Trends in Pharmacological Sciences

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Review

Combining Allosteric and Orthosteric Drugs to Overcome Drug Resistance

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Historically, most drugs target protein orthosteric sites. The gradual emergence of resistance hampers their therapeutic effectiveness, posing a challenge to drug development. Coadministration of allosteric and orthosteric drugs provides a revolutionary strategy to circumvent drug resistance, as drugs targeting the topologically distinct allosteric sites can restore or even enhance the efficacy of orthosteric drugs. Here, we comprehensively review the latest successful examples of such combination treatments against drug resistance, with a focus on their modes of action and the underlying structural mechanisms. Our work supplies an innovative insight into such promising methodology against the recalcitrant drug resistance conundrum and will be instructive for future clinical therapeutics.

Coadministration of Allosteric and Orthosteric Drugs: A Novel Strategy to Tackle Drug Resistance

As a detrimental problem in modern biomedicine, the inevitable emergence of drug resistance poses a great challenge to pharmaceutics and threatens global health [1–7]. It is a sophisticated evolving process involving multiple events on the molecular pathway, and even organismal levels. Based on the underlying mechanism, drug resistance is classified as **target-dependent resistance** (see Glossary), caused by changes within the original targets, and **pathway-dependent resistance**, resulting from alterations in the relevant collateral pathways) [4,8,9]. Different methodologies are applicable for tackling different types of resistance; for example, structure-based drug design is more suitable against target-dependent resistance, whereas the multitargeted approach of polypharmacology may be a better choice against pathway-dependent resistance [10–12]. Many excellent reviews have already discussed various approaches to combat resistance, but most of them only focus on either drug optimization or combined treatments with different types of therapies [5,12–14].

Stemming from rapid developments in structural biology and protein allostery research [15–20], allosteric drugs can resensitize resistant targets and selectively target resistance-related signaling pathways, thereby restoring the efficacy of **orthosteric** drugs. Moreover, compared with monotherapy, double- or even multiple-drugging strategies can cover a broader therapeutic spectrum, achieve synergistic effects, and contribute to better clinical outcomes. Thus, combination therapy with allosteric and orthosteric drugs provides an unprecedented opportunity to overcome the notorious problem of drug resistance and boost the development of next-generation pharmacological agents.

With the goal of forwarding this notion, this review first outlines typical examples of combined treatments to combat clinically relevant resistance, reviewing their modes of action and future perspectives in detail. Then, it discusses the current state of this combinatorial strategy, presenting the existent obstacles as well as possible solutions. This review highlights a promising tactic for overcoming the long-standing conundrum of drug resistance and will hopefully be instructive for future drug discovery and development.

Highlights

Traditional drugs target protein orthosteric sites; drug resistance inevitably develops due to Darwinian selection pressure posed by the therapeutics.

Drug resistance triggers disease relapse and causes great losses to the drug discovery processes. It also constitutes a key threat to global health and a major challenge to modern medicine.

Allosteric drugs target sites distinct from those of orthosteric drugs. They modulate the functions of orthosteric drugs and can resensitize resistant targets. Double targeting at allosteric and orthosteric sites can overcome resistance and improve pharmacological effectiveness.

Combinatorial treatments with allosteric and orthosteric drugs provide a novel strategy against drug resistance.

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Overcoming Target-Dependent Resistance

BCR-ABL Kinase

The quintessential example of the combined use of allosteric and orthosteric drugs is the targeting of BCR-ABL kinase for the treatment of chronic myeloid leukemia (CML). CML used to be one of the most fatal malignancies and still comprises about 15% of all diagnosed adult leukemia cases [21,22]. Present in more than 95% of CML patients, the fusion oncoprotein BCR-ABL is constitutively active, inducing abnormal cell proliferation and leukosis [22,23]. As such, it has been validated as an important target for CML interventions. Orthosteric drugs, such as imatinib, inhibit the aberrant kinase activity of BCR-ABL by competing with ATP for binding to the ATP-binding sites of the protein. Their discovery revolutionized CML therapeutics, decreasing its annual mortality from 10–20% to 1–2% and ameliorating its prognosis [21,24,25]. Nevertheless, the gradual emergence of resistant mutants leads to loss of response and disease relapse. Most of these mutations affect the ATP-binding site or the activation loop within BCR-ABL. Mutations of the gatekeeper residue T315 are among the most challenging ones. They hinder inhibitor binding through steric clash, thus disrupting protein–ligand interactions. The T315I mutation confers resistance to all orthosteric BCR-ABL inhibitors except ponatinib, whereas the latest reported single mutations, T315L and T315M, as well as double mutants such as T315I/Y253H can also obstruct the effect of this drug [26–28]. To address these recalcitrant problems, researchers have raised the possibility of combining allosteric and orthosteric inhibitors to overcome drug resistance.

The first successful attempt to identify novel allosteric pharmacological agents that act synergistically with orthosteric ligands was reported by Adrian *et al.* [29], who used a fluorescence-based proliferation assay to screen several combined compound libraries. GNF-1 was identified as a lead hit, the further medicinal chemical modification of which yielded the more potent GNF-2 (Figure 1A), which could significantly enhance the effect of imatinib on the BCR-ABL-expressing cell line, Ba/F3.p210.

Following this stream, Zhang *et al.* conducted another round of optimizations and obtained an improved compound, GNF-5 (Figure 1B) [30]. Mutagenesis and solution NMR studies suggested that inhibitors of this class act allosterically by binding to the C-terminal myristate pocket of BCR-ABL. This hypothesis was later confirmed by the structure analysis of a BCR-ABL/GNF-2 cocrystal (Figure 1C). Within the myristoyl cavity, GNF-2 interacts with BCR-ABL mainly via hydrophobic interactions and its trifluoromethyl moiety is deeply buried there. Several water-mediated hydrogen bonds are also formed between GNF-2 and neighboring BCR-ABL residues (Figure 1D).

