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# Evolution-informed rescue drug design: exploiting acquired resistance to develop more selective and resistance-durable targeted therapies

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**Abstract**
Acquired resistance to targeted anticancer drugs presents an evolutionary paradox: mutations that allow cancer cells to escape an initial inhibitor may simultaneously create molecular vulnerabilities that enable the development of more selective and potentially more resistance-durable successor drugs. This review examines whether such mutations can resolve a fundamental conflict in drug design: functionally indispensable protein regions are attractive targets for limiting resistance, but their conservation across normal proteins can make selective inhibition difficult. We propose a two-drug strategy in which Drug 1 is the initial anticancer inhibitor and Drug 2 is a rescue inhibitor designed against resistance mutations anticipated before clinical emergence. Drug 2 would exploit a structural feature created by resistance to Drug 1 for selective recognition, while deriving most of its binding strength from experimentally verified, functionally constrained contacts that cannot readily mutate without compromising essential protein function. Published studies of substrate-envelope design, prospective resistance screening, mutant-selective inhibitors, and EGFR resistance mechanisms provide the scientific foundation for this hypothesis, although none of the studies examined establishes the proposed combination. Accelerated resistance evolution, mutagenesis, and CRISPR-based editing could deliberately generate and characterize resistant variants *in vitro*, allowing Drug 2 to be developed and evaluated in parallel with Drug 1 rather than after clinical treatment failure. This prospective approach could also introduce an evolution-informed pharmaceutical development and patenting paradigm in which rescue inhibitors are designed against experimentally characterized molecular targets before those resistance states appear in patients. The hypothesis requires experimental validation of mutant-specific selectivity, functional anchoring, and protection against subsequent resistance. Its central proposition is that the evolutionary process responsible for failure of an initial treatment may itself create the molecular conditions for a more effective successor therapy.

**Keywords**
Evolution-informed drug design, Acquired drug resistance, Targeted cancer therapy, Resistance mutations, Mutant-selective inhibitors, Cancer drug discovery, Evolutionary selection, Functional constraints, Artificial Intelligence in drug discovery, Resistance-durable therapeutics

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## 1. Introduction

Targeted anticancer drugs inhibit proteins that promote cancer cell survival and proliferation. However, treatment can select for cancer cells carrying mutations that reduce their sensitivity to the drug. These changes may arise before treatment or develop during tumor growth. Their importance depends on both the molecular change and the conditions experienced by the cancer cells. EGFR, a signaling protein involved in some lung cancers, illustrates how different drugs acting at the same binding site can encounter different resistance mutations (1,2).

A long-standing question in drug design is whether targeting specific protein-binding pockets can reduce the emergence of resistance. Conserved, functionally essential regions are promising targets because mutations in these regions may compromise protein function. However, structural characteristics alone cannot predict resistance, which also depends on drug–target interactions, the tumor's initial genotype, and treatment conditions.

This review examines whether resistance mutations can create structural differences between tumor proteins and their normal counterparts that enable the design of rescue inhibitors combining mutant selectivity with binding to functionally essential regions. Such inhibitors must distinguish the resistant tumor protein from both the normal version of the target and its paralogues (related proteins encoded by different genes). The proposed approach involves developing and testing rescue drugs in laboratory models before resistance emerges clinically, rather than deliberately inducing resistance in patients.

The review hence proposes a strategy involving two inhibitors: Drug 1, the initial anticancer drug targeting a protein essential for tumor growth, and Drug 2, a rescue inhibitor designed in anticipation of mutations that confer resistance to Drug 1. The central hypothesis is that the evolutionary process responsible for resistance to Drug 1 may create new molecular features that allow Drug 2 to inhibit the resistant protein more selectively while relying on functionally indispensable binding interactions that could reduce further resistance.

This article critically examines selected published studies and proposes a novel research direction for resistance-informed drug design. It does not present an exhaustive literature review, original structural data, or a validated resistance prediction model. An AI-generated compilation of structural information was used solely to identify candidate sources, with unverified entries and measurements excluded from the analysis. The discussion is grounded in published evidence, while the proposed experimental approach remains to be validated.

## 2. Why escape mutation counts alone cannot predict resistance

### **2.1** The number of resistance mutations does not predict the likelihood of resistance

Cancer cells can develop resistance to targeted drugs through mutations that alter the drug's binding to its target protein while preserving enough protein function for the cells to survive and proliferate. However, not all mutations are equally likely to occur, and not all resistant cells are equally capable of multiplying during treatment.

The number of mutations that allow cancer cells to resist a particular drug is referred to here as *escape diversity*, denoted by $k$. For example, if laboratory experiments identify 40 different mutations that permit cancer cells to survive exposure to Drug A, its measured escape diversity is 40. If only one such mutation is identified for Drug B, its measured escape diversity is one. These counts depend on the mutations tested, the experimental conditions, and the criteria used to define resistance.

It might appear that Drug B is less likely to encounter resistance because cancer cells have fewer ways to escape its effects. However, this conclusion is not necessarily correct. The single mutation conferring resistance to Drug B might arise frequently and allow resistant cells to proliferate rapidly. Conversely, the 40 mutations conferring resistance to Drug A might be relatively unlikely to arise or might impair cancer cell growth. Consequently, resistance to Drug B could emerge more readily than resistance to Drug A, despite its substantially smaller escape diversity.

Two factors are therefore particularly important: mutation accessibility, or how readily a resistance mutation can arise, and resistant-cell fitness, or how effectively cells carrying that mutation survive and proliferate under treatment. The likelihood of resistance also depends on the number of cancer cells, the presence of resistant cells before treatment, drug exposure, and the selective advantage conferred by each mutation (3,4).

Thus, counting resistance mutations identifies the number of experimentally observed escape routes but does not establish how likely those routes are to produce treatment failure. A meaningful assessment must consider both the accessibility of individual mutations and the capacity of the resulting resistant cells to expand. This distinction is important for designing successor inhibitors because eliminating numerous rare or poorly tolerated escape mutations may offer less protection than preventing a single readily accessible mutation that confers a strong growth advantage (3,4).

**2.2** A useful distinction without a new prediction score

A schematic expression makes the counting problem clear: $S_{eff} = \sum_{g \in A} \mu_g \, p_{est,g}$ (eq1). Here, $\mu_g$ is the context-dependent rate at which genotype $g$ is generated, and $p_{est,g}$ is its probability of establishing a growing lineage under the stated conditions. Unlike $k$, this expression gives variants different weights. If $\mu_g$ is defined per cell division, the sum has a corresponding per-division basis; it is not itself a patient's probability of resistance. Estimating a whole-tumor outcome would additionally require population size, time, pre-existing variants, and other population information.

The expression is included only to explain why counting variants is insufficient. This article does not calculate $S_{eff}$ or offer it as an early-stage clinical tool. Its terms cannot be reliably filled in from a protein structure or an ordinary screening experiment alone. This review also does not combine structural features into constraint and permissiveness indices. Placing uncertain measurements on two axes does not make them a reproducible ranking method. Conservation, flexibility, and contact geometry remain useful questions to investigate, rather than components of a new score.

**2.3** Interpreting and reporting resistance mutation screens

Deep mutational scanning allows researchers to test large numbers of genetic variants simultaneously to identify mutations that confer resistance to a particular drug. However, these experiments reveal resistance under defined laboratory conditions rather than all possible routes to resistance in patients. An increase in the abundance of a particular variant during treatment does not necessarily establish that the mutation directly interferes with drug binding. It may instead reflect changes in protein expression, cellular growth, or other biological processes. Distinguishing these mechanisms requires comparisons between treated and untreated cells, measurements of protein abundance and function, and independent validation of individual mutations. For example, a published EGFR resistance screen identified amino-acid substitutions requiring more than one nucleotide change, illustrating that experimentally detectable resistance mutations are not necessarily equally accessible through natural mutation (5).

Resistance screens should therefore report the total number of identified escape variants ($k$), the identity of each variant, its degree of drug resistance, and its effects on cellular growth. Importantly, mutations achievable through a single nucleotide substitution should be distinguished from those requiring multiple nucleotide changes, since the latter may be less readily generated during tumor evolution. Where reliable mutation-rate estimates are available, these can be used to calculate a mutation-accessibility-weighted measure of escape potential by summing the estimated generation rates of qualifying resistance genotypes. Such a measure provides more information than simply counting mutations, but it does not account for whether resistant cells will successfully establish and expand within a tumor. The underlying mutation-rate estimates and their uncertainties must therefore be reported. When reliable rates are unavailable, mutations should be classified by nucleotide accessibility without assigning speculative probabilities. Although tumor mutational signatures provide information about the types and relative frequencies of mutations occurring in cancer, they do not independently establish the absolute rates at which particular resistance mutations arise (6).

