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COVALENT KINASE INHIBITORS: DESIGN PRINCIPLES AND CLINICAL EXPERIENCE

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Abstract

Covalent kinase inhibitors combine a reversible binding step with bond formation to a nucleophilic residue, usually a cysteine, and several now have established clinical roles. This mini review summarises the published design principles, including two-step kinetics, warhead selection and tuning, structure-guided targeting of cysteines and proteome-wide selectivity profiling. It then outlines the clinical record of inhibitors of the ErbB family, Bruton's tyrosine kinase and the covalent KRAS G12C programme, alongside resistance and safety findings. The literature supports durable target occupancy and good selectivity when reactivity is kept low, yet resistance at the target cysteine recurs and head-to-head evidence against reversible drugs is scarce. No new experiments or data are reported.

1. INTRODUCTION

Most kinase inhibitors on the market bind reversibly to the ATP pocket and rely on affinity and sustained plasma exposure to keep the enzyme switched off. A smaller group takes a different route. After docking in the pocket, an electrophilic group on the inhibitor forms a bond with a nucleophilic residue, usually a cysteine, and the enzyme stays inactive until it is resynthesised. Covalent inhibition was long regarded with suspicion in drug discovery, because reactive molecules can modify off-target proteins and trigger toxicity or immune reactions [1, 2]. The picture changed as medicinal chemists learned to place weakly reactive groups

exactly where they were needed, and as several covalent kinase inhibitors reached the clinic with convincing benefit [1, 3, 4].

This mini review summarises the design principles that have emerged from that work and the clinical experience with the main agents up to 2021. It covers the logic of two-step covalent binding, the choice and tuning of warheads, the use of structure to select a target cysteine, selectivity profiling across the proteome, and the clinical record of inhibitors of the epidermal growth factor receptor (EGFR), related ErbB kinases, Bruton's tyrosine kinase (BTK) and the GTPase KRAS, which borrows the same chemistry. Resistance and safety are discussed beside the efficacy data. It is a narrative review of published work and reports no new experiments. Most of the evidence comes from a few well-studied targets, so general conclusions about the approach are drawn with some caution.

2. RATIONALE AND KINETICS

The kinetics of a targeted covalent inhibitor are best described in two steps. The molecule first forms a reversible complex with the enzyme, characterised by an inhibition constant, and then the warhead reacts with the nucleophile at a rate that reflects both its intrinsic reactivity and how well the binding pose places it near the target residue [1, 2]. Potency is therefore best expressed as the ratio of the inactivation rate constant to the inhibition constant, because a single IC_{50} shifts with incubation time and says little about the mechanism [2, 5]. The practical advantage is a duration of action that outlasts pharmacokinetics. Since the target stays inactivated until new protein is made, the pharmacodynamic effect can persist after the drug has left plasma, which allows lower or less frequent dosing and, in principle, less systemic exposure to off-target interactions [1, 4].

Covalent binding also changes how selectivity is achieved. A reversible inhibitor must discriminate between kinases through shape and electrostatics alone, in pockets that are highly conserved across the family. A covalent inhibitor faces two filters in sequence: the scaffold must first recognise the pocket well enough to hold the warhead in position, and only then can the bond form, which requires a nucleophile in the right place [1, 2, 6]. Selectivity can therefore exceed what affinity alone would predict. The same property can rescue potency against targets with shallow or featureless pockets, where reversible binding energy is hard to build [4, 7]. Selectivity is not guaranteed, however, since any kinase with an accessible cysteine near the pocket is a candidate off-target.

Covalent drugs are not new. Aspirin, penicillin and omeprazole all act by forming bonds with their targets, and most were found by chance rather than by design [1, 4]. In kinases, the idea of deliberately designing irreversible inhibitors took shape in the early 2000s. A structural bioinformatics study identified kinases carrying a cysteine at a particular position in the pocket, then used it to build selective irreversible inhibitors of one of them, RSK2, and showed that the approach could discriminate between related enzymes [6]. Quinazoline acrylamides directed at a cysteine in the EGFR pocket followed, and the design logic was reviewed for the kinase family as a whole [3, 8]. That insight turned covalent binding from an accident into a strategy.

