1 kinase

The v-Akt murine thymoma viral oncogene homolog (Akt) kinase, a serine/threonine kinase, has a pivotal role in various cellular functions, including cell proliferation, gene expression, metabolism, and survival [37]. Akt is an essential member of the phosphoinositide 3-kinase (PI3K) signaling pathway, a pathway that is frequently hyperactivated in a range of cancers, including breast, colorectal, and ovarian [38]. Akt1 has a classic kinase architecture comprising a KD, an N-terminal pleckstrin homology (PH) domain, and a C-terminal regulatory module (RM) (Fig. 2a). Similar to ABL, the PH-KD interaction also contributes to two distinct conformations of Akt1; an autoinhibited and closed PH-in state formed by interdomain PH–KD contacts, which blocks the access of phosphoinositide-dependent kinase 1 to phosphorylate T308 at the activation loop, and an open PH-out state with impaired PH–KD interactions, which exposes T308 for phosphorylation and subsequently leads to Akt1 activation.

Given the conserved orthosteric ATP sites throughout the human kinome, allosteric inhibition of the kinase provides a option for enhanced selectivity and reduced off-target toxicity [39]. As a result, several allosteric inhibitors (e.g., Inhibitor VIII and GNE-929) have been designed to target the intact PH–KD interface (Fig. 2a) and stabilize the kinase in its autoinhibited PH-in state. Accordingly, perturbation of the crucial interdomain PH–KD interaction shifts the dynamic equilibrium of Akt1 from the closed to open state, leading to a structural alteration at the allosteric ligand-binding site and reducing its sensitivity to allosteric inhibitors [40,41].

A recent study scanned 394 clinical tumor specimens representing multiple tumor types and discovered allosteric drug-resistance mutations that disturb interdomain interactions, encompassing E17K and L52R in the PH domain and D323H in the KD (Fig. 2a) [40]. These three somatic mutations disrupt hydrogen bonds and create steric conflicts that impede the docking of PH, thus hampering PH–KD interactions. Further biochemical assays revealed that, although they retain their sensitivity to orthosteric ATP-competitive inhibitors, E17K, L52R, and D323H mutants give rise to elevated IC_{50} of Inhibitor VIII, indicating their mutation-driven drug resistance against allosteric inhibitors [40]. As an example, the acidic Glu17 is situated in the phosphoinositide-binding pocket, forming a crucial ionic interaction with the nearby basic Lys14 (Fig. 2a). However, the Lys17 substitution in the E17K mutant interrupts the ionic interaction and leads to an alteration from negative to neutral in the surface charge around the allosteric pocket [41]. Thus, the E17K mutation destabilizes the PH–KD interface and induces allosteric drug resistance via perturbation of interdomain interactions.

IDH

The IDH family, containing isozymes IDH1, IDH2, and IDH3, is a family of key metabolic enzymes. IDH functions to convert isocitrate into α-ketoglutarate (α-KG) in the tricarboxylic acid cycle [42]. However, previous studies revealed that arginine mutations (R172 and R140 in IDH2, and R132 in IDH1) occurring at the orthosteric isocitrate binding site lead to gain of function of IDH, representing a mechanism of oncogenesis. Hyperactivation of these orthosteric mutants (e.g., R140Q or R132C) (Fig. 2b) converts α-KG into (D)-2-hydroxyglutarate (2-HG), resulting in the accumulation of 2-HG, a functional oncometabolite. Therefore, multiple somatic mutations at R172, R140, and R132 have been observed in various tumor types, including AML and myelodysplastic syndrome [42,43].

IDH2 is activated as an obligate homodimer, thus revealing the IDH2 dimer interface to be a promising allosteric target (Fig. 2b) to therapeutically block the enzyme. Enasidenib (AG-221), a potent allosteric inhibitor that binds to the IDH2 dimer interface (Fig. 2b), can effectively block the conversion of α-KG to 2-HG caused by orthosteric mutations. Currently, it is in a Phase II clinical trial in patients with AML (ClinicalTrials.gov: NCT02677922). Although the IDH2 dimer presents a symmetrical binding interface, whereas enasidenib features an asymmetrical structure, the identical Gln316 residue on both sides of the interface forms different, yet exquisite hydrogen bonds with enasidenib (Fig. 2b), giving rise to its high affinity.