Together, these measurements distinguish three important characteristics of resistance: the number of possible escape mutations, the likelihood of their generation, and the ability of the resulting resistant cells to proliferate. This distinction is essential when evaluating whether a proposed rescue inhibitor meaningfully restricts the emergence of resistance rather than merely reducing the number of escape variants detected in a laboratory screen.

## 3. Structural mechanisms of drug resistance and implications for inhibitor design

**3.1** Why some mutations disrupt drug binding without compromising protein function

Many anticancer drugs bind to regions of proteins that are essential for tumor cell survival and proliferation. However, the molecular interactions required for drug binding are not always identical to those required for the protein's normal biological function. Consequently, certain mutations can substantially weaken drug binding while

preserving the protein's ability to function. For example, a drug may depend on a particular amino-acid side chain, chemical bond, or local structural arrangement that is not equally important for the binding of the protein's natural substrate or for its catalytic activity. Mutations affecting these interactions can therefore confer drug resistance without eliminating the biological activity that supports tumor growth.
This distinction helps explain why certain resistance mutations are observed in cancer patients while others are not. For a tumor that depends on a particular protein to survive and grow, a mutation that prevents drug binding but also destroys the protein's essential function offers no survival advantage. In contrast, a mutation that prevents the drug from working while allowing the protein to continue functioning enables cancer cells to survive treatment and multiply. Consequently, the resistance mutations identified in patients tend to be those that both reduce drug effectiveness and preserve sufficient protein function. Their prevalence therefore reflects natural selection during treatment, rather than evidence that other mutations cannot occur in the protein's binding pocket.

Not all resistance mechanisms involve altered drug binding. Cancer cells may also become resistant by reducing expression of the target protein or by activating alternative survival pathways that diminish their dependence on it. For example, a CRISPR base-editing study identified PARP1 start-loss variants associated with resistance to PARP inhibitors, suggesting that reduced production of the target protein can confer resistance without directly changing its drug-binding interactions (7). Thus, the absence of particular mutations in patient samples cannot, by itself, establish that those mutations are structurally impossible or that the affected residues are functionally indispensable.

Prospective genetic editing screens can help distinguish mutations that confer resistance from those that compromise protein function. In one such study, substitutions introducing chemically different amino acids at EGFR residues T790 and C797 were depleted rather than enriched during screening. The investigators interpreted this depletion as evidence that these substitutions impaired EGFR function, potentially explaining their absence among clinically observed resistance mutations (7). These findings illustrate why resistance screens should examine both drug sensitivity and preservation of target function, rather than considering drug binding alone.

The central implication for drug design is that an inhibitor may be more difficult to escape if its binding depends on protein interactions that cannot readily be altered without compromising essential function. However, identifying such interactions requires experimental evidence of functional constraint, rather than simply observing which mutations occur in resistant tumors.

Structural studies provide valuable information about how drugs interact with their protein targets, but the proximity of an amino-acid residue to a bound drug does not establish how important that residue is for binding or protein function. Similarly, a small structural change can produce a substantial change in drug sensitivity, while a larger structural alteration may have relatively little effect. Meaningful comparisons must therefore consider the

specific oncogenic mutations already present in the target protein, rather than assuming that the starting protein is identical to its normal wild-type counterpart. Differences in bound ligands, experimental conditions, protein constructs, and conformational states must also be considered when comparing structures. Ultimately, structural observations must be supported by binding measurements and functional experiments to establish how particular mutations influence drug resistance (1,9).

**Table 1.** Published examples of different resistance mechanisms

| Target and drug | Resistance change | Conclusion |
|---|---|---|
| EGFR with gefitinib | T790M alters competition with ATP | Resistance need not mean that the drug can no longer fit in the pocket (1). |
| EGFR with osimertinib | C797S removes the cysteine used for covalent attachment | A drug can depend on a chemical anchor that kinase function does not require (2). |
| BCR-ABL1 with earlier ATP-site inhibitors | T315I changes gatekeeper interactions | A successor inhibitor can accommodate a resistant target; this approach already has precedent (8). |
| BCL2 with venetoclax | G101V shifts a neighbouring contact network | The mutated residue need not directly contact the drug to reduce its binding (9,10). |
| ALK with lorlatinib | L1198F in a C1156Y background changes drug preference | A resistant state can regain sensitivity to an earlier drug, but this is specific to the genotype and drugs tested (11). |

**3.2** The substrate-envelope approach and the challenge of drug selectivity

One strategy for reducing drug resistance is to design inhibitors that bind to regions of a protein that are essential for its normal biological function. This principle underlies the *substrate-envelope approach*, originally developed for protease inhibitors. Proteases are enzymes that recognize and cleave specific protein substrates. Although these substrates may differ in their amino-acid sequences, they often occupy a common three-dimensional space within the enzyme's binding site. This shared space is called the substrate envelope. By designing an inhibitor that fits predominantly within this envelope, researchers aim to make drug binding dependent on interactions that the enzyme must preserve to process its natural substrates. Consequently, mutations that prevent inhibitor binding may also impair enzyme function, reducing the likelihood that such mutations will confer a viable resistance advantage. Studies involving HIV-1 and hepatitis C proteases support this strategy, while structural investigations of SARS-CoV-2 main protease have explored its potential for anticipating resistance mutations (12–14). However, these findings do not establish that the approach will be equally effective against human cancer targets.

Applying this principle to protein kinases presents additional challenges. Kinases catalyse the transfer of phosphate groups from ATP to other molecules, and many anticancer drugs inhibit this activity by occupying the ATP-binding pocket. Because ATP binding is essential for kinase function, interactions within this pocket may provide opportunities to design inhibitors that are difficult to escape through

mutation. However, ATP-binding regions are highly conserved across many human kinases. An inhibitor that relies heavily on these shared structural features may therefore inhibit not only the intended cancer-associated kinase but also other kinases required for normal cellular function. Furthermore, occupying the same binding region as ATP does not necessarily mean that an inhibitor depends on the same molecular interactions. A mutation may disrupt inhibitor binding while preserving ATP binding and catalytic activity. Thus, targeting conserved regions or protein-backbone interactions does not automatically prevent resistance.

This creates a fundamental drug-design challenge: the protein regions most attractive for preventing resistance may also be the least suitable for achieving selectivity. An inhibitor that binds strongly to functionally indispensable but highly conserved regions may be difficult for cancer cells to escape through mutation, yet it may also interfere with essential proteins in normal cells. Conversely, an inhibitor designed to recognize a highly distinctive structural feature of the cancer-associated protein may achieve greater selectivity but become vulnerable to mutations that eliminate that feature without compromising protein function.

The difficulty of distinguishing cancer cells from normal cells is also evident with antimetabolite chemotherapy, which interferes with metabolic processes required by both. However, these drugs do not affect all cells equally; their therapeutic effects and toxicities depend on differences in cellular proliferation, drug uptake, metabolism, and exposure (15). Similarly, inhibitors designed around conserved protein interactions may still achieve useful selectivity, but this cannot be assumed from structural similarity alone.

This apparent conflict between resistance avoidance and drug selectivity gives rise to an evolutionary paradox. Drug 1 may be unable to rely predominantly on functionally indispensable protein interactions because these interactions are shared with normal proteins. However, treatment with Drug 1 can select cancer cells carrying mutations that prevent its inhibition while preserving sufficient protein function for continued growth. In escaping Drug 1, these cells may acquire structural features that distinguish their target protein from its normal counterparts. The very mutation responsible for resistance could therefore provide the selectivity that was previously difficult to achieve. Drug 2 could exploit this newly acquired feature for selective recognition while relying predominantly on functionally indispensable interactions for inhibition. Thus, the evolutionary process that defeats Drug 1 may create the molecular conditions for a more selective and potentially more resistance-durable Drug 2. This possibility forms the central hypothesis of the proposed research.