Hydrogen-exchange mass spectrometry unveiled that the topology of the orthosteric ATP-binding site of BCR-ABL changes in response to GNF-2 binding. Further biochemical and cellular studies, later extended to the murine xenograft model, suggested that the combination of GNF-series compounds with orthosteric drugs such as imatinib or nilotinib is able to potently inhibit previously drug-resistant BCR-ABL mutants such as T315I [29,30], highlighting the promising therapeutic potential of such combinations.

Despite the effectiveness of the GNF-series inhibitors in experimental studies, none of them has yet reached the stage of clinical development. To identify new compound scaffolds and develop better drug molecules, Wylie *et al.* utilized fragment-based NMR screening and optimized the resulting candidates via *in silico* docking and crystallography studies [31]. These efforts led to the discovery of ABL001 (Figure 2A), which can inhibit BCR-ABL mutants synergistically with orthosteric drugs. Analysis of the crystal structure of BCR-ABL^{T315I} in complex with both nilotinib and ABL001 confirmed that ABL001 also binds to the allosteric myristoyl pocket (Figure 2A),

Glossary

Allosteric site: a pocket spatially and topologically distinct from orthosteric sites. Perturbations at allosteric sites caused by factors such as mutations or binding by ligands known as allosteric modulators can modulate the activity of orthosteric sites.

Allosteric modulator: a compound that binds to allosteric sites and modulates the activity of orthosteric sites.

Orthosteric site: a pocket where endogenous ligands and substrates bind.

Orthosteric modulator: a compound that binds to orthosteric sites and modulates the activity of target proteins through direct competition with endogenous ligands or substrate molecules.

Pathway-dependent resistance (off-target resistance): drug resistance resulting from the abnormal activation or rewiring of the compensatory pathways, which can bypass the requirements for the original drug targets, enable pathogenic signal transduction, and reduce therapeutic agent efficacy.

Target-dependent resistance (on-target resistance): drug resistance triggered by changes in the original protein targets, such as mutations or other lesions that modify their biological and medicinal properties by either inducing direct steric clash or altering the structural ensemble. These changes hinder the binding or the functions of drug molecules, making them ineffective.

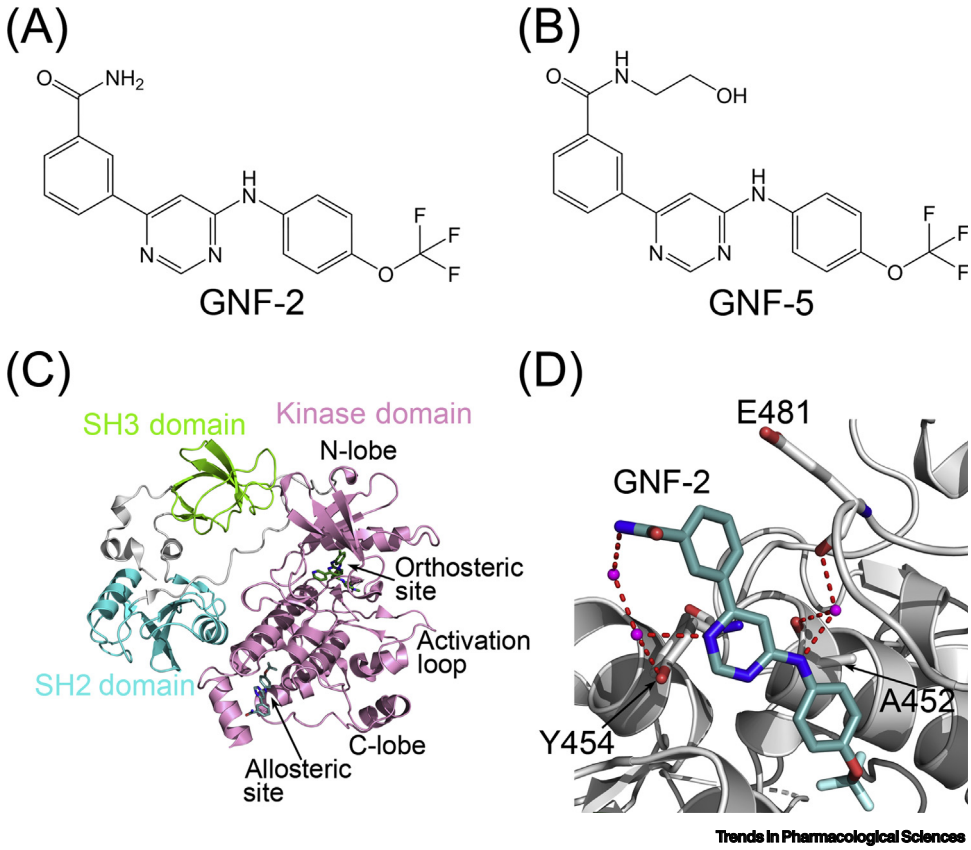

Figure 1. The GNF-series of Allosteric BCR-ABL Inhibitors. (A) Chemical structure of GNF-2. (B) Chemical structure of GNF-5. (C) Structural overview of BCR-ABL in complex with the orthosteric inhibitor, imatinib, and the allosteric inhibitor, GNF-2. While the structures of imatinib and GNF-2 were from the BCR-ABL structure in PDB ID: 3K5V, these were aligned with the full-length BCR-ABL structure from PDB ID: 2FO0 for better visualization. (D) Interaction details between GNF-2 and BCR-ABL (PDB ID: 3K5V). Hydrogen bonds are depicted as red broken lines, whereas water molecules are shown as magenta spheres.