However, allosteric dimer–interface mutations occur and render enasidenib ineffective in a fraction of patients after a few months of treatment. A recent study of two patients with gained enasidenib resistance showed that both Q316E and I319M led to a progressive increase in 2-HG levels and, consequently, caused cancer relapse. Further structural modeling illustrated that the Q316E mutation diminishes hydrogen bonds between IDH and enasidenib (Fig. 2b), whereas the I319M mutation creates steric hindrance because of the bulky side chain of methionine [44]. Both mutations obstruct enasidenib binding and induce drug resistance in patients.

MEK

The RAS-RAF-MEK1/2-ERK pathway is activated by a variety of extracellular stimuli, leading to distinct intracellular responses, including cell proliferation, survival, and migration. Mutations within the RAS-RAF-MEK1/2-ERK pathway have been identified as vital drivers of cancer development [45,46]. Whereas MEK is not a leading cause of mutation-driven oncogenesis, RAF and RAS kinase mutations are frequently discovered in solid tumors. BRAF mutants are conserved in ~20% of all cancers and in ~40–60% of melanomas, and KRAS is conserved mutated in ~55% of metastatic colorectal cancer and in 20–30% of lung adenocarcinomas [45].

Although MEK itself is not a frequently mutated kinase, RAF or RAS mutations hyperactivate downstream MEK, thus amplifying MAPK signaling. Hence, MEK represents an ideal target for cancer therapies by blocking this pathway. Currently available MEK inhibitors are all allosteric [47], including trametinib, cobimetinib (Fig. 3), and binimetinib. These drugs are approved by the US Food and Drug Administration (FDA) either alone or in combination with the BRAF inhibitor vemurafenib to treat patients harboring BRAF V600E mutated melanoma [45]. Allosteric inhibitors bind to the pocket formed by the displacement of α-helix C away from the activation loop and stabilize the MEK1 at the αC-out inactive conformation (Fig. 3). Although these drugs have markedly improved patient survival, allosteric mutations occur and confer drug resistance with two distinct mechanisms: directly reduced binding affinity or through altered α-helix C conformation. A recent study revealed that the 'gatekeeper' MEK1 V211D mutation situated within the arylamine binding pocket of the allosteric site (Fig. 3) perturbs the binding of multiple allosteric inhibitors [48,49]. Template modeling and molecular dynamics simulations illustrated that the substitution of hydrophobic V211 with nonhydrophobic