**3.3** Established approaches to predicting and overcoming drug resistance

Mutant-selective successor inhibitors are established. Earlier studies developed inhibitors active against EGFR T790M, including osimertinib, while reducing activity against wild-type EGFR (16,17). Ponatinib was developed to inhibit BCR-ABL1 T315I (8). Mutant-selective allosteric EGFR inhibition provides another approach, although the cited EAI045 study required combination with cetuximab for effective activity in its cellular

and mouse models (18). These drugs are precedents for targeting resistant states, not proof of the complete design proposed here.

Forecasting resistance before it appears in patients is older and better developed than the rest of this proposal, and the relevant work should be stated plainly. Chemical mutagenesis screens in cultured Ba/F3 cells were used twenty years ago to compare the resistance mutation spectra emerging under imatinib, dasatinib and nilotinib, alone and in pairs; the recovered spectra reproduced the mutations seen in patients with clinical imatinib resistance, and the combinations suppressed the outgrowth of resistant clones at lower concentrations than the single agents (19). CRISPR base editing has since made this systematic. One study used a guide library predicted to install 32,476 variants across 11 cancer genes to identify resistance mechanisms for ten targeted drugs prospectively, and its authors explicitly suggest using such screens to guide the design of new inhibitors against the resistant proteins the screens identify (7). Editing at the endogenous gene, rather than from an introduced cDNA copy, is also established: prime editing has been used to generate 97% of the possible single-nucleotide variants in the ABL1 kinase domain and to profile them against five inhibitors (20), and comparable saturation profiling of drug-resistant variants has been reported for other targets (21). The earlier EGFR study cited above used a cDNA library in mouse Ba/F3 cells (5), but that is a feature of one study rather than a remaining gap in the field.

Work on evolutionary steering and the ALK example show that a resistant state can sometimes create a second vulnerability (11,22). Together, these studies establish the scientific foundations for a further advance: deliberately exploiting an evolutionarily selected resistance mutation as the molecular feature that enables a successor inhibitor to combine mutant selectivity with binding interactions concentrated on functionally indispensable regions. The proposed contribution is therefore the mechanistic integration of established approaches into a strategy designed to achieve both selectivity and resistance durability.

## 4. Exploiting evolutionary resistance for selective and resistance-durable drug design

### **4.1** Using predicted resistance mutations to design selective rescue inhibitors

The evolutionary paradox identified in Section 3.2 suggests a practical drug-design strategy: use resistance mutations selected by an initial inhibitor to identify molecular features that can guide the development of a more selective and potentially more resistance-durable successor inhibitor. This strategy requires two complementary design objectives—selective recognition of the resistant tumor protein and predominant reliance on functionally indispensable binding interactions that could restrict subsequent evolutionary escape.

To explain this approach, consider two drugs: [1] Drug 1 (initial inhibitor): The original anticancer drug, which inhibits a protein essential for tumor cell survival or proliferation. During treatment, some cancer cells may acquire or already possess mutations that reduce their sensitivity to Drug 1 while preserving sufficient protein function for continued growth. [2] Drug 2 (rescue inhibitor): A second inhibitor specifically designed to remain effective against

the protein carrying a resistance mutation to Drug 1. Unlike a conventional successor inhibitor, Drug 2 would be designed to derive most of its binding strength from functionally indispensable regions of the protein, while exploiting the resistance mutation to distinguish the tumor protein from its normal counterparts.

The proposed strategy begins by identifying mutations that could confer resistance to Drug 1 before they emerge clinically. Laboratory mutation screens could identify candidate mutations, while structural and functional experiments would determine whether the resulting mutant proteins remain biologically active and possess molecular features that could be exploited by Drug 2. Researchers could then develop and evaluate candidate rescue inhibitors in advance of clinical resistance.

The key innovation is to assign two distinct functions to different molecular interactions within Drug 2. First, interactions with functionally indispensable regions would provide strong inhibition while potentially limiting further resistance, because mutations that disrupt these interactions may also compromise the protein's essential activity. Second, interactions with a structural feature created by the resistance mutation would provide selectivity, allowing Drug 2 to preferentially inhibit the resistant tumor protein while sparing the normal version of the target and closely related proteins.

For example, a resistance mutation might create a new binding pocket (a *neo-pocket*), alter the shape or electrical charge of an existing pocket, or change the protein's three-dimensional conformation. Drug 2 could exploit one of these differences to recognize the resistant protein. However, not every resistance mutation produces a useful recognition feature. Some mutations may simply obstruct drug binding without creating an alternative interaction that can be exploited. Furthermore, a structural feature should be considered tumor-specific only after demonstrating that it is absent from the relevant normal protein and its paralogues.

Although predicting resistance mutations and developing mutant-selective successor inhibitors are already established approaches (Section 3.3), the proposed strategy differs by deliberately separating the molecular requirements for resistance avoidance and tumor selectivity. Rather than relying predominantly on a mutation-specific interaction that might itself be lost through further mutation, Drug 2 would use that interaction primarily for selective recognition while obtaining most of its binding strength from experimentally verified, functionally constrained regions.

If achievable, this approach could produce rescue inhibitors that both overcome resistance to Drug 1 and make subsequent resistance more difficult to acquire. Whether these two properties can be combined without compromising drug potency or selectivity remains the central experimental question of the proposed research.

**4.2** Combining functionally constrained binding with mutant selectivity

The proposed Drug 1–Drug 2 strategy depends on achieving two complementary objectives: strong inhibition through interactions essential to the target protein's function and selective

recognition of the resistance mutation generated or enriched during treatment with Drug 1. These objectives address the evolutionary paradox introduced in Section 3.2. Drug 1 may be unable to exploit highly conserved functional regions without also inhibiting normal proteins. However, a resistance mutation selected under Drug 1 treatment may create an additional structural or chemical distinction that enables Drug 2 to recognize the resistant tumor protein more selectively while relying predominantly on those conserved interactions for inhibition.

The effectiveness of this strategy depends on the relative contributions of these interactions. If Drug 2 binds too strongly to conserved regions shared with normal proteins, recognition of the resistance mutation may not provide sufficient selectivity to avoid unacceptable toxicity. Conversely, if Drug 2 depends predominantly on the mutation-specific interaction for its inhibitory activity, a subsequent mutation could disrupt that interaction while preserving essential protein function, allowing cancer cells to escape once again. The objective is therefore to design Drug 2 so that the resistance-associated feature provides molecular discrimination, while functionally indispensable interactions provide the principal basis for sustained inhibition. This division of molecular functions could allow Drug 2 to achieve a combination of selectivity and resistance durability that was difficult to obtain against the original tumor protein.

Establishing this mechanism requires more than identifying favourable contacts in a protein structure. The number of observed contacts or the results of molecular docking cannot reliably determine how much each interaction contributes to binding strength. Researchers would need to compare Drug 2 with structurally related compounds lacking its mutant-recognition feature, measure their binding and inhibitory activities against the resistant protein and its corresponding non-resistant forms, and experimentally assess the functional importance of the contacted residues. These comparisons would help determine whether mutant recognition improves selectivity and whether interactions with functionally constrained regions reduce the availability of further resistance mutations. Because binding interactions are influenced by molecular conformation, solvent effects, and protein dynamics, their energetic contributions cannot necessarily be separated into completely independent components.

An additional advantage may arise if Drug 2 retains activity against both the original tumor protein and its resistant variants, potentially allowing it to inhibit mixed cancer-cell populations. However, such broader activity must not compromise selectivity over normal proteins. Where the original tumor target is structurally indistinguishable from its normal counterpart, direct molecular recognition alone cannot provide tumor-specific inhibition. Furthermore, tumors may contain multiple resistant clones carrying different mutations, some of which may not possess the recognition feature targeted by Drug 2. The proposed strategy must therefore be evaluated against both individual resistant variants and heterogeneous tumor populations.

The central experimental question is whether a resistance mutation selected by Drug 1 can provide sufficient additional selectivity to

permit Drug 2 to rely more heavily on functionally indispensable binding interactions without increasing toxicity. If demonstrated, this would establish a mechanistic basis for the evolutionary paradox: the molecular adaptation that allows cancer cells to escape Drug 1 could create the opportunity for Drug 2 to achieve greater selectivity and potentially greater resistance durability than was achievable against the original target.

**4.3** A worked example: EGFR T790M

The argument above can be evaluated more clearly using a specific molecular target. EGFR in non-small-cell lung cancer provides a useful example because the drugs, resistance mutations, and structural mechanisms have been extensively studied, and because a mutant-selective successor drug already exists for comparison.

