Targeted Covalent Inhibitors: A Risk-Benefit Perspective

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Covalent Drugs are not New!

Aspirin - 1897

Inhibits cyclooxygenase enzymes through acetylation of Ser residues (Ser_{530} for COX-1 and Ser_{516} for COX-2) proximal to their active sites.

Penicillin - 1928

Acylates active site Ser residue of bacterial DD-transpeptidase and blocks cell wall biosynthesis.

Omerpazole - 1989

Converted in the stomach to a reactive sulfenic acid that binds to the H^+/K^+-ATPase in parietal cells and inhibits gastric acid secretion.

Clopidogrel - 1991

A prodrug that is metabolized to a reactive sulfenic acid that covalently modifies the P2Y_{12} receptor on the surface of blood platelets and blocks platelet aggregation.

What is a Targeted Covalent Inhibitor?

• A small molecule ligand that is designed \textit{a priori} to covalently modify, and thereby inhibit the function of, a specific protein target. Inhibitors of this type can act by reversible or irreversible mechanisms.

• The resulting “protein silencing” takes place in two steps:
  o Non-covalent association of high affinity ligand to protein target
  o Covalent reaction between electrophilic “warhead” on ligand and specific nucleophilic center on protein

• Typically, the chemistry relies upon Michael addition of a poorly conserved Cys-SH on the protein to an acrylamide-based warhead on the inhibitor.
Potential Advantages of Targeted Covalent Inhibitors

**High Potency**
- Potentially very high potency with complete blockade of target
- High ‘biochemical efficiency’ associated with non-equilibrium binding mechanism
- Low dose translates to decreased potential for off-target effects

**Extended Duration of Action**
- Dependent upon turnover of target protein versus PK of drug
- Mechanism compatible with relatively high drug clearance
- “Uncoupling” of PK and PD can translate to less frequent dosing

**Applicability to Some Previously “Undruggable” Targets**
- Targets with shallow binding sites; disruptors of protein-protein interactions

**Ability to Directly Measure Target Occupancy and Selectivity**
- Activity-based probes allow for assessment of selectivity, and of time- and dose-dependent target occupancy in vitro and in vivo
- Development of target occupancy / biological effect relationships

**Potential to Avoid Some Resistance Mechanisms**
- Drug-resistant forms of EGFR (eg T790M) respond to irreversible inhibitors

Perceived Drawback of (Irreversible) Targeted Covalent Inhibitor Approach

“The formation of a covalent bond between a small molecule drug and its target protein has been largely avoided as a design strategy due to risks associated with immunogenic responses to covalently modified proteins”

- This concern derives largely from studies on chemically reactive drug metabolites
- Caveat is that most reactive drug metabolites are highly reactive electrophiles, in contrast to the weakly reactive warheads found in targeted covalent inhibitors

Reversible Targeting of Noncatalytic Cysteines with “Chemically Tuned” Electrophiles

Reversible covalent inhibitor of RSK2

In a nutshell, the therapeutic applicability or the success of irreversible binding kinase inhibitors is dependent on whether or not the covalent bond can be confined solely to the protein kinase of interest.


“Derisking” Irreversible Covalent Drugs

• **Selectivity of binding** to target protein is key
  - Can be assessed experimentally by ‘activity-based protein profiling’, proteomics mass spectrometry, X-ray structure, etc
    

• **Low dose** is important
  - Reduced body burden of parent drug and metabolites
    

• **Minimal formation of reactive metabolites**
  - Ability to control reactivity of covalent drug
    
Recently Approved Irreversible Tyrosine Kinase Inhibitors

Ibrutinib (IMBRUVICA)

- Blocks B-cell antigen receptor signaling by irreversibly inhibiting Bruton’s tyrosine kinase (Btk)
- FDA approval for mantle-cell lymphoma (Nov 2013) and chronic lymphocytic leukemia (Feb 2014)
- $T_{\text{max}}$ 1-2 hr, $T_{\frac{1}{2}}$ 4-6 hr, $V_{d_{\text{ss}}}$ ~10,000L
- >90% Occupancy @ 24hr after 175mg dose; recommended therapeutic dose = 420 mg/day
- Clearance mechanism: CYP3A4-mediated oxidation, excretion of metabolites in feces
- DDI with ketoconazole gave 24-fold increase in ibrutinib AUC$_p$

Recently Approved Irreversible Tyrosine Kinase Inhibitors
Afatinib (GILOTRIF)

- Irreversible covalent inhibitor of EGFR and HER2
- FDA approval (2013) for metastatic NSCLC with EGFR mutations / deletions
- $T_{\text{max}}$ 3-5 hr, $T_{1/2}$ ~ 34 hr, $V_d\text{ss}$ ~4,500L
- Recommended therapeutic dose = 40 mg/day
- Clearance mechanism: elimination unchanged into feces (metabolism to thiol adducts minor)
- Covalent binding to blood proteins

P. Stopfer et al., Cancer Chemother. Pharmacol., 69, 1051-1061 (2012); D. W. Bowles et al., Drugs Today (Barc), 49, 523-535 (2013)
Key Considerations in Adopting the Targeted Covalent Inhibitor Approach in Drug Discovery

- **Strong bioinformatics support is critical in target selection**
  - Non-catalytic, poorly conserved, accessible, suitably-positioned and oriented nucleophile on target

- **Slow turnover rate of target protein**
  - Duration of PD effect is a function of *de novo* protein re-synthesis

- **Need to optimize potency and selectivity in vitro**
  - Ligand: Enzyme kinetics ($k_{inact}/K_i$, not IC$_{50}$)
  - Protein: Proteomics MS to confirm site of modification, and assess off-target reactions

- **Need to assess potency and selectivity in vivo**
  - Activity-based protein profiling, photoaffinity labeling, PAGE and quantitative MS

- **Traditional in vitro ADME and in vivo PK screens**
  - Less useful in compound selection than measurements of target modification and occupancy
  - PK/PD relationships replaced by target occupancy/PD relationships
  - GST-Mediated conjugation with GSH likely to be a common route of clearance

- **Regulatory**
  - Similar safety requirements to traditional NCE

Conclusions

Over 40 FDA-approved drugs inhibit their target via a covalent mechanism.

The most rapidly growing therapeutic area for targeted covalent inhibitors is oncology.

Slow turnover proteins with appropriately positioned nucleophilic centers are attractive targets for the covalent inhibitor approach.

Highly selective, low-dose inhibitors that are resistant to metabolic activation should have acceptable safety profiles.