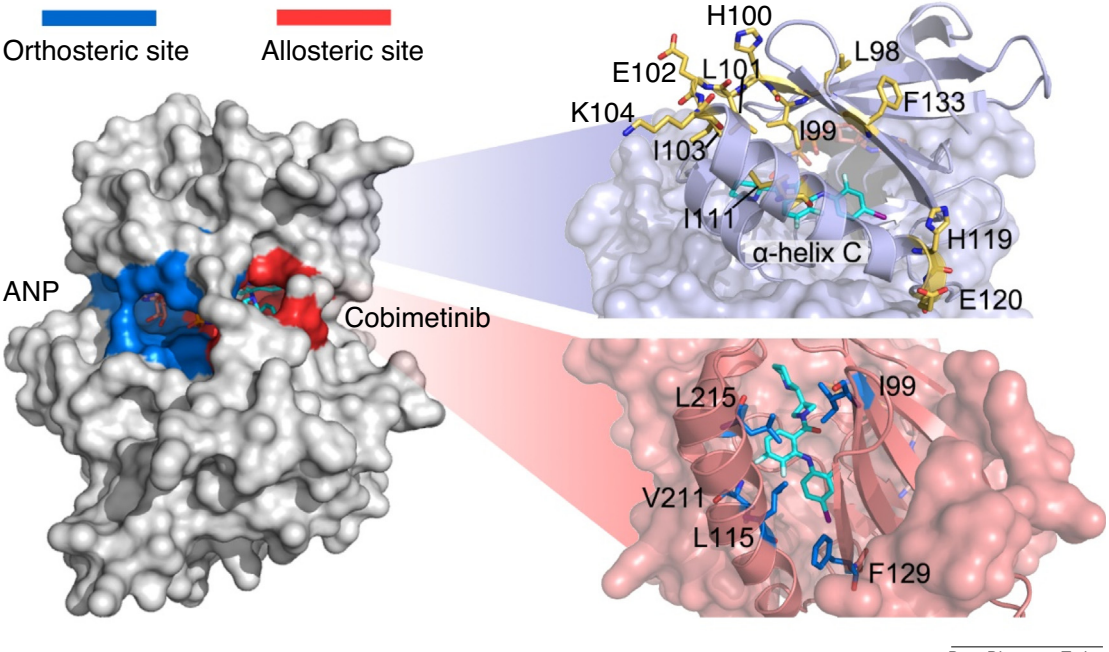

FIGURE 3

Surface representation of the X-ray crystal structure of MAPK/ERK kinase (MEK) bound to cobimetinib (teal sticks) and phosphominophosphonic acid-adenylate ester (ANP) (deep-pink sticks) [Protein Databank (PDB) ID: 4LMN], with the adjacent orthosteric and allosteric sites highlighted in blue and red, respectively. Enlarged image represents α-helix C and β15 in cartoon. The enlarged image of the MEK1 allosteric binding pocket (dark-pink surface) depicts the sites of mutations clustering within the allosteric binding pocket (blue sticks) (Ile99, Leu115, Phe129, Val211, and Leu215).The enlarged image of α-helix C (light-blue surface) shows the sites of mutations located along and adjacent to the α-helix C or in the β3-αC loop (yellow sticks) (Ile103, Lys104, Ile111, His119, Glu120, Phe133, and ^{98}LIHLEIK^{104}).

D211 eliminated the hydrophobic carbon atoms that interact with inhibitors [48]. Furthermore, similar mutations that directly cluster within the arylamine binding pocket were revealed, encompassing I99T, L115P/R, F129L, and L215P (Fig. 3) [49].

Besides directly interfering mutations, a host of mutations located along and adjacent to the α-helix C (e.g., I103N, K104N, I111N, H119P, E120D, and F133L) (Fig. 3) lead to drug resistance via the alteration of α-helix C conformation [49]. Furthermore, mutants with deletions at the β3-αC loop bounded by amino acids ^{98}LIHLEIK^{104} (e.g., ΔL98-I103, ΔE102-I103, ΔI99-K104, and ΔI103-K104) also confer drug resistance (Fig. 3) [47]. The inactive αC-out conformation provides the binding pocket for allosteric inhibitors. However, the conformational shift from the αC-in state to the αC-out state requires a minimum length of the β3-αC loop for movement. Consequently, the deletion of amino acids 98–104 stabilizes the active αC-in conformation, closing the binding pocket, and decreasing the affinity to allosteric inhibitors.

Fortunately, despite worsening allosteric drug resistance, the allosteric mutants discussed earlier remain sensitive to ATP-competing orthosteric inhibitors [47]. Accordingly, the newly developed orthosteric inhibitors present a rational therapeutic strategy for patients who develop drug resistance after treatment with allosteric inhibitors.

SHP2

SHP2 is a key downstream regulator in the signaling pathways of a variety of growth factors and cytokines and has a crucial role in cell proliferation and survival mainly via the activation of RAS–ERK signaling pathway [50]. However, hyperactivation of SHP2 caused by upstream oncogenic mutated protein tyrosine kinases (PTKs) has been discovered in several cancers types, including leukemia and multiple solid tumors [50,51]. Therefore, instead of traditionally targeting mutated protein kinases, suppression of SHP2 activity restrains tumor growth and presents a prominent target for cancer treatment.