Gefitinib and erlotinib are reversible inhibitors that occupy the ATP-binding cleft of EGFR and are effective in tumors carrying activating EGFR mutations. The most common acquired resistance mutation is T790M, a substitution at the gatekeeper position of that cleft. Its mechanism is not simply the steric obstruction that the structural change might suggest. Direct binding measurements showed that T790M EGFR still binds gefitinib with low-nanomolar affinity, approximately 4.6 nM for T790M alone and 10.9 nM for the L858R/T790M double mutant. The more important change involves competition with ATP: adding T790M to L858R lowers the *Km* for ATP from approximately 148 μM to 8.4 μM, increasing the kinase's effective affinity for ATP. Consequently, ATP competes more effectively with gefitinib while the kinase retains its signalling activity (1). T790M therefore illustrates the unequal dependency described in Section 3.1: the mutation substantially reduces the drug's effectiveness while preserving the protein's essential function.

T790M also changes the chemistry of the binding pocket by replacing a small polar threonine with a larger hydrophobic methionine at a position bordering the ATP-binding cleft. This creates an additional molecular distinction between the resistant tumor kinase and wild-type EGFR, which remains expressed in normal tissues, including the skin and gastrointestinal tract. However, T790M is not the only distinction. The tumor kinase already carries an activating mutation, and the cited study examined both T790M alone and the L858R/T790M double mutant; the latter provides the background for this example (1). Activating mutations can alter kinase conformation and contribute independently to mutant selectivity. Thus, T790M provides an additional recognition feature rather than the sole basis for distinguishing tumor EGFR from wild-type EGFR.

This distinction is important to the design problem described in Section 3.2. The ATP-binding region contains interactions essential to kinase function and is therefore an attractive region for inhibitors intended to make subsequent resistance more costly. However, many of these interactions are conserved across the kinase family, making selective inhibition difficult. A resistance mutation within this conserved region may help resolve that conflict by creating a distinctive recognition feature while leaving essential functional interactions available for inhibitor binding. In the proposed Drug 1–Drug 2 strategy, the mutation would

contribute to Drug 2 selectivity, while functionally indispensable contacts would provide the principal basis for inhibition and potentially constrain further resistance.

The feasibility of exploiting a resistance mutation for selectivity has already been demonstrated. Mutant-selective irreversible inhibitors were developed that inhibit T790M-containing EGFR substantially more potently than wild-type EGFR (17). Osimertinib, a third-generation inhibitor developed using this principle, inhibits EGFR carrying sensitising mutations and T790M while showing greater selectivity over wild-type EGFR than first-generation inhibitors (16). This selectivity can improve the therapeutic window by reducing inhibition of normal EGFR and its associated skin and gastrointestinal toxicities. The use of a resistance mutation to achieve drug selectivity is therefore an established principle. The additional proposition examined in this review is whether that selectivity can be combined with predominant reliance on functionally indispensable binding interactions to make subsequent resistance more difficult.

Osimertinib's potency against mutant EGFR depends substantially on forming a covalent bond with cysteine 797, a residue that the kinase does not require for ATP binding or signalling. Substitution of this residue through C797S is a documented mechanism of resistance (2). This might suggest that a noncovalent inhibitor relying on protein-backbone interactions would be more resistant to subsequent escape. However, an inhibitor with these characteristics has already been tested, and its results illustrate why this assumption is insufficient.

BLU-945 is a reversible, wild-type-sparing fourth-generation EGFR inhibitor developed to remain active against T790M, including when combined with C797S. Unlike osimertinib, it does not depend on a covalent bond with C797. Instead, it forms hydrogen bonds with the protein backbone at the hinge region: one with the backbone carbonyl of Q791 and another with the backbone NH of M793. Nevertheless, the deep mutational scan cited earlier identified substantially more candidate resistance mutations against BLU-945 than against osimertinib. These mutations clustered around the hinge region and ATP-binding pocket, including Q791. The Q791L mutation was individually validated: in a resistant EGFR background inhibited at approximately 1.7 nM, the addition of Q791L increased the concentration required for half-maximal inhibition to more than 100 nM (5). Thus, avoiding a covalent anchor and forming backbone hydrogen bonds do not, by themselves, prevent resistance.

The same study provides an important observation supporting the present proposal. BLU-945 also contacts K745, a catalytic lysine that forms a salt bridge with E762 and is important for phosphoryl transfer. No mutations at K745 were recovered in the resistance screen. The authors attributed this observation to the residue's functional importance and suggested that mutational screening could guide inhibitor design toward functionally constrained contact points and away from more mutation-tolerant positions, such as Q791 and L718 (5). Although the absence of recovered K745 mutations is consistent with functional constraint, independent functional measurements are needed to establish which substitutions impair

protein activity and which remain functional without conferring resistance. This distinction is central to the proposed strategy: a backbone contact is not necessarily a functionally constrained contact. Resistance may be more difficult when an inhibitor depends on interactions that the target protein cannot readily alter without compromising its essential function. Such constraints must be established experimentally rather than inferred from structural contacts alone.

The remaining research question is therefore more specific than whether mutant-selective, noncovalent inhibitors can be developed. BLU-945 demonstrates that an inhibitor can recognize resistant EGFR, avoid dependence on C797, and retain selectivity over wild-type EGFR. However, it does not establish whether concentrating binding interactions on independently verified, functionally constrained residues can reduce subsequent resistance while preserving mutant selectivity. This is the additional principle proposed for Drug 2: the resistance-associated mutation provides molecular discrimination, while indispensable protein interactions provide the principal basis for inhibition. The experiments outlined in Section 6 would test this principle using matched compounds that differ in their dependence on contacts identified as potentially constrained through mutational screening and subsequently confirmed through direct functional measurements. Each compound would be evaluated for its selectivity over wild-type EGFR and related kinases, its activity at achievable drug exposures, and the number and characteristics of resistance mutations that emerge.

Two limitations of the EGFR example remain important. First, whether a compound can derive most of its binding strength from functionally constrained positions such as K745 while retaining sufficient mutant selectivity is a structural and medicinal-chemistry question that has not yet been resolved. As discussed in Section 4.2, strong binding to conserved functional regions could compromise the selectivity gained through mutant recognition. Second, this example focuses on tumors carrying T790M, whereas resistance may also arise through C797S, L718X—which the same scan identified as a liability shared by osimertinib and BLU-945—or mechanisms outside EGFR, as discussed in Section 7. BLU-945 itself is no longer in clinical development (5), illustrating that favourable molecular binding characteristics alone do not establish clinical utility.

The significance of the EGFR example is that it brings the proposed Drug 1–Drug 2 strategy into sharper focus. Existing inhibitors establish that resistance mutations can be exploited for selectivity, while the BLU-945 results demonstrate why noncovalent binding and backbone interactions alone are insufficient to prevent further escape. The proposed advance is to combine mutant-specific recognition with experimentally verified functional constraints, potentially allowing the mutation responsible for failure of Drug 1 to become the selectivity feature that makes a more resistance-durable Drug 2 possible.

## 5. Experimental validation of evolution-informed rescue drug design

**5.1** Identify and validate evolutionarily selected

resistance mutations

The first objective is to identify resistance mutations that are selected during exposure to Drug 1 and determine whether they create molecular features that Drug 2 could exploit for selective recognition. This is the critical first step in testing the evolutionary paradox proposed in Section 3.2: can the evolutionary process that allows tumor cells to escape Drug 1 create a molecular vulnerability that enables the development of a more selective and potentially more resistance-durable Drug 2?

An established target such as EGFR provides a suitable starting point because its structures, resistance mutations, and mutational-screening data are available (1,5). Researchers would begin with tumor cells carrying a defined oncogenic background that is initially sensitive to a specified Drug 1. Multiple independent populations would then be exposed to Drug 1 under controlled laboratory conditions, allowing resistant cells to emerge through mutation and selection rather than through the deliberate introduction of predetermined resistance mutations. Drug exposure could be adjusted to reproduce relevant selection pressures and accelerate the emergence of resistant populations. Untreated control populations would be maintained to distinguish changes associated with Drug 1 selection from background genetic changes.

