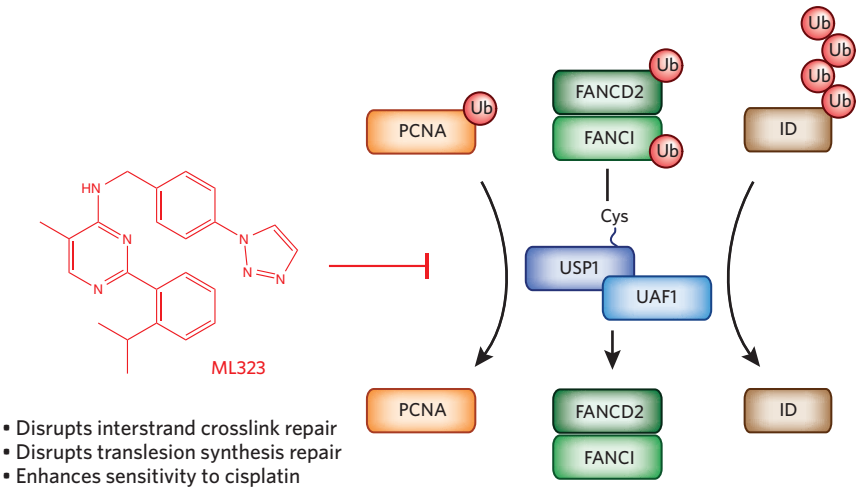

Figure 1 | ML323 is a specific inhibitor of the USP1–UAF1 deubiquitylating complex. Inhibition of USP1–UAF1 by ML323 increases the ubiquitination of FANCI, FANCD2, PCNA and inhibitors of DNA binding transcriptional repressors (ID), thus interfering with interstrand crosslink and translesion synthesis DNA repair. Consequently, treatment with ML323 sensitizes cancer cells to chemotherapeutic drugs, such as the crosslinking agent cisplatin, that overburden DNA repair pathways. Ub, ubiquitin.

of USP1. ML323 acts on USP1–UAF1 through an allosteric mechanism by binding a site distant from the DUB's catalytic center. This feature of ML323 mirrors recently discovered molecules that inhibited the ubiquitination enzymes such as CDC34 by an allosteric, rather than competitive, mechanism[10]. The ubiquitin pathway thus appears to offer rich opportunities for the development of allosteric modulators, which should improve the probability of isolating specific small molecules against enzymes of this class. Indeed, the specific activity of ML323 is an exciting discovery that might spur the isolation of allosteric inhibitors against other validated DUB targets, such as USP7 (also known as HAUSP).

With ML323 in hand, the authors could investigate the dynamics of ubiquitin-dependent signaling in DNA repair[2]. As expected from phenotypes of *USP1* deletion, acute inhibition of this DUB complex increased the appearance of monoubiquitinated FANCD2. Loss of USP1–UAF1 activity also interfered with the formation of FANCD2 foci, highlighting the requirement for reversible ubiquitination in this repair pathway. In a similar manner, ML323 increased the levels of monoubiquitinated PCNA, the persistence of which compromised the functionality of translesion synthesis repair. These data demonstrate that dynamic ubiquitination, as achieved by the USP1–UAF1 complex, is critical for translesion synthesis and interstrand crosslink DNA repair (**Fig. 1**).

The mechanistic studies were bolstered by survival experiments in which ML323 sensitized cancer cells to treatment with cisplatin, which was dependent on the presence of FANCD2, or to UV irradiation, which required the presence of translesion synthesis polymerases[2]. The effects of ML323 in these medically relevant settings were dependent on the presence of USP1–UAF1, underscoring the on-target effect of this drug. Inhibition of USP1–UAF1 could thus be exploited for chemotherapeutic strategies that limit the emergence of cancer cell populations resistant to cisplatin.

Together, the work of Liang *et al.*[2] reveals ML323 as a promising basis for the development of new hemotherapeutics. In addition, ML323 offers an exciting tool for basic research, as it opens up a path to probing the dynamics of ubiquitin-dependent signaling in DNA repair, enabling researchers to refine our understanding of one of the central pathways essential for eukaryotic life. ■

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Competing financial interests

The authors declare no competing financial interests.

DRUG DISCOVERY

Follow your lead

Probe molecules with systemic activity remain rare, and, as was realized in the pre-molecular era, even their off-target activity can illuminate biology. A study reporting a screen of over 600 kinase inhibitors found nine with bromodomain inhibitory activity and has implications for mechanism and compound optimization.

