

# Induced proximity-based therapeutic modalities

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## Abstract

Proximity is a key component of nearly all regulatory pathways within biological systems. Over the past few decades, the rapid development of induced proximity modalities has allowed for therapeutic intervention beyond classical occupancy-driven pharmacology. These modalities comprise multispecific small molecules or biologic agents that co-opt native biological pathways by inducing an interaction between biomolecules. Small-molecule ‘molecular glues’ modify protein surfaces to induce non-native interactions or to stabilize existing protein–protein interactions. They have been in the clinic since the 1980s but have more recently been shown to enable targeted protein degradation or inhibition and have been rationally designed to achieve this. Early discoveries on molecular glues spearheaded the development of next-generation heterobifunctional modalities for targeted protein degradation, such as proteolysis-targeting chimeras, which are seeing early-stage clinical success. Here, we aim to survey the field of induced proximity with a focus on potential therapeutic applications. We discuss the emergence of novel approaches to control cellular processes beyond protein degradation, including post-translational modifications, cellular localization and transcriptional activation. Some of these approaches are showing preclinical efficacy in various disease models.

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## Introduction

Traditionally, small-molecule drug discovery has been dominated by occupancy-driven pharmacology, in which inhibitors or agonists bind directly to a target of interest in a well-defined pocket. Although this approach has yielded many therapies, most of the proteome cannot be therapeutically accessed because an estimated 80% of proteins lack a chemical ligand<sup>1</sup>. Despite recent advances in expanding the ligandable proteome, including annotation of fragment ligands for over 2,000 proteins, estimates suggest that only approximately 10% of ligandable proteins (3% of the total proteome) have been drugged by approved, relevant therapies<sup>1,2</sup>. ‘Undruggable’ protein targets can either lack a well-defined ligand-binding pocket or contain ligand-binding sites that do not lie at a protein interface, active site or other regulatory site, so that ligand discovery alone is insufficient for therapeutic intervention. A powerful alternative to occupancy-driven pharmacology has emerged. ‘Event’-driven pharmacology bypasses the need for a functional inhibitor or active-site binder because an event triggers the modulation of protein function or levels. The event is facilitated by induced proximity, in which a small-molecule or biological agent (biologic) induces complexation of two macromolecules, resulting in co-optation of their native pathways and functional modulation of the target. By bringing together unique macromolecular combinations, these agents have the potential to access the large swath of the undruggable proteome to generate next-generation disease therapies.

Induced proximity modalities can be divided into two main categories, molecular glues and bifunctionals, and they can encompass both small molecules and biologics (Fig. 1). Molecular glues are monovalent molecules that remodel protein surfaces to increase the affinity of two macromolecules, such as two proteins, and result in functional modulation of the target depending on the recruited macromolecule. Most commonly, ‘glueing’ of an E3 ubiquitin ligase prompts ubiquitination and degradation of a non-substrate target protein (or ‘neosubstrate’) through the proteasome. Alternatively, the formation of a non-native ternary complex can lead to inhibition or activation of a target protein or the disruption or enhancement of native protein–protein interactions. Heterobifunctional molecules consist of two separate protein-targeting entities, each of which has a high affinity for its respective protein target, linked together by either a chemical linker or direct conjugation. These larger drugs, exemplified by proteolysis-targeting chimeras (PROTACs), have been shown to induce degradation, stabilization, post-translational modification,

localization, inhibition and transcriptional activation (Fig. 1). Historically, glues and bifunctionals have also been differentiated based on size and the presence of a linker. Still, it has recently become clear that these compounds exist on a continuum and might be better differentiated by their interaction at the protein interface and the resulting cooperativity of the induced ternary complex.

Induced proximity has been clinically successful for decades – approved drugs such as thalidomide, cyclosporin A (CsA) and anagrelide have been retrospectively characterized as molecular glues. However, prospective molecular glue and PROTAC degrader discovery has expanded rapidly in the past 10 years. Over 60 monovalent and bivalent drugs, primarily consisting of protein degraders and some protein inhibitors, are currently in clinical trials against a wide range of indications in oncology, inflammatory disease and neurodegenerative disease.