The resistant populations would be sequenced to identify mutations that arise or become enriched during treatment. Sequencing before exposure, where sufficiently sensitive, could also establish whether particular resistant variants were already present at low frequencies. This distinction is important because acquired resistance may result either from new mutations or from the expansion of pre-existing resistant clones. Both represent evolutionary responses to Drug 1 selection. Comparing independent populations would reveal whether particular mutations repeatedly emerge or become enriched, providing evidence of reproducible evolutionary pathways rather than isolated experimental events.

A further experiment would test whether these evolutionary pathways are reproducible across different tumor models. Drug 1 selection would be repeated in several independently derived tumor-cell lines or patient-derived organoids carrying the relevant oncogenic target. The resulting resistant populations would be sequenced and compared to determine whether the same mutations, or mutations affecting the same structural regions, recur across different genetic backgrounds. Recurrent selection would strengthen the case that these changes represent accessible resistance pathways, while differences between models would reveal the extent to which resistance depends on cellular context. The frequency of a mutation across laboratory experiments would not, by itself, establish its probability of emerging in patients.

The experimentally selected mutations would then be compared with available clinical resistance data from patients treated with Drug 1. Particular attention would be given to mutations detected after treatment that were absent or present at lower frequencies before treatment. Agreement between independently evolved laboratory resistance and clinically observed resistance would provide stronger evidence that the experimental system captures biologically relevant evolutionary trajectories.

Where Drug 1 is still under development and clinical resistance data are unavailable, the laboratory findings would remain prospective predictions to be evaluated when such data become available. This comparison would therefore strengthen the selection of Drug 2 targets without requiring researchers to wait for clinical resistance before beginning their development.

Candidate mutations would next undergo functional validation. CRISPR-based methods could introduce individual mutations into the endogenous target gene of initially sensitive tumor cells or patient-derived organoids (7,20,21). The edited cells would be compared with the parental population, independent edited clones, mock-edited controls, and cells in which the mutation had been reverted. Sequencing, protein measurements, functional assays, and Drug 1 sensitivity testing would establish whether each mutation independently causes resistance while preserving the target activity required for tumor survival. In this experiment, CRISPR would validate mutations identified through evolutionary selection rather than substitute for that selection process.

The validated mutations would then be examined for their potential usefulness in Drug 2 design. A suitable candidate must satisfy three requirements: it must confer resistance to Drug 1, preserve sufficient target function for tumor survival, and create an exploitable structural or chemical distinction between the resistant tumor protein and the corresponding normal protein. Such distinctions could include changes in binding-pocket geometry, charge, hydrophobicity, or accessible protein conformation. Mutations that confer resistance but do not create a useful recognition feature would remain important for understanding Drug 1 failure but would not necessarily provide suitable targets for the proposed Drug 2 strategy.

For the most promising variants, structural and functional studies would identify two complementary classes of potential Drug 2 interactions: mutation-associated features capable of providing selective recognition and functionally indispensable protein contacts capable of supporting inhibition while constraining further resistance. Candidate constrained contacts would require direct functional validation rather than being identified solely through structural conservation or molecular docking. The objective would be to determine whether a resistance mutation selected by Drug 1 creates sufficient molecular discrimination to allow Drug 2 to depend more strongly on functionally indispensable binding interactions without losing selectivity over normal proteins.

Figure 1 summarizes the progression from experimentally evolved resistance to prospective Drug 2 design.

The intended outcome is not simply a catalog of possible resistance mutations, but a set of experimentally validated and evolutionarily credible molecular targets for Drug 2. Repeated selection across independent laboratory populations, recurrence across tumor models, and agreement with clinical resistance data would progressively strengthen confidence in the relevance of each target. Researchers could begin developing Drug 2 against resistance pathways that Drug 1 has demonstrably selected, potentially before those pathways

become clinically prevalent. It would thereby convert the evolutionary consequences of Drug 1 treatment from an unexpected obstacle into a prospective source of molecular targets for successor drug development.

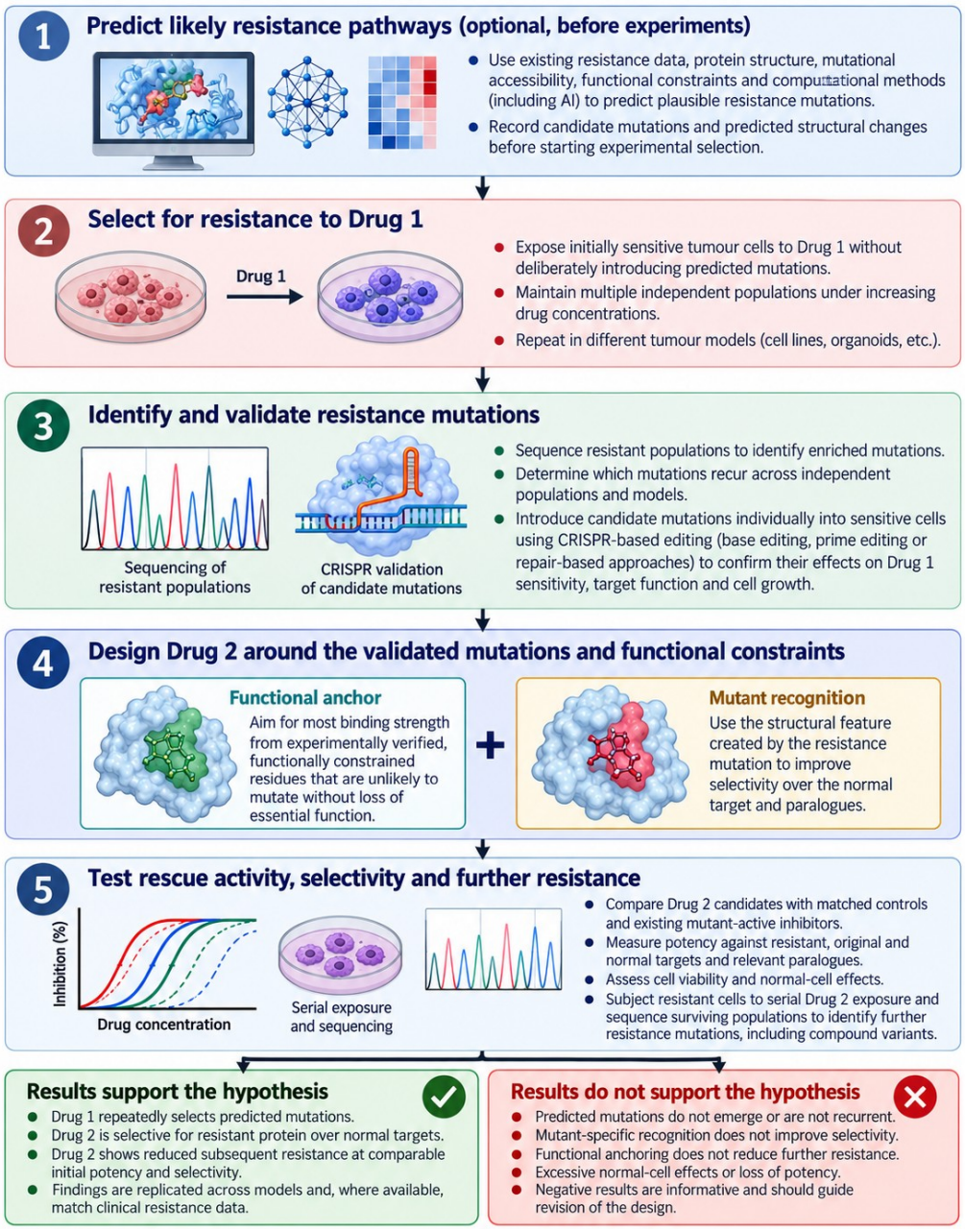



**Figure 1.** Proposed workflow for evolution-informed rescue drug design. The workflow integrates computational prediction, experimental evolution, sequencing and CRISPR validation to identify resistance mutations that arise under Drug 1 selection, and uses these validated mutations to guide the design and testing of Drug 2. Each stage requires experimental validation; mutation emergence and accessibility should be reported separately, and neither is a probability of resistance.

Table 2 identifies the cell models and controls required to establish resistance causation, preserved target function, and mutant-specific recognition. The comparative inhibitor experiments described in subsequent sections would then determine whether these features can be combined in a single compound.