Warhead position within the kinase also differs between targets, and this shapes what chemistry works. In EGFR and BTK the cysteine lies at the lip of the pocket and is reached from the solvent-facing side, which lets a flexible acrylamide extend from the hinge-binding core. In other kinases the nucleophile sits near the glycine-rich loop or deeper in the back of the pocket, where the approach angle is different and the reversible scaffold must be reshaped to deliver the warhead [9, 10]. Classification of these positions has become a design tool in its own right. A medicinal chemist can look at a kinase, find which of the known cysteine positions it has, and start from the templates that have worked for others with the same arrangement [7, 10].

A kinase can only be targeted this way if it carries a suitably placed nucleophile. Analyses of the human kinome suggest that a large minority of kinases have a cysteine somewhere near the ATP site, in several

distinct positions, and that the position at the lip of the pocket, which corresponds to Cys797 in EGFR and Cys481 in BTK, is the best exploited so far [9, 10]. Mapping of this kind is useful in two directions. It shows which kinases might respond to the same chemistry, and it flags kinases that are likely to be hit by accident, which becomes a selectivity question later in the programme [9, 10].

3. DESIGN PRINCIPLES

Acrylamides dominate. They are weak Michael acceptors that react slowly with free thiols in solution and become reactive mainly when the scaffold holds them close to the target cysteine, which is the central trick of the approach [2, 7]. Alternatives include vinyl sulfonamides, chloroacetamides, alkynamides and cyanoacrylamides, although chloroacetamides are generally too reactive for systemic use [2, 11]. Reactivity is adjusted through substituents. A dimethylaminomethyl group on the crotonamide of afatinib, for instance, is thought to raise reactivity and assist the reaction through intramolecular base catalysis, whereas substitution at the alpha or beta carbon of an acrylamide generally lowers it [11, 12].

Intrinsic reactivity is usually measured by the half-life of the compound in the presence of glutathione, sometimes backed by computed activation energies, and programmes aim for the lowest reactivity that still allows efficient target engagement [11, 13]. A warhead that is too reactive consumes glutathione, binds plasma proteins such as albumin, and risks idiosyncratic toxicity through haptenisation. One that is too unreactive never forms the bond [13, 14]. Medicinal chemists therefore optimise the reversible binding first, so that most of the potency comes from non-covalent contacts, and add the electrophile as a final contributor. This ordering is one reason modern covalent inhibitors tend to look like well-behaved reversible drugs with a small reactive addition.

Structure-guided design is illustrated by the path to osimertinib. Reversible EGFR inhibitors lose potency against the T790M gatekeeper mutation, which restores the receptor's affinity for ATP, and early irreversible compounds that did hit T790M also inhibited wild-type EGFR, producing rash and diarrhoea [8, 15]. Pyrimidine-based, mutant-selective compounds placed an acrylamide to reach Cys797 while gaining much of their binding energy from contacts in the mutant pocket, which gave a wide margin of selectivity over the wild-type receptor [8, 15, 16]. The same logic, using a covalent bond to compensate for a weakened reversible interaction, now guides work on other resistant targets.

Geometry matters as much as chemistry. For the bond to form, the electrophilic carbon must approach the sulfur at a suitable angle and distance, and the linker between scaffold and warhead has to allow that without strain. Crystal structures of reversible analogues are used to judge whether a cysteine is reachable, and covalent docking and molecular dynamics are increasingly used to rank warhead positions before synthesis [7, 8, 11]. Small changes in linker length or ring conformation can move activity by orders of magnitude, which is why series are often explored with several warhead attachment points. Co-crystal structures of the final adduct provide confirmation that the intended residue is modified and that the pocket has not rearranged in unexpected ways [8, 13].