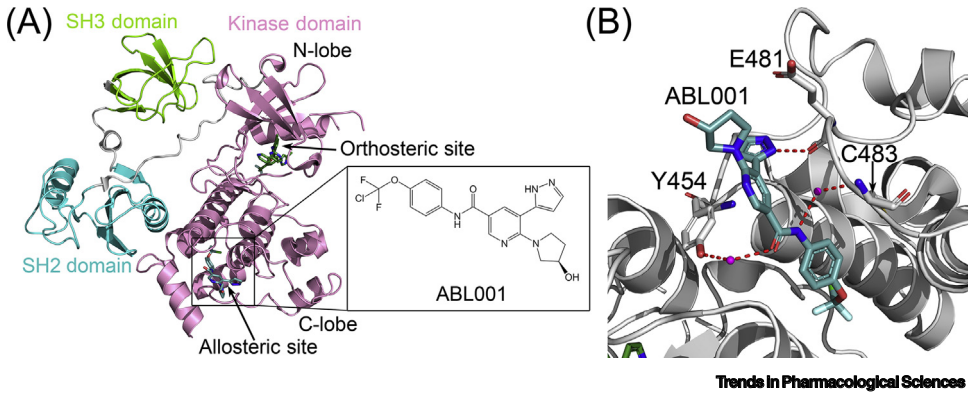

Figure 2. Allosteric BCR-ABL Inhibitor ABL001. (A) Structural overview of BCR-ABL in complex with the orthosteric inhibitor, nilotinib, and the allosteric inhibitor, ABL001 (PDB ID: 5MO4). (B) Interaction details between ABL001 and BCR-ABL (PDB ID: 5MO4). Hydrogen bonds are depicted as red broken lines, whereas water molecules are shown as magenta spheres.

where it adopts an identical binding mode to the GNF-series inhibitors; mostly forming hydrophobic interactions with BCR-ABL. The chloro-difluoro-methoxy-phenyl group of ABL001 projects deep into the binding cavity and the pyrazole moiety is hydrogen-bonded to E481. The neighboring Y454 and C483 residues form two water-mediated hydrogen bonds with ABL001 (Figure 2B). *In vitro* cell growth inhibition experiments showed that ABL001 acts synergistically with classic therapeutic agents such as imatinib, dasatinib, and nilotinib, which could help overcome resistance to these agents. Remarkably, the simultaneous treatment of xenograft mice with ABL001 and nilotinib completely eradicated tumor burden, without inducing drug resistance or relapse, which were observed in traditional treatments. Even after 3 months, no tumor regrowth or remission was observed, implying that the dual-agent treatment yields a durable, complete regression of the malignancy [31]. Recently, researchers discovered that treating resistant BCR-ABL^{T315I} cell clones with the latest-generation orthosteric drug, ponatinib, led to the gradual emergence of secondary mutations such as Y253H and E255V, resulting in resistance. Coadministration of ABL001 with ponatinib tackled this recalcitrant problem, not only restoring the efficacy but also increasing the effectiveness of ponatinib [28].

These findings, combined with its enhanced pharmacological properties such as high selectivity as well as its improved pharmacokinetics and pharmacodynamics, validate ABL001 as the best allosteric synergist candidate for orthosteric BCR-ABL drugs so far and a promising pharmacological modality for tackling drug-resistant BCR-ABL mutants. There is an ongoing clinical trial (Clinical Trial Number: NCT02081378[i]) to evaluate the possibility of combining ABL001 with imatinib, nilotinib, or dasatinib for treating CML.

Nonstructural Protein (NS)5A

Hepatitis C virus (HCV) infection is a notorious global health threat affecting more than 150 million people [32,33]. Mediating multiple stages during the HCV replication cycle and infection processes, NS5A first dimerizes and then incorporates into a helical polymer to execute its pathological functions [34–36]. It has thus been established as a promising target for antiviral agent development [37,38].

The first class of HCV NS5A inhibitors was reported by Gao *et al.* [37]. Using high-throughput screening and further lead optimization, they identified daclatasvir (also known as DCV, Daklinza, or BMS-790052) (Figure 3A). Structural studies showed that the binding site of daclatasvir is located at the NS5A dimer ridge (residues F37, Q62, Y93, F37', Q62', and Y93'; apostrophes denote residues from the second monomer of the NS5A dimer), adjacent to the N-terminus of Domain I within NS5A, opposite to its RNA-binding domain (Figure 3B). Upon ligand binding, the natural packing and protein–protein interactions between the NS5A dimers are compromised, disrupting the structure of the NS5A polymer. This, in turn, disrupts formation of the virus replication complex, thus hindering HCV pathogenesis. A series of experimental and clinical studies has demonstrated the potency of daclatasvir, with EC_{50} values of 50 and 9 pM for HCV replicon genotypes 1a and 1b, respectively. However, clinical specimen analyses reported drug resistance stemming from the L31V and Y93H mutations of NS5A; two of the commonest genetic alterations in HCV genotypes 1a and 1b [39]. Despite the fact that knowledge on the mutant protein structures is still lacking, these mutations are believed to induce conformational rearrangements in the daclatasvir-bound NS5A that restore the structure and function of the NS5A helical polymers, thereby hampering inhibition by daclatasvir.

