EDITORS' pick

Principia Biopharma

A University of California, San Francisco, startup offers a reversible twist on covalent drugs.

Covalent drugs, largely ignored for decades, are staging a comeback. Five irreversible covalent kinase inhibitors have advanced to phase 3 in recent years, with Boehringer Ingelheim's afatinib now under review by US and European regulatory agencies for treating lung cancer. Meanwhile, Principia Biopharma, a startup dedicated to covalent drug development, is offering a unique twist on covalent drugs—reversibility.

Covalent drugs typically bond irreversibly and, consequently, pharma has mostly avoided them because of the potential for unpredicted toxicities or immune reactions. Of reversible drugs, the vast majority bind noncovalently, relying on ionic bonds, hydrogen bonds, hydrophobic bonds or van der Waals forces—all generally weaker than covalent bonds, in which the drug and target share a pair of electrons.

Principia's reversible approach preserves the main advantage of covalency, a stronger bond, and thus minimal necessary drug exposure, without its main theoretical drawback, lasting off-target adduct formation. The covalent drug's electrophile—the electron-accepting element—has the potential to interact with nucleophiles (electron donors) on unpredictable nontarget proteins. For example, the amino acid cysteine, which contains a nucleophilic thiol, is the favored site for covalent drugs, so an irreversible covalent drug could potentially bind tightly to cysteines present on any of hundreds of proteins, with such adducts circulating freely after the proteins degrade. Designers of irreversible covalent drugs have countered by engineering compounds with high target specificity and weak nucleophiles, but have yet to put the safety concerns fully to rest.

That's where Principia's reversible compounds could fill an important niche. Reversible covalent drugs that target noncatalytic cysteines already exist, but in most cases their reversibility was accidental. Principia co-founder, Jack Taunton, a chemical biologist at the University of California, San Francisco, has made reversible compounds by design. After Taunton designed a selective irreversible inhibitor of ribosomal S6 kinase (RSK; *Science* **308**, 1318–1321, 2005), his graduate student Michael Cohen systematically made various electrophilic additions to the same scaffold. Cohen created one compound with a doubly active electrophile—a nitrile attached to an

acrylamide—expecting irreversible binding. Surprisingly, mass spectrometry showed none. Taunton immediately suspected a reversible reaction, which experiments confirmed (*Nat. Chem. Biol.* **8**, 471–476, 2012).

Principia vice president for drug discovery, David Goldstein, explains that the additional reactivity conferred by the nitrile destabilizes the reaction products, lowering the barrier for the reverse reaction. So instead of the irreversible inhibitor binding once, there exists an equilibrium process, a constant 'on-off.' Taunton says that few medicinal chemists, himself included, would have predicted this covalent reaction to be reversible under physiologic conditions, especially with such rapid reaction times. Taunton and Richard Miller founded Principia in 2009 to develop such reversible covalent compounds for autoimmune disease and cancer. The company has secured \$36.3 million in series A funding.

Principia's reversible covalent kinase inhibitors work by first binding noncovalently, placing the electrophile close to a noncatalytic cysteine, thus forcing them to interact, share electrons and bind covalently. Over 100 kinases have cysteines near enough to the active site to make such covalent binding possible, and, in theory, are targetable by Taunton's doubly activated cyanoacrylamide, as well as other chemistries. Principia claims to already have made potent, selective inhibitors against Bruton's tyrosine kinase (Btk), interleukin 2 tyrosine kinase, fibroblast growth factor receptor, RSK and Janus kinase 3.

Like all covalent compounds, Principia's molecules have potential for enhanced potency and selectivity over noncovalent drugs. Tight binding confers potency, and the coincident noncovalent and covalent bonds confer great specificity for the target. It's possible that reversibility confers even greater selectivity. Taunton speculates that reversible drugs, coming on and off the target, are more likely to sense subtle differences in its structure, whereas irreversible inhibitors are kinetically trapped once the covalent bond has formed.

For potency, Principia's medicinal chemists fine-tune their reversible compounds to bind targets quickly but come off slowly. In this way, the drug can clear from circulation, minimizing potential side effects, but remain

bound to its target. This advantage for reversible covalent drugs, however, diminishes if the target protein resynthesizes quickly. This could limit Principia's choice of targets. The resynthesis rate of most drug targets, Taunton says, is unknown.

In practice, "quick on, slow off" requires an electrophile reactive enough to bind the target tightly, but not so reactive that it will bind to nontarget cysteines at the expected drug concentration. Even if this delicate "sweet spot" is found, off-target drug encounters will happen, explains Goldstein, but they're nonproductive—the drug comes right off.

Nathanael Gray, an organic chemist at Harvard University in Cambridge, Massachusetts, considers Principia's approach a creative response to the toxicity concerns of irreversible compounds. But he emphasizes the challenge facing the company in building molecules with reactivity "hot" enough to bind tightly to a target yet not so reactive as to engage off-target proteins. Preclinical assays for determining off-target effects are lacking, Gray says, because there aren't any specific adducts to look for. So toxicity is hard to predict in advance.

Juswinder Singh, a computational chemist at Celgene Avilomics Research in Bedford, New Jersey, considers much of the general toxicity concern unfounded because it's based on studies of noncovalent drugs that are metabolized into covalent compounds. Targeted covalent inhibitors, he says, are fine-tuned for specificity. But Singh questions the ability of even slow-off reversible covalent compounds to match the efficacy of irreversible inhibitors. It's too early, he says, to know.

Even if reversible inhibitors prove superior overall, Principia will face competition. New York-based Pfizer reported preclinical data for its own reversible covalent Btk inhibitor at the 2013 annual meeting of the American Chemical Society in New Orleans, on 9 April. Meanwhile, the most advanced Principia compound is scheduled to begin human clinical testing before the end of the year.

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Jack Taunton, Principia Founder.