Not every programme wants a permanent bond. Cyanoacrylamides form thiol adducts that can reverse, and chemically tuned versions were used to target noncatalytic cysteines with adjustable residence time, with a hope of reducing haptenisation and permanent off-target modification [17, 18]. Reversible covalent BTK inhibitors have been described with residence times ranging from minutes to days, depending on substituents [18]. Selectivity can be assessed across the proteome by chemoproteomic methods in which a broad cysteine-reactive probe competes with the test compound in lysates or live cells, and these methods have revealed unexpected off-targets as well as new ligandable cysteines [19, 20]. Such profiling is becoming a routine part of the design cycle.

Confirmation of the covalent mechanism is part of good practice. Intact-protein mass spectrometry shows the mass shift of the

adduct, peptide mapping identifies the modified residue, and a cysteine-to-serine mutant of the target should lose time-dependent inhibition [2, 13]. Washout experiments in cells test whether pharmacodynamic effect outlasts the compound. These checks take little time and prevent a programme from mistaking a slowly reversible inhibitor, or a nonspecific reactive compound, for a designed covalent one.

4. CLINICAL EXPERIENCE

The first approved covalent kinase inhibitors target the ErbB family. Afatinib, an anilinoquinazoline with a crotonamide warhead, binds EGFR, human epidermal growth factor receptor 2 (HER2) and HER4 irreversibly, and in a phase III trial in EGFR-mutant lung adenocarcinoma it improved progression-free survival compared with cisplatin and pemetrexed [12, 21]. Neratinib, which targets HER2 and EGFR, was characterised as an irreversible inhibitor in preclinical models and, in the ExteNET trial, improved invasive disease-free survival when given after trastuzumab-based adjuvant therapy to women with early HER2-positive breast cancer [22, 23]. Diarrhoea, rash and stomatitis are the characteristic adverse effects, reflecting inhibition of wild-type EGFR in skin and gut [21, 23]. The main findings for these and later agents are listed in table 1, and fig. 1 shows how the entries divide by theme.

Osimertinib, the best-documented agent in the class, was first licensed for T790M-positive disease after progression on earlier inhibitors. Phase I and III studies showed high response rates and longer progression-free survival than platinum-pemetrexed chemotherapy in that setting [24, 25]. The FLAURA trial then moved the drug to first-line use in EGFR-mutated disease and found longer progression-free survival than gefitinib or erlotinib, with fewer serious adverse events, as expected from selectivity for mutant over wild-type receptor [26]. Activity against central nervous system disease was also notable. Rash and diarrhoea occurred but were generally milder [25, 26]. This is the strongest clinical support so far for the claim that a well-designed covalent inhibitor can be selective as well as durable.

In B-cell malignancies, ibrutinib set the template. It binds Cys481 of BTK, and early preclinical work showed that it blocked B-cell receptor signalling and was active in models of autoimmune disease and lymphoma [27, 28]. Clinical trials in relapsed chronic lymphocytic leukaemia (CLL) and mantle-cell lymphoma (MCL) reported high response rates with an oral once-daily regimen and durable remissions, which changed the treatment of these diseases [29, 30]. Ibrutinib also inhibits other kinases that carry a cysteine at the equivalent position, including EGFR, ITK and TEC family members, and these off-target effects are thought to contribute to rash, diarrhoea, bleeding and atrial fibrillation [14, 31]. Acalabrutinib, designed to be more selective, showed high response rates in relapsed CLL in an early-phase study with favourable tolerability at that stage [31].

Table 1: Covalent kinase inhibitors and design strategies: published findings

Inhibitor or strategy	Published finding	Ref.
Afatinib EGFR, HER2, HER4	Anilinoquinazoline with crotonamide warhead; longer progression-free survival than platinum chemotherapy in EGFR-mutant lung adenocarcinoma.	[12, 21]
Neratinib HER2, EGFR	Irreversible inhibitor; improved invasive disease-free survival as extended adjuvant therapy in early HER2-positive breast cancer.	[22, 23]
Osimertinib EGFR T790M	Mutant-selective pyrimidine reaching Cys797; superior to platinum-pemetrexed after progression and to first-generation agents first-line.	[16, 25, 26]
Ibrutinib BTK	Binds Cys481; high response rates and durable remissions in relapsed CLL and mantle-cell lymphoma.	[28, 29, 30]
Acalabrutinib BTK	Designed for greater selectivity than ibrutinib; high response rates in relapsed CLL with favourable early tolerability.	[31]