SHP2 is a nonreceptor phosphatase comprising a protein tyrosine phosphatase (PTP) domain and two preceding Src homology 2 domains (N-SH2 and C-SH2 domains) (Fig. 4). SHP2 is observed mainly in two interconverting conformations: in the autoinhibited state, the N-SH2 domain docks to the PTP domain, forming an N-SH2/PTP interface (Fig. 4); in the extended, activated state, the N-SH2 twists away from the PTP domain, releasing the autoinhibitory interface. When the N-SH2 and C-SH2 domains bind to the appropriately spaced phosphotyrosine residues in upstream proteins, SHP2 is activated and shifts towards the extended state. Thus, the linker between the two SH2 domains rotates and SHP2 turns to the N-SH2-open conformation, rendering the active site at PTP for substrate recognition and catalytic functions [50].

However, because of the high polarity and solvated nature of the catalytic site at PTP, efforts to design orthosteric inhibitors face challenges. To discover alternative allosteric drugs targeting the 'undruggable' enzyme, a study screened a library of 100 000 compounds [52]. SHP099 (Fig. 4) was identified as a potent allosteric inhibitor that binds to a tunnel-like site comprising all three domains (Fig. 4), locking SHP2 in the autoinhibited conformation.

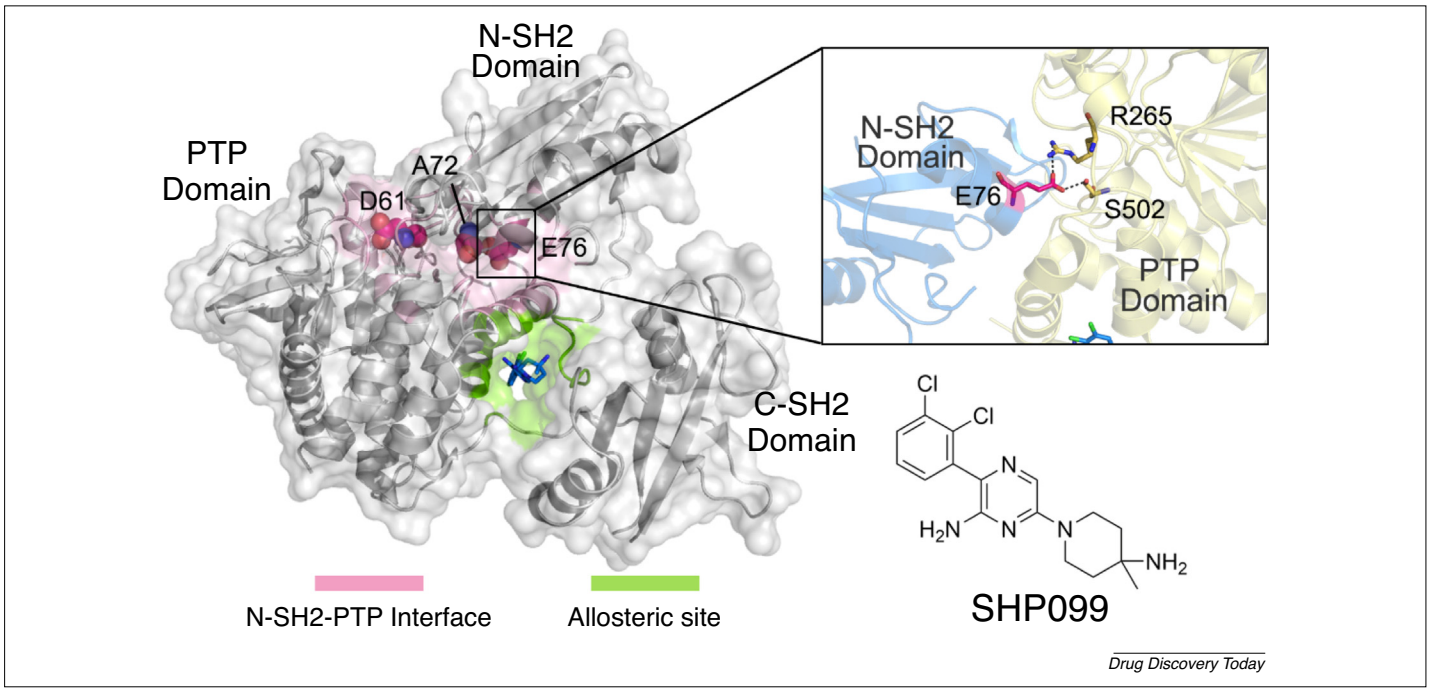

FIGURE 4

An X-ray crystal structure of SRC homology 2 domain-containing phosphatase 2 (SHP2) complexed with SHP099 [Protein Databank (PDB) ID: 5EHR], containing the protein tyrosine phosphatase (PTP) domain, and Src homology 2 domains (N-SH2 and C-SH2 domains), with the allosteric site and N-SH2–PTP interface highlighted in green and pink, respectively. The wild-type residues affected by allosteric drug-resistance mutations (Asp61, Ala72, and Glu 76) at the N-SH2–PTP interface are shown in pink spheres. The enlarged image depicts a hydrogen bond (E76–S502) and a salt bridge (E76–R265) between the N-SH2 (blue cartoon) and PTP domain (yellow cartoon).

Moreover, SHP099 features high selectivity, showing no activity against SHP1, the closest homolog of SHP2 [52].