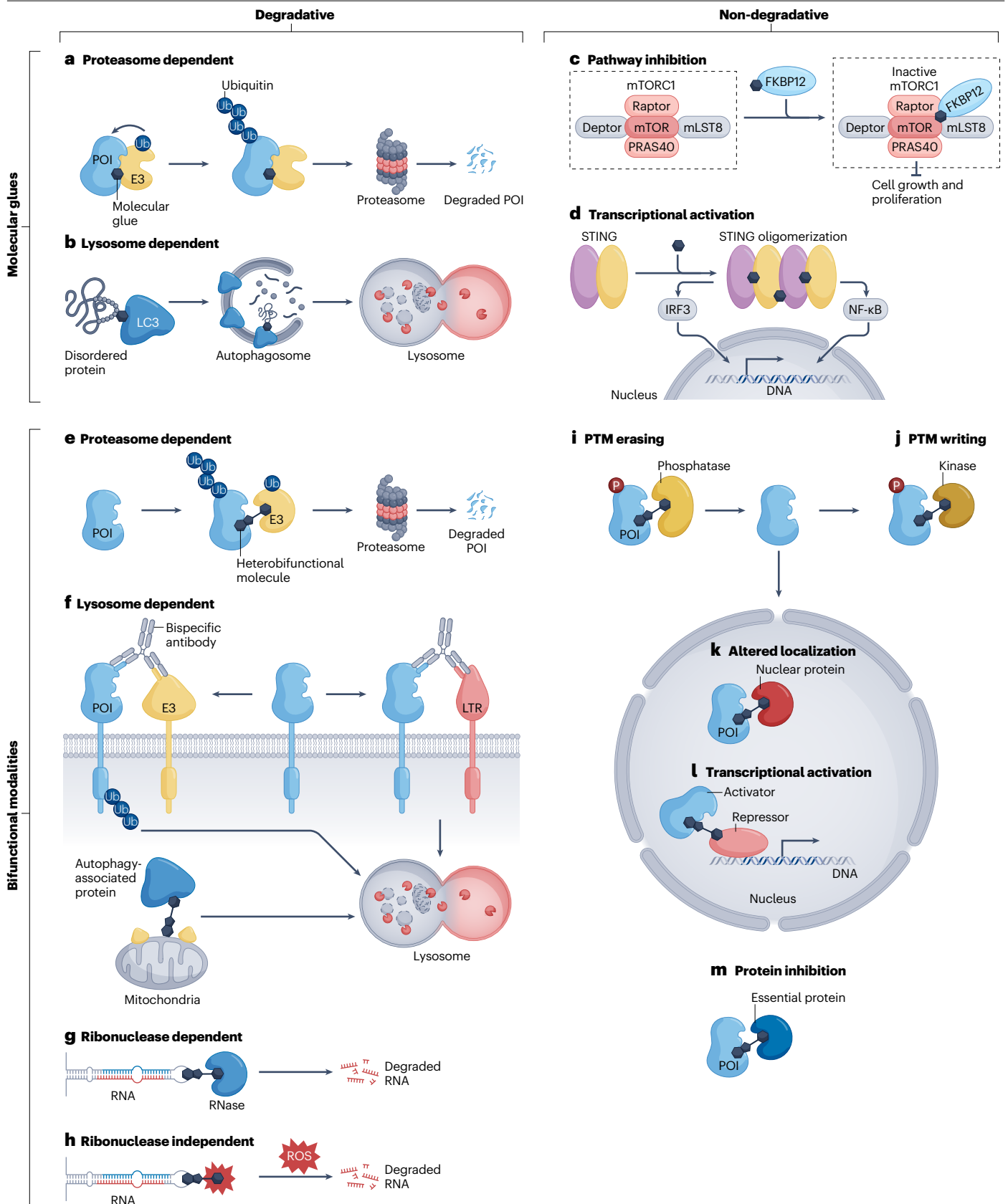
In this Review, we will give an overview of seminal discoveries in the field of induced proximity pharmacology and describe monovalent and bivalent compounds as well as the most recent developments in both degradative and non-degradative modalities. We will describe key preclinical and clinical findings, and highlight the ability of induced proximity drugs to open new avenues for accessing previously undruggable targets. Although our focus will primarily be on small molecules, we will touch on some biologic modalities, including antibody–degrader conjugates and some bispecific antibodies, which are otherwise reviewed in detail elsewhere<sup>3,4</sup>.