To tackle drug resistance, Sun *et al.* developed a synergist, Syn-395 (Figure 3A), for daclatasvir [40]. Syn-395 was shown to act allosterically as it did not compete with daclatasvir for binding on NS5A; researchers hypothesized that it binds adjacent to the orthosteric ligand, causing a

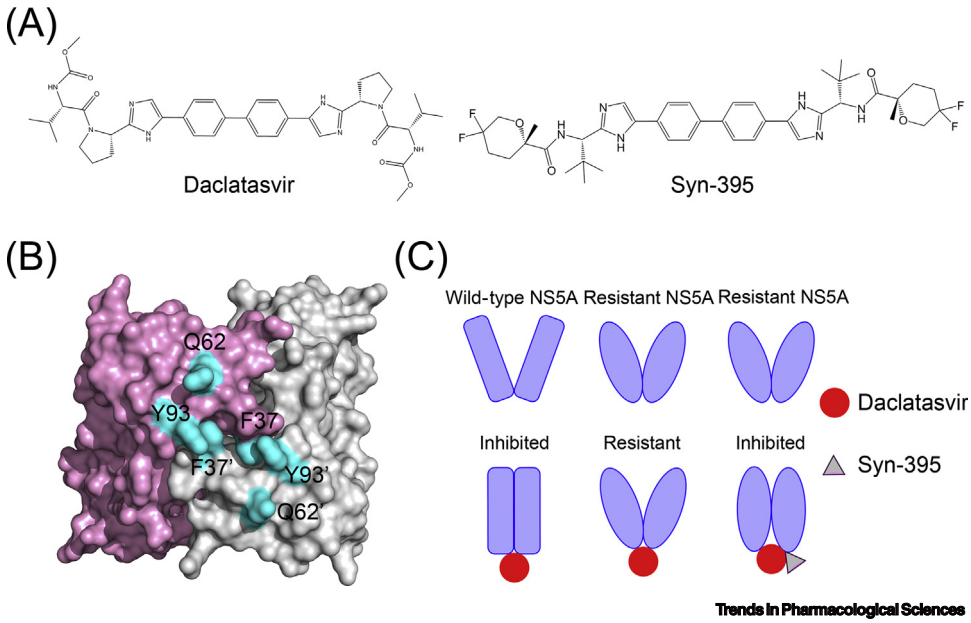

Figure 3. Synergistic Inhibition of Nonstructural protein (NS)5A by Daclatasvir and Syn-395. (A) Chemical structures of the orthosteric NS5A inhibitor, daclatasvir, and its allosteric synergist, Syn-395. (B) Structural overview of the binding sites on NS5A of daclatasvir and Syn-395. Syn-395 binds to the NS5A dimer ridge consisting of F37, Q62, Y93, F37', Q62', and Y93'; apostrophes denote residues from the second monomer of the NS5A dimer. Daclatasvir binds adjacent to the Syn-395's orthosteric binding site. Note that no co-crystal structure of NS5A and inhibitors is currently available. The two NS5A monomer within the dimer system are shown in pink and grey respectively. (C) Model of the synergistic inhibition of NS5A by daclatasvir and Syn-395. Daclatasvir binds to wild-type NS5A and induces structural alterations that inhibit its function. Upon drug-resistant mutations, structural alterations triggered by daclatasvir are compromised, resulting in a loss of efficacy. Syn-395 allosterically co-binds on the NS5A mutant adjacent to daclatasvir and restores the latter's ability to inflict structural changes, thus resensitizing NS5A to daclatasvir.

conformational change that is transmitted to adjacent dimers along the polymer and potentiates the structural alterations triggered by daclatasvir (Figure 3C). Upon coadministration, Syn-395 remarkably enhanced the potency of daclatasvir against NS5A mutants (more than 1000-fold) [40], highlighting the unprecedented therapeutic potential of this combination against drug-resistant HCV.

Epidermal Growth Factor Receptor (EGFR)

EGFR is a well-recognized target for non-small-cell lung cancer (NSCLC) therapies, as its mutation rate among patients is as high as 30% [41,42]. The first generation of EGFR drugs approved for clinical applications, such as gefitinib, erlotinib, and lapatinib, were orthosteric inhibitors targeting the substrate-binding pocket of the receptor (Box 1) [43,44]. They are widely used as EGFR-targeted therapeutics for NSCLC, but resistance frequently emerges due to double mutations such as L858R/T790M [45].

To confront this problem, To *et al.* combined another orthosteric drug, osimertinib, with the newly discovered allosteric inhibitor, JBJ-04-125-02 (see Figure I in Box 1) [46]. Researchers first demonstrated that these two compounds could indeed co-bind on EGFR using a biotinylated protein interaction pull-down assay. A crystallographic study confirmed that JBJ-04-125-02 resensitized the resistant EGFR mutant to osimertinib by inducing its conformational rearrangement (Box 1). Cell viability and apoptosis assays confirmed that JBJ-04-125-02 significantly enhances osimertinib potency. Furthermore, an *N*-ethyl-*N*-nitrosourea mutagenesis assay found that no

Box 1. Combinatory Strategy to Drug the Resistant EGFR

All orthosteric drugs for EGFR are based on an anilinoquinazoline scaffold and inhibit EGFR through competitive inhibition. As the first-line EGFR-targeted therapy, they are effective against wild-type and L858R EGFR. However, novel mutations such as T790M result in drug resistance. A double mutant harboring L858R and T790M has increased affinity for the substrate ATP, resulting in a loss of response to the orthosteric drugs.

Crystallography and structural modeling studies revealed that JBJ-04-125-02 is situated in a pocket opposite the substrate-binding site created by the outward displacement of the αC helix (Figure IA), confirming its allosteric mode of action. JBJ-04-125-02 forms several hydrogen bonds with the adjacent EGFR residues, such as K745, E749, D855, and F856, whereas its phenyl ring forms a π–π stacking interaction with F723, a residue within the kinase P loop (Figure IB). The 4-piperazinophenyl moiety within the compound extends outwards into the exterior solvent along the αC helix and is hydrogen-bonded to E865, thereby arranging the neighboring kinase activation loop into a more ordered conformation and stabilizing it (Figure IC).