Inhibitor or strategy	Published finding	Ref.
EGFR C797S osimertinib	Loss of the target cysteine removes the covalent anchor and mediates acquired resistance.	[32]
BTK C481S ibrutinib	Serine substitution converts irreversible binding to weak reversible binding; main reported relapse mechanism in CLL.	[33]
AMG 510 KRAS G12C	Binds the mutant cysteine in the GDP-bound state; antitumour activity and immune effects in preclinical models.	[34, 35]
Cyanoacrylamides reversible covalent	Reversible thiol adducts give residence times tunable from minutes to days.	[17, 18]
Chemoproteomics selectivity	Competition with cysteine-reactive probes maps off-targets across the proteome in cells and lysates.	[19, 20]

Dose selection for covalent drugs draws on target occupancy as well as plasma exposure. For ibrutinib, occupancy of BTK in peripheral cells was measured with probe-based assays during early trials, and doses that gave near-complete and sustained occupancy were carried forward, a rationale quite different from the exposure-matching used for reversible drugs [28, 29]. The approach has limits. Occupancy in blood does not necessarily reflect occupancy in lymph nodes or tumour tissue, and it says nothing about downstream signalling in resistant clones. Even so, it shows how pharmacodynamic endpoints can be built into early development of a covalent agent when a suitable probe exists [1, 28].

Pharmacokinetic features of the approved agents are worth setting beside their clinical effects. Several are absorbed rapidly, metabolised extensively and have short plasma half-lives, so that exposure in plasma is brief and peaks sharply. For a reversible drug that profile would be a handicap, since inhibition would fall away between doses. For a covalent drug it is acceptable, because inhibition depends on the inactivated protein and not on the plasma concentration [1, 4, 14]. The same profile helps reduce the chance of sustained exposure to reactive species or metabolites, and it explains why a once-daily regimen suffices even when elimination is fast [1, 29].

A third area is KRAS, long regarded as undruggable. The G12C mutation introduces a cysteine next to the nucleotide-binding pocket, and compounds were found that bound it covalently in the inactive GDP-bound state, trapping the protein [34]. Later optimisation produced AMG 510, which showed antitumour activity in mouse models, was reported to induce antitumour immunity, and moved into early clinical testing [35]. The chemistry is borrowed from kinase programmes: a modest acrylamide, structure-guided placement and mutant selectivity, since the cysteine exists only in the tumour protein [34, 35]. Although KRAS is a GTPase and not a kinase, the example shows how far the design principles have travelled.

5. RESISTANCE AND SAFETY

Covalent binding does not prevent resistance. The commonest change reported in EGFR after osimertinib is the C797S mutation, which removes the cysteine and with it the covalent anchor [32]. In CLL, relapse on ibrutinib is most often linked to a C481S substitution in BTK, which converts the irreversible interaction into a weaker reversible one, or to gain-of-function mutations in PLCG2 downstream of the kinase [33]. These patterns illustrate a general weakness. Dependence on a single residue means that one point mutation can remove the advantage of the strategy [32, 33].

Resistance also arises without touching the cysteine. In EGFR-mutant lung cancer, bypass signalling and histological transformation are described after osimertinib, and the pattern of resistance depends on the line of therapy in which the drug was used [25, 26, 32]. In CLL the clonal architecture matters: subclones carrying C481S or PLCG2 changes may be present at low frequency before relapse, which raises the prospect of earlier detection by sequential sampling [33]. The practical consequence is that a covalent inhibitor is not a single-step solution, and the biology of the tumour still

sets how long the benefit lasts. Planning for the next line of treatment, including re-biopsy or sequencing at progression, is part of using these drugs well.