## Molecular glues

The term ‘molecular glue’ was first defined in the 1980s as a body that brings two or more proteins together, although the earliest uses of the term referred to proteins that were necessary for other proteins to interact<sup>5,6</sup>. It was not until 1992 that the term was used to define a small molecule that induces a new binding interaction between proteins<sup>7</sup>. The term has further been associated with small molecules that can be leveraged to influence cellular pathways for therapeutic applications; however, molecular glues are fundamental to natural processes. The discovery of natural molecular glues such as auxin – a plant hormone that induces binding of TIR1, a substrate receptor for the SKP1–Cullin–F-box ubiquitin ligase complex, to its target proteins – was foundational to the initial understanding of small-molecule modulation of protein abundance<sup>8</sup>. Several molecular glues are already approved for use in the clinic or are in clinical trials, including degraders, inhibitors and stabilizers (Table 1 and Fig. 2).

**Fig. 1 | Summary of induced proximity modalities. Degradative molecular glues:** **a**, A molecular glue that recruits a protein of interest (POI) to an E3 ligase results in ubiquitination (Ub) and proteasomal degradation of the target POI. **b**, A glue between a disordered protein aggregate and the LC3 autophagosome protein prompts autophagosome formation and lysosomal degradation of the disordered protein. **Non-degradative molecular glues:** **c**, A glue such as the rapalog everolimus promotes an interaction between the mTORC1 component mammalian target of rapamycin (mTOR) and the immunophilin FKBP12, resulting in inactivation of mTORC1 and inhibition of cell proliferation. **d**, A glue prompts oligomerization of the innate immunity regulator stimulator of interferon genes (STING), resulting in activation of IRF3 and NF- $\kappa$ B-dependent transcription. **Degradative bifunctional modalities:** **e**, Heterobifunctional molecules can induce proximity between a POI and an E3 ligase to promote proteasomal degradation. **f**, Bispecific antibodies can induce binding of a POI to either a membrane E3 ligase (left) or a lysosomal-trafficking receptor (LTR; right) to induce endosomal uptake and Ub-mediated lysosomal degradation of the

POI. Heterobifunctional molecules targeting an autophagy-associated protein and mitochondria induce mitochondrial degradation through autophagosome formation. **g**, RNA ligands appended to a ligand that recruits RNase prompt RNA degradation. **h**, RNA ligands appended to a reactive moiety generating localized reactive oxygen species (ROS) induce RNA degradation. **Non-degradative bifunctional modalities:** **i**, Bifunctional molecules can prompt the erasure of post-translational modifications (PTMs), including the removal of phosphorylation by recruiting a phosphatase. **j**, Bifunctional molecules can induce PTM writing, including phosphorylation through recruitment of kinases. **k**, Subcellular localization can be altered with bifunctional molecules that link a cytoplasmic POI to a nuclear-resident protein and sequester the POI in the nucleus. **l**, Heterobifunctional molecules can recruit a transcriptional activator to a transcriptional repressor, leading to activation of transcription. **m**, Small-molecule-induced binding between an essential protein and POI can inhibit or sequester the essential protein, resulting in apoptosis.