**Table 2.** Cell models and controls for the proposed study

| Cell population | Purpose |
|---|---|
| Parental tumor cells | Establish sensitivity to Drug 1 in the original oncogenic background. |
| Separate edited variants A, B, and C | Test each candidate resistance change and its possible rescue inhibitor. |
| Independent clones and mock-edited cells | Check whether the result depends on editing or clone-specific differences. |
| Reversion control | Restore the original sequence and test whether the phenotype reverses. |
| Normal human cells or organoids | Measure effects on normal tissues in the chosen models. |
| Models or assays for close paralogues | Check selectivity beyond the normal version of the target. |

**5.2** Distinguish evolutionarily selected resistance from functional validation

The resistance experiments described in Section 5.1 would produce populations of tumor cells that survive Drug 1 exposure through evolutionary selection. Sequencing these populations, together with their untreated controls and starting populations, would identify mutations that emerge or become enriched during treatment. Their recurrence across independent experiments and different tumor models would provide evidence of reproducible resistance pathways. However, survival under Drug 1 does not by itself establish that an identified mutation causes resistance, because other genetic changes or cellular adaptations may contribute to the observed phenotype.

The mutations identified through evolutionary selection would therefore undergo individual functional validation. CRISPR-based editing could reproduce each mutation in the original drug-sensitive tumor background, allowing researchers to measure its effect on Drug 1 sensitivity, target function, and cellular growth. Reverting the mutation in resistant cells would provide an additional test of causation. Published editing screens demonstrate the usefulness of this approach, while also showing that some substitutions at established resistance-associated positions impair cellular fitness rather than promote resistance (7). Enrichment or depletion in an editing screen must therefore be interpreted alongside editing efficiency, starting representation, and untreated controls. Importantly, an engineered mutation that confers resistance is not necessarily one that would emerge through natural evolution; the preceding Drug 1 selection experiments provide the evidence of evolutionary accessibility (5,23).

Mutations that repeatedly emerge under Drug 1 selection, independently confer resistance, and preserve sufficient target function would be prioritized for further investigation. Researchers would then determine whether each mutation creates a structural or chemical feature that Drug

2 could exploit for selective recognition. Measurements should include resistance magnitude, target activity, growth with and without Drug 1, nucleotide accessibility, recurrence across independent experiments and tumor models, and agreement with available clinical resistance data. These findings should be reported separately rather than combined into a single resistance-prediction score.

The purpose of this stage is to distinguish three questions: whether a mutation is selected during evolution under Drug 1, whether it actually causes resistance, and whether it creates a useful molecular target for Drug 2. Only mutations supported by the relevant evolutionary and functional evidence would advance to the structural and rescue-drug experiments described in Section 5.3.

**Table 3.** Interpreting results from the screen

| Observed result | Interpretation |
|---|---|
| Drug resistant with retained target function | Candidate on-target escape; confirm the mechanism and cellular context. |
| Drug resistant with impaired target function | Investigate residual function, bypass mechanisms, or assay artifacts. Do not automatically label the variant evolutionarily impossible. |
| Drug sensitive with retained target function | Not an escape variant at the tested exposure and duration. |
| Drug sensitive with impaired target function | A damaging substitution in this assay. One such result does not prove that every change at that residue is intolerable. |

**5.3** Design and test rescue inhibitors against evolutionarily selected resistance mutations

The mutations identified through evolutionary selection in Section 5.1 and validated in Section 5.2 would provide the molecular targets for Drug 2 development. For each promising mutation, researchers would compare the structures of the original and resistant proteins, including their interactions with Drug 1 and their natural ligands. Where experimental structures are available, the exact protein sequences, bound molecules, and structural conditions should be recorded. Computational models may help identify potential binding features but should be distinguished from experimentally established structures. As discussed in Section 4.2, structural contacts and docking scores alone cannot establish which interactions contribute most strongly to inhibitor binding.

The central design objective would be to exploit two complementary molecular features. First, the resistance mutation selected under Drug 1 would provide a distinctive recognition feature capable of differentiating the resistant tumor protein from the corresponding normal protein. Second, Drug 2 would be designed to depend predominantly on interactions with functionally indispensable regions of the target, where further mutations would be more likely to compromise essential protein activity. The functional importance of these regions would be established experimentally rather than inferred

solely from structural conservation. The hypothesis is that combining mutation-specific recognition with functionally constrained binding could produce a rescue inhibitor that is both selective and less susceptible to subsequent resistance.

Candidate Drug 2 compounds would be evaluated against the evolutionarily selected resistant protein, the original oncogenic protein, wild-type protein expressed in normal cells, and relevant paralogues. Existing mutant-active inhibitors would provide reference comparisons. Matched compounds lacking the proposed mutation-recognition feature would help establish whether that feature contributes to selectivity. Additional matched compounds designed to differ in their dependence on functionally constrained contacts would test whether those interactions reduce the emergence of subsequent resistance. These comparisons would require biochemical binding and inhibition measurements alongside cellular assays, because tumor-cell killing alone cannot establish the proposed molecular mechanism.

The most important comparison would be between inhibitors with similar initial activity against the resistant tumor protein but different binding strategies. If a Drug 2 candidate that relies more strongly on experimentally verified functional constraints produces fewer viable escape mutations than a comparator with similar potency and selectivity, this would support the proposed resistance-durability mechanism. Conversely, if resistance emerges just as readily, or if stronger functional anchoring compromises selectivity over normal proteins, the proposed advantage would not be demonstrated. Serial Drug 2 exposure and sequencing of surviving populations would reveal both the number and identity of subsequent resistance mutations, including possible compensatory changes elsewhere in the protein.

These experiments should also determine whether Drug 2 retains activity against the original tumor population as well as its resistant descendants. Such activity could be advantageous in heterogeneous tumors, provided that selectivity over normal proteins is maintained. However, the primary objective remains to establish whether a mutation selected by Drug 1 can supply the molecular discrimination needed to exploit functionally indispensable binding interactions that were difficult to target selectively in the original protein.

**Table 4.** Desired activity profile to be tested

| Protein or cell model | Drug 1 | Proposed Drug 2 |
|---|---|---|
| Original tumor target | Confirm strong inhibition | Retain activity if compatible with selectivity. |
| Validated Drug 1 escape mutant | Confirm reduced activity | Seek strong inhibition. |
| Normal wild-type target | Measure actual activity | Seek substantially less inhibition than of the resistant target. |
| Close paralogues | Measure actual activity | Seek low activity at the intended exposure. |

Table 4 presents the proposed comparisons and the conclusions that each can support. Together, these experiments would test whether the evolutionary escape from Drug 1 creates a practical molecular design advantage for Drug 2, rather than merely another target for conventional inhibitor development.

The decisive result would be a Drug 2 that exploits a naturally selected resistance mutation for selectivity while demonstrating greater resistance durability through experimentally verified functional anchoring. Such a finding would provide direct experimental support for the evolutionary paradox underlying this review.

**5.4** AI-assisted prediction and the practical feasibility of evolution-informed drug design

The experimental strategy described above establishes how resistance mutations could be identified, validated, and exploited for Drug 2 design. A complementary opportunity is to predict these mutations before experimental selection begins, allowing computational forecasts to be tested against independently observed evolutionary outcomes. Artificial intelligence could integrate existing resistance data, protein structures, mutational accessibility, and functional constraints to prioritize plausible escape pathways and accelerate the development of prospective rescue inhibitors.

An illustration of the growing role of predictive computational systems in pharmaceutical development is the BeatSoC Virtual Clinical Trial (BVCT) platform developed by BioinvestGPT (27). BVCT uses computational modelling and biomedical knowledge to predict clinical-trial outcomes and estimate treatment effects relative to specified comparators. The developer reports 95.6% accuracy across 705 prospectively scored clinical-trial readouts as of September 2026. Although these results are company-reported and require independent evaluation, they illustrate an emerging approach in which computational predictions are generated before clinical outcomes become known and subsequently compared with observed results.

The present proposal applies a related predictive principle to a different biological question: which resistance mutations are likely to arise or become enriched through natural evolutionary processes under the selective pressure of Drug 1? An AI system could integrate protein structure, mutation accessibility, functional constraints, drug-binding properties, existing resistance data, and results from independent experimental-evolution studies to identify and rank plausible evolutionary escape pathways. These predictions could then be tested by exposing initially sensitive tumor populations to Drug 1 without deliberately introducing the predicted mutations. Repeated emergence of the predicted variants across independent experiments and tumor models, followed by agreement with clinical resistance observations, would establish whether the system has learned evolutionarily relevant patterns rather than merely identifying mutations capable of producing resistance.