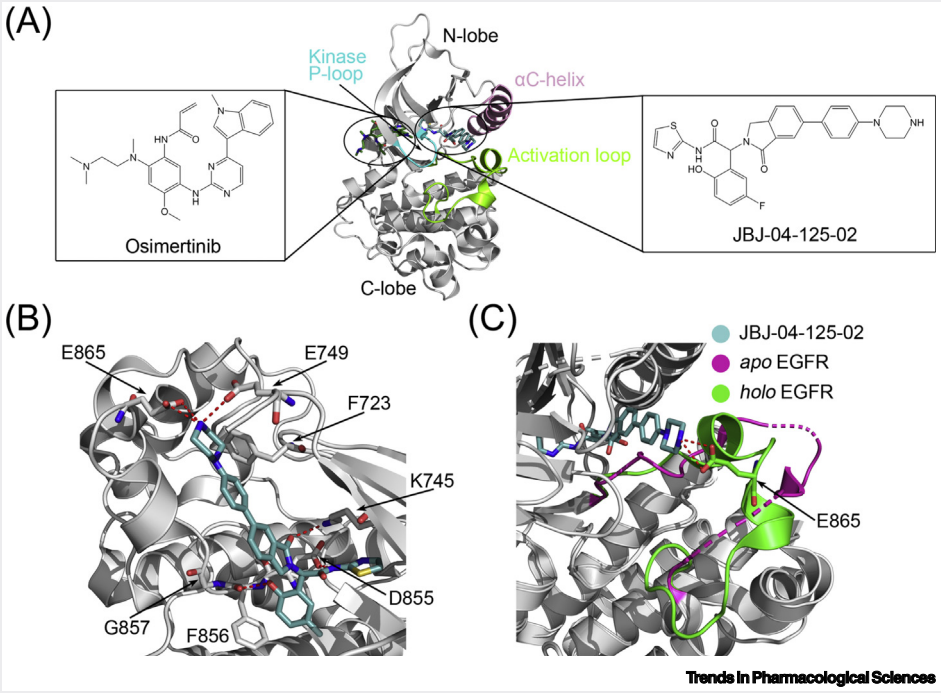

Figure I. (A) Structural overview of the epidermal growth factor receptor (EGFR) in complex with the orthosteric inhibitor, osimertinib, and the allosteric inhibitor, JBJ-04-125-02. EGFR and JBJ-04-125-02 were extracted from 6DUK (PDB ID), whereas osimertinib was aligned from 4ZAU. (B) Interaction details between JBJ-04-125-02 and EGFR. Hydrogen bonds are depicted as red broken lines. (C) Allosteric binding of JBJ-04-125-02 formed two hydrogen bonds with E865 and induced the structural rearrangement of the activation loop.

resistant clones emerged upon dual EGFR inhibition by osimertinib/JBJ-04-125-02. In addition, *in vivo* studies revealed that the two-drug treatment induces tumor regression more effectively and results in longer median overall survival times [46]. All these findings mark the great translational potential of osimertinib/JBJ-04-125-02 therapy against drug-resistant EGFR mutants.

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

Allosteric synergists may also find application as adjuvants in cystic fibrosis (CF) treatments targeting CFTR. CFTR consists of two nucleotide-binding domains (NBD1 and NBD2), two membrane-spanning domains (MSD1 and MSD2), and one regulatory domain (R) (Figure 4), which allosterically interact with each other and cooperatively function as an anion channel for water and ion transportation across epithelial cell membranes [47]. One of the most common