**Table 1 | Summary of induced proximity drugs in the clinic and in clinical trials**

Compound	Protein target	Binding partner	Mechanism	Company	Indications	Clinical Stage
<b>Molecular glues</b>						
Cyclosporin A <sup>a</sup>	Calcineurin	Cyclophilin	Inhibition	Sandoz/Novartis	Prophylaxis of organ transplant rejection, RA, PsO	Approved (1983)
Voclosporin <sup>a</sup>	Calcineurin	Cyclophilin	Inhibition	Aurinia Pharmaceuticals	Lupus nephritis	Approved (2021)
FK506 <sup>a</sup>	Calcineurin	FKBP12	Inhibition	Fujisawa Pharmaceutical	Prophylaxis of organ transplant rejection	Approved (1994)
Rapamycin <sup>a</sup>	mTOR	FKBP12	Inhibition	Pfizer	Prophylaxis of renal transplant rejection, LAM	Approved (1999)
Temsirolimus <sup>a</sup>	mTOR	FKBP12	Inhibition	Wyeth Pharmaceuticals	aRCC	Approved (2007)
Everolimus <sup>a</sup>	mTOR	FKBP12	Inhibition	Novartis	Breast cancer, RCC, neuroendocrine tumours, RAM, SEGA, partial-onset seizures, organ transplant rejection	Approved (2016)
Paclitaxel <sup>a</sup>	α-Tubulin	β-Tubulin	Stabilization	BMS	Ovarian cancer, breast cancer, NSCLC, Kaposi's sarcoma	Approved (1993)
Docetaxel <sup>a</sup>	α-Tubulin	β-Tubulin	Stabilization	Sanofi-Aventis	Breast cancer, NSCLC, gastric cancer, head and neck cancer, prostate cancer, renal impairment	Approved (2004)
Trametinib <sup>a</sup>	KSR	MEK1/MEK2	Inhibition	Novartis	NSCLC, thyroid cancer, BRAF V600E-mutant solid tumours	Approved (2013)
Tafamidis <sup>a</sup>	Transthyretin	Transthyretin	Stabilization	Pfizer	ATTR-CM	Approved (2019)
Lenacapavir <sup>a</sup>	P24 capsid monomers	P24 capsid monomers	Inhibition	Gilead	HIV/AIDS	Approved (2022)
Thalidomide <sup>a</sup>	IKZF1/3; SALL4	CRBN	Degradation	Chemie Grunenthal	ENL	Approved (1998)
Lenalidomide <sup>a</sup>	IKZF1/3; CK1α	CRBN	Degradation	BMS/Celgene	MM, MCL, follicular lymphoma	Approved (2006)
Pomalidomide <sup>a</sup>	IKZF1/3	CRBN	Degradation	BMS/Celgene	MM, Kaposi's sarcoma	Approved (2013)
Mezigdomide <sup>a</sup> (CC-92480)	IKZF1/3; ZFP91-99	CRBN	Degradation	BMS/Celgene	RRMM, MM, renal impairment	Phase III
Iberdomide <sup>a</sup> (CC-220)	IKZF1/3	CRBN	Degradation	BMS/Celgene	MM, RRMM, PCM, lymphoma	Phase III
Golcadomide <sup>a</sup> (CC-99282)	IKZF1/3	CRBN	Degradation	BMS/Celgene	NHL, T cell and B cell lymphomas	Phase III
KPG-818	IKZF1/3	CRBN	Degradation	Kangpu	SLE, MM, lymphoma	Phase II
KPG-121	IKZF1/3; CK1α	CRBN	Degradation	Kangpu	mCRPC	Phase II
Avadomide <sup>a</sup> (CC-122)	IKZF1/3	CRBN	Degradation	BMS/Celgene	NHL, melanoma	Phase I-II
Cemsidomide <sup>a</sup> (CFT-7455)	IKZF1/3	CRBN	Degradation	C4 Therapeutics	MM, NHL	Phase I-II
BTX-1188	IKZF1/3, GSPT1	CRBN	Degradation	BioTheryX	Haematological and solid malignancies	Phase I
ICP-490	IKZF1/3	CRBN	Degradation	InnoCare Pharma Limited	MM, DLBCL	Phase I
GT-919	IKZF1/3	CRBN	Degradation	Gluetacs	RRMM	Phase I
GLB-002	IKZF1/3	NA	Degradation	Glubio	MM, NHL	Phase I
GLB-001	CK1α	NA	Degradation	Glubio	AML, MDS	Phase I
BMS-986397 <sup>a</sup>	CK1α	CRBN	Degradation	BMS	AML	Phase I
NVP-DKY709 <sup>a</sup>	IKZF2	CRBN	Degradation	Novartis Pharmaceuticals	Solid tumours	Phase I (discontinued)
PLX-4545 <sup>a</sup>	IKZF2	CRBN	Degradation	Plexium	Solid tumours	Phase I
Helios CELMoD	IKZF2	CRBN	Degradation	BMS	Solid tumours	Phase I
RMC-6236 <sup>a</sup>	Pan-RAS(ON)	Cyclophilin	Inhibition	Revolution Medicines	NSCLC, PDAC	Phase III