Importantly, predicting clinical-trial success and predicting the molecular evolution of drug resistance are distinct tasks. BVCT does not itself establish that naturally selected resistance mutations can be forecast accurately. Nevertheless, it illustrates how prospective

computational prediction can be incorporated into pharmaceutical research and evaluated against subsequent observations. The same principle could be applied to resistance evolution, using experimental and clinical evidence to test and progressively improve predictive performance.

The significance of this integration is that Drug 2 development need not begin only after Drug 1 fails in patients. AI-assisted prediction, combined with accelerated experimental evolution and functional validation, could identify credible future resistance states while Drug 1 remains under development. Drug 2 could then be designed against the molecular vulnerabilities created by those states, potentially allowing both inhibitors to advance through development in parallel. This would transform acquired resistance from a largely reactive problem into a prospectively investigated and potentially exploitable feature of targeted cancer therapy.

**5.5** Accelerated resistance evolution, parallel drug development, and a new patenting paradigm

The ability to anticipate and experimentally reproduce resistance pathways has implications beyond inhibitor design: it could change the sequence of pharmaceutical development itself. Rather than initiating Drug 2 discovery only after Drug 1 encounters clinical resistance, researchers could use accelerated laboratory evolution to identify resistant populations while Drug 1 remains under development. Mutagenesis and CRISPR-based editing would complement this process by investigating individual mutations and their functional consequences, while experimental selection would establish which resistance states can emerge and expand under Drug 1 exposure.

Once viable resistance mutations have been identified, their structural and functional consequences could guide the parallel development of rescue inhibitors (Drug 2). Each rescue inhibitor would be designed to recognize a molecular feature associated with resistance to Drug 1 while deriving most of its binding strength from functionally indispensable regions of the target protein. Drug 1 and Drug 2 could therefore undergo overlapping temporal development and preclinical evaluation, with Drug 2 potentially available for further clinical development before resistance to Drug 1 becomes widespread. Importantly, this strategy would not require artificially inducing resistance in patients; the evolutionary experiments would be confined to laboratory models.

This approach also raises an important question about the conventional patenting and commercialization of anticancer drugs. Drug 2 could be designed to inhibit a mutant protein that does not yet exist in any identified patient, or whose future clinical emergence remains uncertain. Although the resistant protein would have been generated and characterized experimentally, its relevance as a future therapeutic target would initially rest on laboratory evidence rather than documented clinical disease. Thus, the development of Drug 2 would be directed toward a molecular target anticipated to arise through evolutionary selection under Drug 1 treatment, rather than one already established as a clinically prevalent therapeutic target.

Existing patent systems can accommodate inventions involving experimentally characterized or predicted molecular targets, and the absence of a naturally occurring clinical mutant does not, by itself, prevent patent protection. Nevertheless, systematic development of rescue inhibitors against anticipated resistance mutations could create a need for a more explicitly prospective, evolution-informed patenting paradigm. Such a framework would need to address how inventions directed against predicted future resistance states are described, experimentally supported, distinguished from existing compounds, and protected when their eventual clinical utility depends on mutations that may or may not emerge.

The commercial implications could be substantial. Under conventional sequential development, the discovery of resistance may initiate another lengthy drug-development program. Under the proposed parallel strategy, the original inhibitor and a portfolio of potential rescue inhibitors could be developed as related but independently evaluated therapeutic candidates. Intellectual-property protection might consequently encompass not only the initial drug but also specifically characterized successor compounds directed against experimentally demonstrated escape variants, potentially allowing developers to anticipate future treatment needs rather than respond to them after clinical failure.

This proposal does not establish that existing patent law is inadequate or that a new legal category of patent is necessary. Rather, it identifies a potential shift in the timing and organization of pharmaceutical innovation: a drug could be designed, tested, and potentially patented against a resistance-associated protein variant before that variant has ever been observed in a patient. The novelty of the scientific strategy would lie in anticipating the evolutionary consequences of Drug 1 and incorporating those consequences into the design of Drug 2 from the outset. Whether such an approach would improve clinical outcomes, reduce development delays, or require changes to intellectual-property practices remains an important subject for further investigation.

## 6. Experimental validation and falsification of the proposed paradigm

The study should define its comparisons before examining the results. The central hypothesis is that resistance mutations selected through evolution under Drug 1 may create molecular features that allow Drug 2 to achieve greater selectivity while relying predominantly on functionally indispensable binding interactions to limit subsequent resistance. Testing this hypothesis requires establishing both the evolutionary relevance of the resistance mutation and the molecular advantages of the proposed rescue inhibitor.

The first question is whether the resistance mutations anticipated during Drug 1 development actually emerge through evolutionary selection. Candidate mutations could be predicted using existing resistance data, protein structures, mutational accessibility, functional constraints, and AI-assisted computational methods. These predictions should be recorded before new experimental-evolution studies begin. Multiple independent populations of initially sensitive tumor cells would then be exposed to Drug 1, and the

resulting resistant populations would be sequenced. The same experiments should be repeated across different tumor models to determine whether predicted mutations recur under selection. CRISPR-based editing and mutation-reversion experiments would subsequently establish whether the mutations identified through evolution independently cause resistance while preserving essential target function. This sequence distinguishes mutations that are merely capable of producing resistance from those that are demonstrably accessible through evolutionary selection.

The predictive component should also undergo independent validation. Known clinical resistance mutations can be used for a clearly labelled retrospective check, but they should not be used both to develop the predictor and to claim independent validation. A prospective test would record candidate variants and design predictions before a new independent experiment. Where clinical resistance data are available, the mutations selected in laboratory experiments should be compared with those observed in patients receiving Drug 1. Agreement across independent laboratory populations, tumor models, and clinical observations would strengthen the evolutionary relevance of the predicted resistance pathways. Screening models can nominate specific variants, whereas a general structural diagram cannot identify individual escape mutations without a separate variant-level method.

The next comparisons would test the proposed molecular architecture of Drug 2. First, does the proposed mutant-recognition group improve selectivity for the resistant target compared with a matched compound lacking it? Second, does the proposed functional anchor help retain activity across additional viable variants compared with an appropriate existing mutant-active inhibitor? Third, does either advantage remain in cells while avoiding excessive normal-cell effects? The required margins should be chosen for the target, assay precision, and feasible exposure before testing; this review does not assign universal numerical cutoffs. These experiments would determine whether the resistance-associated feature supplies useful molecular discrimination while functionally indispensable contacts provide a measurable advantage against subsequent escape. Candidate Drug 2 inhibitors should be compared with appropriate existing inhibitors at comparable initial potency, selectivity, and drug exposure, so that differences in subsequent resistance can be attributed more confidently to their dependence on functionally indispensable binding interactions.

The main comparison should report rescue potency, selectivity, the measured escape variants, and their accessibility separately. Counts are comparable only when screening coverage and assay conditions are comparable; an untested variant is not a negative result. A smaller $k$ alone is not evidence of a more durable treatment. A compound that reduces escape diversity but loses its normal-cell safety margin would not satisfy the hypothesis. Equally, a selective compound that is readily escaped through another mutation affecting its new recognition feature would not establish the intended resistance advantage. A failure to identify a useful mutant-specific feature among evolutionarily selected resistance mutations would also be an informative negative result.

Further resistance needs direct testing. A Drug 1 escape mutation A may acquire a second change B that defeats Drug 2. Two drugs with no shared single-mutation escape route can still be defeated by a compound genotype. The relevant question is whether cells carrying complete genotypes grow under the actual combined or sequential treatment. Serial exposure to Drug 2, followed by sequencing and functional testing of surviving populations, would establish whether dependence on experimentally verified, functionally constrained contacts reduces the emergence of viable secondary resistance mutations. BCR-ABL1 studies support the value of combining different binding modes in particular models, but do not make every such combination resistance-proof (24,25).

Later studies could compare Drug 1 alone, Drug 2 alone, simultaneous treatment, and a justified sequential schedule. Alternation or "waxing and waning" exposure should be considered only if measured drug responses provide a reason to test it. Continuous combination is an important comparator. Drug concentrations or inhibitory effects cannot simply be added to assume adequate treatment, particularly when drugs compete for the same site. Exposure, target occupancy, toxicity, and mixed resistant populations would need to be considered (3,22). These comparisons would also establish whether prospective availability of Drug 2 provides an advantage over waiting for resistance to emerge before introducing a successor inhibitor.