Despite its high potency and low off-target toxicity, the treatment of SHP099 faces the allosteric drug resistance. An in vitro study demonstrated that gain-of-function mutations situated in the middle of the N-SH2/PTP interface, showing less sensitivity to SHP099 compared with the wild-type SHP2 [52]. Further inspection of COSMIC database illustrated that D61, E76, and A72 were most frequently mutated allosteric residues in human cancer (e.g., D61Y, A72V, and E76K; Fig. 4). Destabilization of the closed conformation and the increased open-state population caused by the disturbance of the N-SH2/PTP interface is the major mechanism of allosteric drug resistance. As an example, E76K mutation disrupts a hydrogen bond (E76-S502) and a salt bridge (E76-R265), which are both important to N-SH2–PTP interactions (Fig. 4), thus destabilizing the assembled state [51]. In addition to causing a population shift in the variants to the active state, the impaired N-SH2–PTP interface in the SHP2 mutants significantly destabilizes the SHP099-binding tunnel formed by all three domains, resulting in decreased sensitivity to SHP099.

Therefore, increased emphasis has been placed on the discovery of novel allosteric inhibitors that overcome common drug resistance issues. For instance, an allosteric inhibitor compound 23 was recently validated as an effective small molecule to target the less common $SHP2^{E76A}$ [51].

Concluding remarks and future perspectives

The structural and mechanistic overview of the five different cases provided earlier reveals the common mechanism of drug resistance associated with allosteric mutations. Generally, allosteric mutations within or outside the allosteric sites can create steric hindrances and/or disrupt the arrangements of binding-determinant residues, leading to drug resistance through two major mechanisms. The first is directly interfering with modulator binding via mutations at, or adjacent to, the target site. For instance, mutations clustering within the arylamine binding pocket in MEK reduce its binding ability to multiple allosteric inhibitors [49]. The other essential mechanism is a population shift in the mutants to an active or inactive state that is unfavored by allosteric modulator binding. In the case of MEK, a host of mutations located along and adjacent to the α-helix C, as well as deletions at the β3-αC loop, lead to a population shift of MEK mutants towards the αC-in active conformation, rendering the mutants insensitive to the binding of allosteric inhibitors [47,49].