Safety questions fall into two groups. The first is on-target and off-target kinase inhibition, which is mostly dose-related and manageable, such as the skin and gut effects of EGFR inhibition or bleeding with BTK inhibition [14, 21, 31]. The second is idiosyncratic toxicity caused by covalent modification of off-target proteins, which is rare but unpredictable. Evidence for it with kinase inhibitors is limited, and the available reviews have not identified a class-wide signal [13, 14]. Medicinal chemists reduce the risk by keeping warhead reactivity low, limiting dose and checking proteome-wide selectivity [13, 20]. Low doses, made possible by prolonged target occupancy, also reduce the total burden of reactive compound in the body [1, 13].

6. LIMITATIONS AND FUTURE DIRECTIONS

Several gaps limit what can be concluded. Almost all clinical data come from EGFR, BTK and, recently, KRAS, so extrapolation to other kinases is speculative. Head-to-head comparisons between covalent and reversible inhibitors of the same target are scarce, which makes it hard to tell how much of a clinical advantage derives from the covalent bond itself and how much from the other properties of the molecule [26, 36]. Reactivity assays and kinome-wide cysteine maps are incomplete, and proteomic selectivity data obtained in lysates may not reflect behaviour in patients [10, 20]. Finally, resistance mutations at the target cysteine are a recurring theme [32, 33].

Other practical problems are less often discussed. Drug-drug interactions can matter for agents cleared by oxidative metabolism, since a change in exposure alters the peak concentration reaching the target, even though total occupancy depends on the cumulative dose rather than on the average level [14, 36]. Special populations such as people with hepatic impairment have been studied less. The quality of the evidence also varies by indication: the pivotal trials in lung cancer and CLL are randomised and large, while the supporting data for other covalent kinase inhibitors rest on single-arm or early-phase studies [26, 29, 31]. Clinicians should keep that gradient in mind when generalising from the best-studied examples.

Future directions include warheads that engage other nucleophilic residues, reversible covalent designs that tune residence time, and combination strategies that pair a covalent drug with an agent active against the expected resistance mutation [2, 7, 18]. Non-covalent approaches to the C797S and C481S variants are also being pursued [32, 33]. Better predictive models of reactivity and kinetics, built on structure and computed activation energies, should reduce the number of analogues synthesised per programme [7, 11]. Perhaps the most useful addition would be systematic reporting of kinetic constants and proteome-wide selectivity in publications, so that compounds can be compared on a common footing [2, 5].

7. CONCLUSION

Covalent kinase inhibitors work by pairing a reversible binding step with a bond to a well-placed nucleophile, most often a cysteine. The published design principles are consistent: optimise reversible affinity first, choose a warhead of low intrinsic reactivity, use structure to position it, and test selectivity across the proteome rather than only within the kinome [2, 7, 20]. Where these rules were followed, as with osimertinib, the result was a selective and durable drug, and the clinical record with afatinib, neratinib, ibrutinib and acalabrutinib shows that the approach can deliver lasting benefit [16, 21, 26, 29].

Limits remain. Resistance at the target cysteine recurs in both EGFR and BTK, the clinical evidence is concentrated on a handful of targets, and direct comparisons with reversible inhibitors are few [32, 33, 36]. Reversible covalent designs, other nucleophilic residues and better kinetic reporting are the most promising ways forward [5, 18]. For pharmacists and clinicians, the practical points are that duration of effect follows target turnover and not plasma half-life, and that adverse effects usually reflect which kinases are hit and not

the reactivity of the warhead [1, 14].

8. ABBREVIATIONS

ATP-Adenosine triphosphate, BTK-Bruton's tyrosine kinase, CLL-Chronic lymphocytic leukaemia, EGFR-Epidermal growth factor receptor, GDP-Guanosine diphosphate, HER2-Human epidermal growth factor receptor 2, IC_{50}-Half-maximal inhibitory concentration, KRAS-Kirsten rat sarcoma viral oncogene homologue, MCL-Mantle-cell lymphoma

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Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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