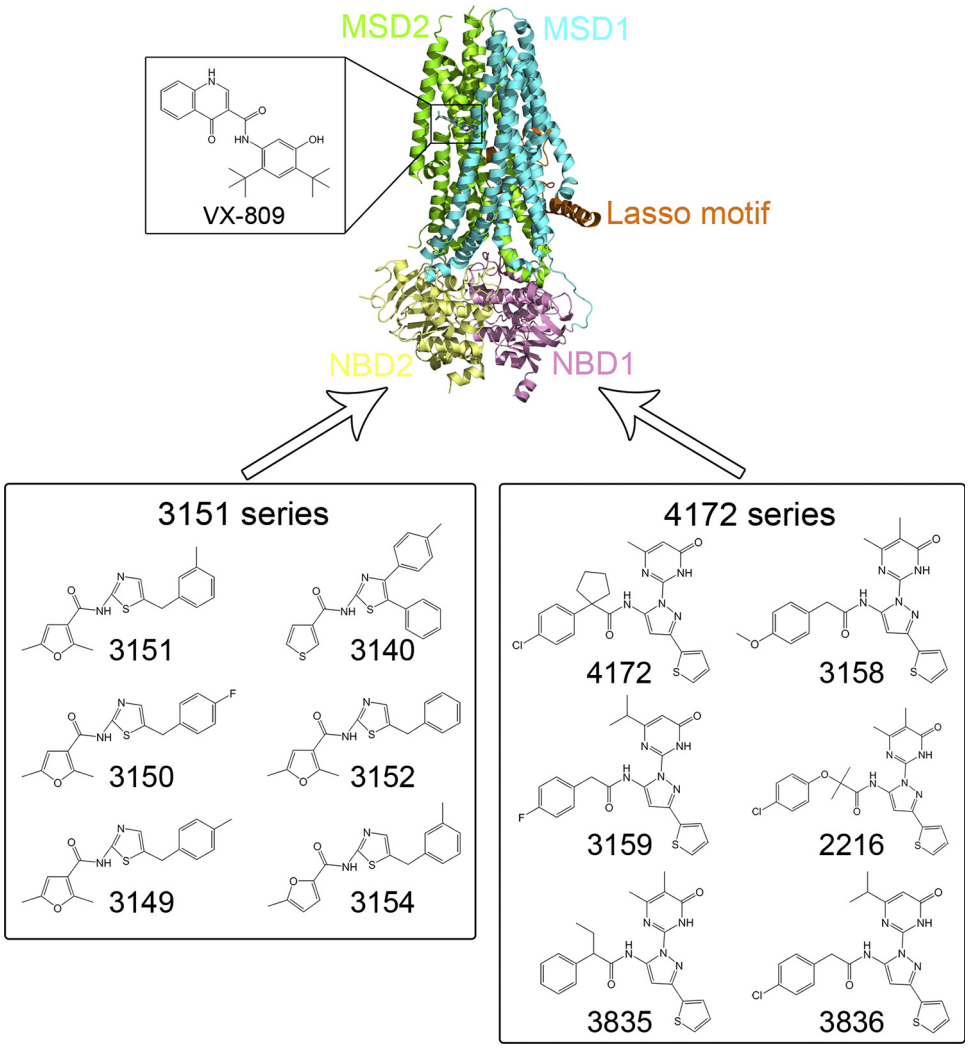

Figure 4. Structural Overview of CFTR in Complex with the Orthosteric Drug VX-809 (PDB ID: 6O2P). Two classes of allosteric ligands, the 4172 and 3151 series, and their respective target domains, NBD1 and NBD2, are also displayed. Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; MSD, membrane-spanning domain; NBD, nucleotide-binding domain.

pathogenic mutations in CFTR is F508del (or Δ508) within the NBD1 domain, which leads to CFTR misfolding [48]. The use of corrector molecules to rescue the structural defects of CFTR mutants is regarded as a promising approach to CF therapeutics. The orthosteric ligand VX-809 (Lumacaftor) has already been approved as a clinical CFTR Δ508-targeted drug (Figure 4) [49,50]. VX-809 reinforces the interplay between different CFTR domains, especially the NBD1–MSD1 and NBD1–MSD2 interactions, stabilizing the overall CFTR protein topology. As a result, VX-809 can rescue CFTR from degradation, partially restoring its levels and function [51,52]. Nevertheless, several newly reported mutations, such as P67L and S492F, lead to drug resistance by decreasing the susceptibility of Δ508 CFTR to VX-809 [53].

Recently, Veit *et al.* designed a triple-corrector combination consisting of VX-809 and two other classes of **allosteric modulators** to restore the efficacy of VX-809 [53]. Using a biochemical

assay with the plasma membrane CFTR level as an indicator of the potency of each compound, researchers screened more than 600 000 molecules from the Novartis academic collection screen (ACS) library [54,55]. In total, 39 compounds with regulatory activity were identified and they were clustered into different subgroups based on their scaffold structure. Further experiments demonstrated that the combination of VX-809 with 3151-series and 4172-series compounds (Figure 4) resensitized the resistant CFTR mutants to VX-809, significantly increasing their levels and rescuing their function. Even though the exact binding sites for the ligands remain to be elucidated, surface plasmon resonance experiments and related structure analyses revealed that neither series of compounds competes with VX-809 for binding but both function through distinct allosteric mechanisms. Specifically, 3151-series compounds interact with NBD2 or its interfaces (Figure 4), whereas 4172-series compounds target NBD1 (Figure 4) and revert its misfolding. The excellent performance of the triple-corrector strategy against the resistant CFTR mutants extended to *in vivo* studies using mouse models, in which coadministration of the above three agents relieved the clinical symptoms of CF [53]. Collectively, these data suggest that combinatorial CFTR-targeted drugging has great translational potential for CF treatment, but more in-depth mechanistic research and lead optimization are needed for the development of effective therapeutics.

Overcoming Pathway-Dependent Resistance

Besides the concomitant use of allosteric and orthosteric drugs that simultaneously bind to the same target, another promising direction in combinatorial treatment is combating drug resistance through the simultaneous targeting of the target proteins themselves as well as their resistance-related signaling pathways. As most components within these pathways are protein kinases, the active sites of which are highly conserved, orthosteric drugs frequently suffer from poor selectivity, leading to off-target adverse effects. However, allosteric drugs possess advantages such as higher specificity, better physiochemical properties, and improved pharmacological performance [19,56–59]; thus, they may represent a more suitable choice for combinatory therapeutics targeting resistance-relevant signaling cascades.

One of the most notorious of such signaling pathways is the Akt/mTOR (mammalian target of rapamycin) pathway [8,60]. It mediates drug resistance to aromatase inhibitors (AIs) and other therapeutic agents [61–63]. Vilquin *et al.* showed that the allosteric Akt inhibitor, MK-2206, or the allosteric mTOR inhibitor, rapamycin, resensitized resistant breast cancer cells to AIs such as anastrozole [61]. Coadministration with MK-2206 has also been shown to rescue the function of bufalin in the treatment of myeloma [62] and of trastuzumab in the treatment of human epidermal growth factor receptor 2-overexpressing breast and gastric cancers [63]. Similar mechanisms and molecules have already entered the clinical trial stage (Clinical Trial Number: NCT01007942[i] and others) and possess great potential for future clinical resistance management. Another critical mediator of drug resistance is the mitogen-activated protein kinase (MEK) signaling pathway, which is associated with the compromised potency of gemcitabine and other therapeutic agents. Synergistic activity was observed upon the cotreatment of pancreatic cancer cell lines with gemcitabine and the allosteric MEK1/2 inhibitor, pimasertib, which suggests that the latter might have the potential to improve the therapeutic performance of the former in this cancer type [64]. Another promising study using melanoma cell lines showed that combination treatment might be able to tackle resistance to the BRAF inhibitor, dabrafenib [65]. SHP2 is an intracellular signaling node involved in the loss of response of the anaplastic lymphoma kinase (ALK) to orthosteric inhibitors such as lorlatinib and ceritinib [66,67]. Recently, researchers discovered that combining SHP099, an SHP2 allosteric inhibitor, with ALK inhibitors can successfully circumvent resistance [68]. Finally, cancer metabolism is also a key player involved in drug resistance [69,70]. Recently, Huang *et al.* discovered that allosterically targeting phosphoglycerate mutase 1 with the inhibitor