Brian K Shoichet

In antediluvian times, before molecular biology remade the world, drug discovery was dominated by a chemical view of biology. Receptors were rarely purified but were characterized by the small molecules that modulated them, and many still bear names conferred by chemical action (for example, the muscarinic and nicotinic receptors, the μ- and κ-opioid receptors). In the Stygian half-light of this chemical world, a lead molecule—by necessity active on a whole tissue or organism—was a crucial biological informant. Investigators such as Black, Janssen and Wermuth would elaborate such leads not only to optimize

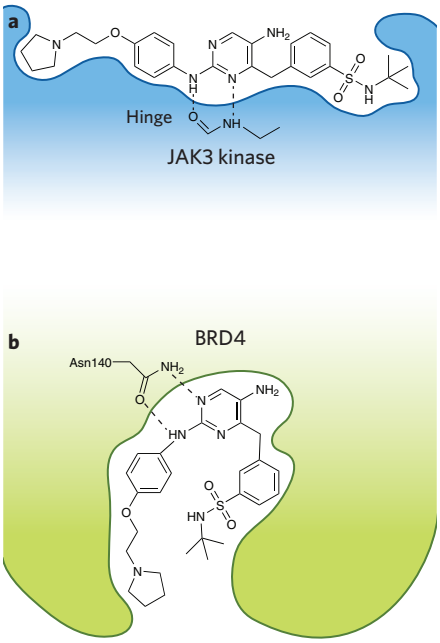

Figure 1 | Polypharmacology at atomic resolution. (**a**,**b**) The binding of federatinib to both JAK3 (**a**) and BRD4 (**b**) uses the same ligand warhead to hydrogen bond with a protein amide—in one from a main chain and in the other from an asparagine side chain—in active sites that otherwise differ.

specific activity but also to characterize other receptors, often by optimizing side activities[1,2]. And so the loop diuretics resemble sulfonamide antibiotics, cimetidine was derived from histamine analogs, and most anti-depressants seem only three degrees of separation from diphenhydramine (Benadryl). In this issue, Ciceri *et al.*[3] return to this classical approach, screening a panel of kinase drugs and leads for activity on a new family of epigenetic targets, the bromodomains.

Bromodomains recognize acetylated lysines, often on histones. Present on nuclear proteins, they help regulate the expression of proteins, including kinases, that are essential for tumor growth. Dysfunction of bromodomain-containing proteins has correspondingly been associated with cancer development. Recently, the BET family of proteins, such as BRD4, has been found to be druggable through binding to their bromodomains, leading to new chemical probes and to therapeutic leads[4]. BET and kinases represent two different pathways for tumor growth, and a compound that hits both might have complementary effects. Therefore, the authors screened 628 kinase inhibitors—including 200 drugs and investigational drugs—for affinity to the first bromodomain of BRD4. Nine were active in the initial screen and by calorimetry were shown to have affinities in the mid-nanomolar to low-micromolar range.

There is no obvious target-based reason for why kinase inhibitors should bind bromodomains, as the two families share little sequence or structural similarity. To investigate recognition at atomic resolution, the structures of BRD4 in complex with the kinase inhibitors BI-2536 (an analog of volasertib) and TG-101348 (federatinib) were determined by crystallography. Intriguingly, the key adenine mimetic warhead in each inhibitor forms hydrogen bonds with amides in both kinases and the bromodomain. For instance, in JAK3 kinase, federatinib is thought to hydrogen bond to the key recognition main chain amide through an amino-pyrimidine donor-acceptor pair. In BRD4, the same group hydrogen bonds with the amide of Asn140. Though the shape of the two active sites differ, and in other regions different inhibitor-protein interactions are made, for this key pharmacophore the two proteins are related (**Fig. 1**). From these crystal structures, it should be possible to design analogs that optimize either kinase-bromodomain polypharmacology or specificity for each family.

In the teeth of the dissimilarity of the targets, this screen for bromodomain binding by kinase inhibitors was remarkably effective. Was this a one-off, or is there a general logic to such polypharmacology? Undoubtedly, part of the activity observed here is explained by the promiscuity of individual molecules. Federatinib, in particular, is unusually dirty even for a kinase inhibitor. More broadly, however, ligands and proteins use a relatively small repertoire of recognition elements, and entirely different scaffolds can bind exactly the same active site residues in a protein, whereas the same drug can bind two unrelated proteins using shared functional groups[5], as seen here. In retrospect, polypharmacology may be explained by detailed structural and biophysical analysis, even if it may not have been anticipated prospectively. The biophysical justification for the recognition of identical molecules by unrelated proteins supports extension of the strategy used by Ciceli and colleagues to other target and ligand classes, even if it cannot currently be predicted.

This brings us back to the pharmacology of the pre-molecular period, where investigators practiced the same strategy though they were fifty years and several Nobel prizes away from examining its biophysical basis. Indeed, researchers then could not be sure that the theoretical constructs that they called 'receptors' were even single proteins—some, like the σ-opioid receptor, turned out to be figments of the ligand-based ontology. But when these drugs were inspired by endogenous metabolites and signaling molecules, which themselves are chemically related, it was perhaps a small step to believe that they could be optimized for activity on multiple receptors. Recent chemoinformatics analysis suggests that thousands of unrelated targets share highly related ligands, with many of the paired receptors sharing canonical signaling molecules[6]. Said another way, many pairs of sequence unrelated targets—ion channels and GPCRs, GPCRs and nuclear hormone receptors, nuclear receptors and transporters—are receptors for exactly the same signaling molecules. Seen this way, drug polypharmacology is not a bug but a feature.

The work of Ciceli reminds us that chemical probes and drugs can lead us to new biology, unanticipated by target similarity but sometimes recapitulating shared pharmacology. Molecules active on whole systems remain rare, as they were in the pre-molecular era. Their ability to penetrate and act on whole systems makes them remarkable informants, if asked the right question. So follow your lead. ■

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