**Table 1 (continued) | Summary of induced proximity drugs in the clinic and in clinical trials**

Compound	Protein target	Binding partner	Mechanism	Company	Indications	Clinical Stage
<b>Molecular glues (continued)</b>						
RMC-6291 <sup>a</sup>	KRAS G12C(ON)	Cyclophilin	Inhibition	Revolution Medicines	Solid tumours	Phase I–II
RMC-9805 <sup>a</sup>	KRAS G12D(ON)	Cyclophilin	Inhibition	Revolution Medicines	KRAS G12D-mutant solid tumours	Phase I
E7820 <sup>a</sup>	RBM39	DCAF15	Degradation	Eisai	AML, MDS, CMML	Phase II
MRT-2359 <sup>a</sup>	GSPT1	CRBN	Degradation	Monte Rosa Therapeutics	NSCLC, SCLC, MYC-driven malignancies	Phase I–II
CC-90009 <sup>a</sup>	GSPT1	CRBN	Degradation	BMS/Celgene	AML, MDS	Phase I (terminated)
Anagrelide <sup>a</sup>	PDE3A	SLFN12	Stabilization	Takeda Pharmaceuticals	Thrombocytopenia, PV, hepatic impairment	Approved (1997)
BAY-2666605 <sup>a</sup>	PDE3A	SLFN12	Degradation	Bayer	Melanoma	Phase I (terminated)
NST-628 <sup>a</sup>	Pan-RAF	MEK1	Inhibition	Nested Therapeutics	RAS-driven and RAF-driven tumours	Phase I
MRT-6160	VAV1	CRBN	Degradation	Monte Rosa Therapeutics	Systemic and neurological autoimmune diseases	Phase I
BMS-986470	ZBTB7A, WIZ	CRBN	Degradation	BMS	Sickle cell disease	Phase I
ATI-450 <sup>a</sup>	MK2	p38α	Inhibition	Aclaris Therapeutics	HS, PsA, RA	Phase II (discontinued)
VVD-130037 <sup>a</sup>	KEAP1	CUL3	Degradation/activation	Vividion Therapeutics	Solid tumours	Phase I
<b>Proteolysis-targeting chimeras</b>						
ARV-471 <sup>b</sup>	ER	CRBN	Degradation	Arvinas/Pfizer	Breast cancer	Phase III
AC0699	ER	NA	Degradation	Accutar Biotech	Breast cancer	Phase I
AC0682	ER	CRBN	Degradation	Accutar Biotech	Breast cancer	Phase I
BMS-986365	AR	CRBN	Degradation	BMS	Prostate cancer	Phase III
ARV-766 <sup>b</sup>	AR, AR-V7	CRBN	Degradation	Arvinas/Novartis Pharmaceuticals	Prostate cancer	Phase II
ARV-110 <sup>b</sup>	AR	CRBN	Degradation	Arvinas	Prostate cancer	Phase II (discontinued)
GT20029	AR	NA	Degradation	Kintor	Androgenetic alopecia, acne vulgaris	Phase II
HP518	AR	NA	Degradation	Hinova	mCRPC, TNBC	Phase I
AC0176	AR	NA	Degradation	Accutar Biotech	Prostate cancer	Phase I
KT-474 <sup>b</sup>	IRAK4	CRBN	Degradation	Kymera/Sanofi	HS, inflammatory disease	Phase II
BGB-45035	IRAK4	CRBN	Degradation	BeiGene	Immunology and inflammation	Phase I
KT-413	IRAK4, IKZF1/3	CRBN	Degradation	Kymera	DLBCL, NHL	Phase I (discontinued)
BGB-16673	BTK	NA	Degradation	BeiGene	B cell malignancies	Phase I–II
NX-5948 <sup>b</sup>	BTK	CRBN	Degradation	Nurix	B cell malignancies, autoimmune disease	Phase I
HSK29116	BTK	NA	Degradation	Haisco	B cell malignancies	Phase I
AC0676	BTK	NA	Degradation	Accutar Biotech	Haematological cancers	Phase I
NX-2127 <sup>b</sup>	BTK, IKZF1/3	CRBN	Degradation	Nurix	B cell malignancies	Phase I
KT-253	MDM2	NA	Degradation	Kymera	ALL, AML, solid tumours	Phase I (discontinued)
KT-333	STAT3	NA	Degradation	Kymera	Liquid and solid tumours, T cell lymphomas	Phase I (discontinued)
KT-621	STAT6	NA	Degradation	Kymera	Allergic and atopic diseases	Phase I
RNK05047	BRD4	HSP90	Degradation	Ranok	Advanced solid tumours, DLBCL	Phase I
CFT-8634 <sup>b</sup>	BRD9	CRBN	Degradation	C4 Therapeutics	Synovial sarcoma, SMARCB1-null solid tumours	Phase I (discontinued)
FHD-609 <sup>b</sup>	BRD9	CRBN	Degradation	Foghorn	Synovial sarcoma, SMARCB1-null solid tumours	Phase I (discontinued)