HKB99 was able to overcome resistance to the orthosteric drug, erlotinib, in NSCLC [71]. To summarize, there are multiple reports of pathway-dependent resistance in which combination with an allosteric agent effectively restored the effectiveness of the orthosteric treatment; this highlights the therapeutic potential of such combinations in combating this type of drug resistance.

Concluding Remarks

In the era of modern biomedicine, drug resistance remains one of the leading problems in the clinic, affecting multiple aspects ranging from anti-infection to anticancer treatments [1–7,72]. Tackling this refractory problem will require multidisciplinary endeavors and collaborations. As allosteric agents have revolutionized drug development, the combination of allosteric and orthosteric drugs provides a novel strategy for overcoming resistance. In the campaign against drug resistance, allosteric drugs function synergistically with orthosteric drugs mainly through two different mechanisms. In double-targeting applications, they co-bind on the recalcitrant drug targets and fine tune **orthosteric sites** through either tailoring of the intrinsic residue networks of proteins or shifting the structural ensemble of the target towards conformational states prone to orthosteric drugging [19,59,73,74]. Alternatively, they might subtly tailor resistance-related pathways, indirectly resensitizing the resistant targets to orthosteric targeting [8,17].

Coadministration strategies have the potential to tame historically resistant targets, redistribute the resistome landscape, and significantly improve therapeutic perspectives. A series of promising allosteric–orthosteric drug combinations that were identified in experimental studies are now undergoing clinical trials; moreover, some have been translated into clinical applications and therapeutics (Table 1). Among the tested regiments, only the two-drug targeting of the BCR-ABL protein for the treatment of relapsed CML and of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) seeks to tackle target-dependent resistance (see underlined text in Table 1); all others focus on pathway-dependent resistance; that is, they target resistance-related signaling, with most attention being paid to the classic Ras–RAF–MEK–ERK pathway and the PI3K–Akt–mTOR pathway, which is consistent with their critical roles in mediating drug resistance in a series of medical conditions.

The limited number of allosteric agents successfully advancing into clinical application reflects the difficulties in identifying novel **allosteric sites** for drug design; that is, the challenge lies at the first stage of the process to develop a combinatorial treatment (see Outstanding Questions). Despite the fact that everything but the active site of a protein can be considered as a putative target for allosteric regulation, information on specific allosteric sites and their corresponding ligands is still lacking for a plethora of proteins. For example, an S904F substitution makes the oncogenic RET kinase, a target in lung adenocarcinoma treatment, resistant to the orthosteric drug, vandetanib [75]. No allosteric modulators have yet been identified for RET, which constitutes a great challenge to drug optimization and combination therapeutics design.

Once based on serendipitous discovery, allosteric drug development has now been revolutionized by structure-based rational design [76,77]. Accumulating allosteric data, including allosteric protein and ligand structures, allosteric pocket sequences and conformations, as well as allosterome analyses [78–81], will deepen our understanding of protein allostery and its regulatory role in the design of orthosteric drugs, thus assisting the development of combinational therapeutics. The application of bioinformatics tools may also aid allosteric drug discovery [76,82,83]. Tools such as AlloDriver [84,85] and AlloMAPS [78] may be applicable for evaluating the allosteric effects of resistant mutants, whereas the utilization of AlloFinder [86], AlloSite (Pro) [87,88], and CavityPlus [89] for allosteric site detection could aid in structure-based drug design and hit-to-lead optimization. As these constitute the theoretical basis for computational allosteric drug

Outstanding Questions

Will resistance still develop even against multidrug regimens? If yes, how will we manage it?

How can we discover more allosteric targets and carry out rational drug design based on their biochemical, structural, and medicinal properties?

Why is double-drugging the same target with allosteric and orthosteric drugs more difficult to translate into clinical use than dual-targeting the resistance-related pathways?

How can we design and select the optimal combinations of allosteric and orthosteric drugs?

Will drug–drug interactions affect the therapeutic performance of the combinatory approaches? If yes, how can we optimize the combinations?

Table 1. Current Clinical Trials of Orthosteric–Allosteric Drug Combination Treatments[a]