Identifying potential mutation sites that alter the affinity of a protein for pre-existing allosteric drugs is a promising strategy to nominate novel targets for further drug design or optimization. One of the major stumbling blocks in new drug design is the prediction of latent allosteric drug-resistance mutations, which is an important process in anti-mutation drug development. Unfortunately, the prediction of allosteric mutations is more laborious than for orthosteric mutations, because orthosteric mutations are limited in catalytic sites. By contrast, allosteric mutations can occur at various positions, including allosteric regulator binding sites, allosteric signaling pathways [26,27], and other residues vital for protein conformational stability. As such, it is difficult to uncover allosteric mutations via experimental approaches [20]. However, recent advances in computational allosteric methods have realized the investigation of allosteric molecular regulation at per-residue resolution [53]. As a result, a host of computational

approaches have recently emerged, aiming to identify allosteric mutations and observe the consequent effects with sequence-, structure-, and dynamics-based methods [54–56].

AlloDriver is a recently developed platform for the identification and analysis of cancer-driver somatic mutations at allosteric sites [3,57,58]. It is based on the mapping of >47 000 somatic missense mutations generated from ~7000 clinical tumor samples across 33 cancer types, to allosteric sites derived from 3D protein structures [28]. AlloDriver can identify unreported cancer-driver mutation sites from clinical samples, aiding the prediction of novel allosteric targets. As an example, AlloDriver was used to discover new targets of human protein tyrosine phosphatase, receptor type K (PTPRK) in head and neck squamous cell carcinoma, and successfully predicted an unreported cancer-driver L1143F mutation at the allosteric site of PTPRK [57], which is a possible target for novel inhibitors to overcome resistance to current allosteric drugs.

Another potential method to predict allosteric drug-resistance mutations is the structure-based statistical mechanical model of allostery (SBSMMA), which allows one to estimate the causality and energetics of allosteric communication caused by perturbations of allosteric ligand binding or mutations [17–19]. SBSMMA enables allosteric site identification by reversing the allosteric signaling through perturbation at the orthosteric site, and further explores the molecular effects of allosteric mutations, aiding the observation of latent drug-resistance mutations. The SBSMMA was implemented in the web server AlloSigMA, which offers a framework for free energy calculation and detects the positive and/or negative effects caused by allosteric mutations [17,59]. AlloMAPS, a database containing comprehensive allosteric signaling maps of ~2000 distinct proteins and protein chains, is also built utilizing SBSMMA and evaluates the allosteric effects of each mutation [18]. A recent study used SBSMMA to predict the allosteric mutations that lead to the deficiency of galactose 1-phosphate uridyltransferase and glucose-6-phosphate dehydrogenase, and the output loss-of-function mutants have been verified by in vitro experiments [19].

Overall, computational allosteric prediction approaches can help to address emerging drug resistance, such as the identification or prediction of allosteric mutation sites by AlloDriver and SBSMMA, respectively. For instance, AlloDriver provides uncovered mutation sites generated from clinical samples, which expands the discovery of novel allosteric targets [28,57]. These identified targets can guide the design of next-generation modulators targeting novel mutated sites.

Besides the identification and prediction of potential drug-resistance mutations, clinical dual-targeting therapy is also a validated approach to overcome emerging drug resistance [34,60]. Several in vivo and in vitro studies of distinct targets revealed that combination dosing with allosteric and orthosteric ligands can firmly stabilize the protein conformation in the wanted state, delaying the emergence of drug-resistance mutations at both allosteric and orthosteric sites. Dual-targeting therapy also prevents drug-resistance emergence and shows excellent therapeutic effects and, thus, is under clinical trials for multiple diseases. For instance, asciminib together with imatinib provides an initial solution for Bcr-Abl drug resistance [34], whereas osimertinib together with JBJ-04-125-02 prevents the emergence of drug-resistance mutations in EGFR [60]. In summary, we expect that the comprehensive mechanistic understanding of allosteric drug-resistance mutations will arouse our awareness of the emerging drug-resistance problem in allosteric modulation and guide future endeavors to overcome such obstacles.

Acknowledgments

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