Testing in additional tumor backgrounds, patient-derived organoids, and appropriate animal models would examine whether an initial result transfers beyond one cell line. Serial selection and sequencing should include compound variants and resistance mechanisms outside the original target. Patient sequencing could later test whether predicted changes actually occur and help guide the clinical evaluation of rescue options. Such findings would support the prospective development of Drug 2 but would not, by themselves, establish clinical safety or efficacy.

The decisive test of the proposed paradigm is whether Drug 1 repeatedly selects resistance mutations that can be anticipated before clinical emergence and subsequently exploited to design a Drug 2 with both improved mutant selectivity and demonstrably greater resistance durability. If these properties are achieved at therapeutically relevant exposures, the results would support the central evolutionary paradox: the molecular adaptation that allows tumor cells to escape Drug 1 can create the opportunity for a more selective and potentially more durable successor therapy. If the predicted mutations do not emerge, the mutant-recognition feature fails to provide selectivity, or functional anchoring does not reduce subsequent resistance, the corresponding component of the hypothesis would not be supported.

## 7. Limitations and significance

The principal contribution of this review is the identification of an evolutionary paradox that may provide a new foundation for anticancer drug design: the mutations responsible for resistance to an initial inhibitor may simultaneously create the molecular features needed to develop a more selective and potentially more resistance-durable successor drug. This proposition addresses a fundamental conflict in targeted therapy. Functionally

indispensable protein regions are attractive binding targets because mutations that disrupt them may also compromise tumor survival. However, these regions are often conserved across normal proteins, making selective inhibition difficult. A resistance mutation that preserves essential protein function while creating a distinctive molecular feature could resolve this conflict by providing Drug 2 with a selective recognition site while allowing its principal binding interactions to depend on functionally constrained regions.

This approach extends established advances in prospective resistance screening, mutant-selective inhibition, and substrate-envelope design (7,8,12–14,16,17,19–21). Its distinguishing contribution is to assign complementary functions to two classes of molecular interactions: mutant-specific features provide selectivity, while functionally indispensable contacts potentially restrict subsequent evolutionary escape. The EGFR examples illustrate the importance of this distinction. Although mutant-selective inhibitors have successfully overcome earlier resistance mutations, subsequent escape remains possible when their activity depends on interactions that can be disrupted without destroying target function. The resistance profile of BLU-945 further demonstrates that noncovalent binding and protein-backbone interactions do not necessarily confer resistance durability (5). These observations motivate a more stringent design principle based on experimentally demonstrated functional constraints rather than structural conservation alone.

The proposal also has implications beyond the molecular architecture of individual inhibitors. Deliberately accelerating resistance evolution in laboratory models, supported by mutagenesis and CRISPR-based editing, could allow researchers to identify and characterize viable resistance states while the original inhibitor remains under development (7,19–21). Drug 2 could then be designed and evaluated against these anticipated molecular targets in parallel with Drug 1. This would shift pharmaceutical development from a predominantly sequential response to acquired resistance toward a prospective strategy in which the evolutionary consequences of treatment are investigated before clinical failure. The possibility of developing and patenting inhibitors against experimentally characterized resistance states not yet observed in patients also raises important questions about how intellectual-property protection and commercial development might accommodate evolution-informed therapeutic portfolios.

The approach has identifiable biological and experimental boundaries. Not every resistance mutation creates a useful recognition feature, and some mutations may produce structural changes that cannot be exploited without compromising potency or selectivity. Resistant tumors may also contain multiple competing clones or escape through target amplification, bypass signalling, altered drug handling, or changes in cellular state rather than mutations in the original drug-binding region. Clinical resistance to KRAS G12C inhibitors illustrates how multiple resistance mechanisms can coexist within the same disease (26). Furthermore, even when Drug 2 binds predominantly to functionally indispensable contacts, distant mutations or conformational changes may

reduce its effectiveness without eliminating essential protein function. These possibilities establish the circumstances under which the proposed strategy may succeed or fail and identify the measurements needed to evaluate it.

An additional consideration is that drug binding depends on more than static molecular geometry. Binding affinity, association and dissociation kinetics, target occupancy, cellular exposure, and conformational dynamics can influence therapeutic effectiveness and the emergence of resistance. Consequently, structural comparisons and computational docking must be complemented by biochemical measurements, functional assays, and evolutionary selection experiments. These investigations would determine whether the proposed separation between mutant recognition and functionally constrained anchoring produces measurable improvements in selectivity and resistance durability.

The broader significance of this proposal is that tumor evolution need not be viewed exclusively as an obstacle to successful treatment. Under the selective pressure imposed by Drug 1, surviving cancer cells may acquire molecular features that make them vulnerable to a Drug 2 that could not have been designed with the same selectivity against the original target. By identifying these potential vulnerabilities before they emerge clinically, researchers could investigate whether the evolutionary process responsible for treatment failure can be anticipated and exploited therapeutically. The proposed strategy thus reframes acquired resistance not merely as a problem requiring another inhibitor, but as a possible source of the molecular information needed to design a better one.

## 8. Conclusion

Acquired resistance to targeted anticancer therapy is conventionally regarded as a consequence of treatment failure. This review proposes a different interpretation: the evolutionary process that defeats an initial inhibitor may simultaneously create the molecular vulnerability needed to develop a more selective and potentially more resistance-durable successor drug. The central paradox is that functionally indispensable protein regions, although attractive targets for preventing resistance, are often conserved across normal proteins, making selective inhibition difficult. However, a mutation that allows cancer cells to escape Drug 1 while preserving essential protein function may create a new structural or chemical feature that distinguishes the resistant protein from its normal counterparts. Drug 2 could exploit this feature for selective recognition while deriving most of its binding strength from functionally constrained interactions that cannot readily be disrupted without compromising protein function.

The proposed innovation is not the prediction of resistance mutations, the development of mutant-selective inhibitors, or substrate-envelope design, all of which have established precedents (7,8,12–14,16,17,19–21). Rather, it is the deliberate separation of two competing molecular requirements: using an evolutionary escape mutation to provide selectivity and functionally indispensable protein interactions to constrain further escape. Existing EGFR inhibitors demonstrate both the feasibility of mutant-selective targeting and the limitations of relying on mutable binding interactions. The resistance profile of BLU-945 further illustrates that avoiding covalent attachment or relying on

protein-backbone contacts does not necessarily prevent subsequent resistance (5). The proposed strategy therefore requires experimentally verified functional constraints rather than structural conservation alone.

This principle also suggests a fundamental change in the timing of anticancer drug development. Instead of waiting for resistance to emerge clinically, researchers could deliberately accelerate resistance evolution *in vitro*, complement evolutionary selection with CRISPR-based mutation screens, and characterize viable escape variants while Drug 1 is still under development (7,19–21). These experimentally generated resistant proteins could then serve as prospective targets for designing and evaluating Drug 2 in parallel with Drug 1. Such an approach could transform resistance from an unforeseen clinical obstacle into an anticipated stage of pharmaceutical development. It could also encourage an evolution-informed patenting paradigm in which successor inhibitors are developed and potentially patented against experimentally characterized resistance-associated proteins before those variants have been observed in patients.

The hypothesis remains experimentally unproven. Not every resistance mutation creates a useful recognition feature, not every functionally constrained interaction can be exploited selectively, and cancer cells may escape through additional mutations or alternative survival pathways. The proposed experiments must therefore establish whether mutant recognition improves selectivity, whether dependence on indispensable contacts reduces further resistance, and whether both advantages can coexist at therapeutically achievable exposures. Nevertheless, these uncertainties define a falsifiable research direction rather than diminish its conceptual significance.

The central proposition is that the evolutionary failure of Drug 1 may create the conditions for the molecular success of Drug 2. If validated, this would shift the objective of resistance-informed drug design from merely anticipating how cancer cells escape treatment to exploiting the new vulnerabilities created by that escape. Instead of treating tumor evolution exclusively as an adversary, anticancer drug development could begin to use its predictable consequences as the foundation for the next generation of targeted therapies.

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A large language model was used to assemble a preliminary list of anticancer drugs with resistance mutations and associated structures, and to assist with drafting and revision. That list was used only to identify candidate sources. Every example retained in this article was checked against the cited primary literature, and the list itself is not reported as evidence. The author remains responsible for the final content.

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