Clinical trial ID	Orthosteric target and modulator	Allosteric target and modulator	Disease to benefit	Stage
NCT01271920	HER2 (pertuzumab, trastuzumab)	Tubulin (docetaxel)	HER2 amplified breast cancer	Approved
NCT01007942	HER2 (trastuzumab), chemotherapy (vinorelbine)	mTOR (everolimus)	HER2 amplified breast cancer	Phase III
NCT00096486	EGFR (gefitinib)	mTOR (everolimus)	Lung cancer	Phase I/II
NCT02321501	ALK (ceritinib)	mTOR (everolimus)	Head and neck cancer and lung cancer	Phase I
NCT02081378	BCR-ABL (imatinib/nilotinib/dasatinib)	BCR-ABL (asciminib)	CML and Ph$^{\pm}$ALL	Phase I
NCT01392521	PI3K (copanlisib)	MEK1/2 (refametinib)	Neoplasms	Phase I
NCT01973309	FZD receptor (vantictumab)	Tubulin (paclitaxel)	Metastatic breast cancer	Phase I
NCT01344031	Aromatase (anastrozole)	Akt (MK-2206)	Breast carcinoma	Phase I
NCT01705340	HER2 (trastuzumab), EGFR (lapatinib)	Akt (MK-2206)	HER2-positive breast and colon cancer	Phase I
NCT01783171	CDKs (dinaciclib)	Akt (MK-2206)	Pancreatic cancer	Phase I
NCT01251861	Androgen receptor (bicalutamide)	Akt (MK-2206)	Prostate cancer	Phase II
NCT00848718	EGFR (erlotinib)	Akt (MK-2206)	Solid tumors	Phase I
NCT01042379	HER2 (trastuzumab)	Akt (MK-2206)	Breast cancers	Phase II
NCT01295632	mTOR (ridaforolimus)	Akt (MK-2206)	Advanced cancers	Phase I
NCT01243762	IGFR1 (dalotuzumab)	Akt (MK-2206)	Neoplasms malignant	Phase I
NCT01072175	BRAF (dabrafenib)	MEK1/2 (Trametinib)	BRAF mutant melanoma and colorectal	Phase I
NCT01958112	Akt (uprosertib)	MEK1/2 (trametinib)	Cervical cancer	Phase II
NCT01248858	mTOR (omipalisib)	MEK1/2 (trametinib)	Solid tumors	Phase I
NCT01155453	PI3K (buparlisib)	MEK1/2 (trametinib)	Advanced solid tumors	Phase I
NCT01476137	Akt (afuresertib)	MEK1/2 (Trametinib)	Cancer	Phase I
NCT01750918	BRAF (dabrafenib), EGFR (panitumumab)	MEK1/2 (trametinib)	Cancer	Phase II
NCT02900664	PD1 (PDR001)	MEK1/2 (trametinib)	Colorectal cancer, triple negative breast cancer, NSCLC	Phase I
NCT03428126	IgG1κ (durvalumab)	MEK1/2 (trametinib)	Malignant neoplasms of digestive organs, colon cancer	Phase II
NCT03087448	ALK (ceritinib)	MEK1/2 (trametinib)	NSCLC	Phase I/II
NCT00996892	PI3K (pictrelisib)	MEK1/2 (cobimetinib)	Dual PI3K/K-Ras mutant colorectal cancer	Phase I
NCT01562275	Akt (ipatasertib)	MEK1/2 (cobimetinib)	Neoplasms	Phase I
NCT03989115	SHP2 (RMC-4630)	MEK1/2 (cobimetinib)	Solid tumors	Phase I/II
NCT01271803	BRAF (vemurafenib)	MEK1/2 (cobimetinib)	Malignant melanoma	Phase I
NCT01988896	PD-L1 (atezolizumab)	MEK1/2 (cobimetinib)	Solid tumors	Phase I
NCT01495988	VEGF (bevacizumab)	MEK1/2 (cobimetinib)	Melanoma	Phase II
NCT03202940	ALK (alectinib)	MEK1/2 (cobimetinib)	NSCLC	Phase I/II
NCT02143466	EGFR (osimertinib)	MEK1/2 (selumetinib)	Advanced NSCLC	Phase I
NCT02025114	EGFR (gefitinib)	MEK1/2 (selumetinib)	NSCLC	Phase I/II
NCT00777309	EGFR (erlotinib)	MET (tivantinib)	NSCLC	Phase II
NCT01513174	EGFR (gefitinib)	PARP (olaparib)	NSCLC	Phase I/II

[a]Abbreviations: ALK, anaplastic lymphoma kinase; CDK, cyclin-dependent kinase; CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; FZD receptor, frizzled receptor; HER2, human epidermal growth factor receptor 2; IGFR1, insulin-like growth factor receptor 1; IgG1κ, immunoglobulin G1 kappa; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NSCLC, non-small-cell lung cancer; PARP, poly (ADP-ribose) polymerase; PD1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; Ph^{+} ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia; PI3K, phosphoinositide 3-kinase; SHP2, tyrosine-protein phosphatase non-receptor type 11 (also known as PTPN11); VEGF, vascular endothelial growth factor.

discovery, coupling them with advances in experimental studies is bound to lead to novel concomitant therapies.

Another obstacle in the development of combinatory therapeutics is choosing the drug combinations themselves (see Outstanding Questions). Historically, experimental methods such as siRNA screening and unbiased chemical screening with compound libraries have been used to identify the targets and the combination cocktails for administration [13,90,91]. However, they unavoidably suffer from limitations such as the relatively long time they require and potential off-target effects. These problems might be ameliorated by complementing the experimental methods with systemic and network-based biology methodology [92,93], as integrating multiomics data and incorporating different mathematical models could facilitate high-throughput testing and analysis of potential drug combinations, quantification of drug–disease relationships, and prediction of the efficacy of adjuvant therapeutics. Beyond identifying novel therapies, such approaches might also resolve the problem of toxicity stemming from inappropriate combinations.

To summarize, despite the potential obstacles and pitfalls, combining allosteric and orthosteric drugs to circumvent drug resistance; that is, one of the critical bottlenecks of modern pharmaceutical research, has already achieved some success. Hence, we are hopeful that coadministration strategies will eventually become standard practice, constituting a powerful weapon in our arsenal against drug resistance, and pave a compelling avenue for future drug discovery.

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Resources

[i]https://clinicaltrials.gov/

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