**Table 1 (continued) | Summary of induced proximity drugs in the clinic and in clinical trials**

Compound	Protein target	Binding partner	Mechanism	Company	Indications	Clinical Stage
<b>Proteolysis-targeting chimeras (continued)</b>						
CFT-1946 <sup>b</sup>	BRAF V600E	NA	Degradation	C4 Therapeutics	NSCLC, melanoma	Phase I
ARV-102	LRRK2	NA	Degradation	Arvinas	PD, PSP	Phase I
DT-2216 <sup>b</sup>	BCL-XL	VHL	Degradation	Dialectic	T cell lymphomas	Phase I
ARV-393	BCL6	NA	Degradation	Arvinas	B cell malignancies	Phase I
BMS-986458	BCL6	CRBN	Degradation	BMS	B cell malignancies	Phase I
CG001419	Pan-TRK	CRBN	Degradation	Cullgen	Solid tumours	Phase I-II
ASP3082	KRAS G12D	NA	Degradation	Astellas	KRAS G12D-mutant cancers	Phase I
ASP4396	KRAS G12D	NA	Degradation	Astellas	KRAS G12D-mutant cancers	Phase I
HSK-40118	EGFR	NA	Degradation	Haisco	NSCLC	Phase I
CFT-8919	EGFR L858R	NA	Degradation	C4 Therapeutics/Betta Pharmaceuticals	EGFR-mutant cancers	Phase I
<b>Other induced proximity therapies</b>						
ORM-5029	HER2, GSPT1	CRBN	Antibody-degrader conjugate	Orum	Breast cancer	Phase I
BMS-986497	CD33, GSPT1	CRBN	Antibody-degrader conjugate	BMS/Orum	AML	Phase I
BHV-1300	IgG	ASPGR	Degradation	Biohaven	Graves' disease, RA	Phase I
BHV-1400	Gd-IgA1	ASPGR	Degradation	Biohaven	IgA nephropathy	Phase I
BHV-1600	β1AR auto-antibodies	ASPGR	Degradation	Biohaven	Peripartum cardiomyopathy	Phase I

Information is from ClinicalTrials.gov and company press releases. ALL, acute lymphocytic lymphoma; AML, acute myeloid leukaemia; AR, androgen receptor; aRCC, advanced renal cell carcinoma; ATTR-CM, transthyretin amyloidosis cardiomyopathy; CMML, chronic myelomonocytic leukaemia; CRBN, cereblon; DLBCL, diffuse large B cell lymphoma; ENL, erythema nodosum leprosum; ER, oestrogen receptor; HS, hidradenitis suppurativa; LAM, lymphangioliomyomatosis; MCL, mantle-cell lymphoma; mCRPC, metastatic castration-resistant prostate cancer; MDS, myelodysplastic syndromes; MM, multiple myeloma; mTOR, mammalian target of rapamycin; NA, undisclosed effector or binding partner; NHL, non-Hodgkin's lymphoma; NSCLC, non-small-cell lung cancer; PCM, plasma cell myeloma; PD, Parkinson's disease; PDAC, pancreatic ductal adenocarcinoma; PsA, psoriatic arthritis; PsO, psoriasis; PSP, progressive supranuclear palsy; PV, polycythemia vera; RA, rheumatoid arthritis; RAM, renal angiomyolipoma; RCC, renal cell carcinoma; RRMM, relapsed/refractory multiple myeloma; SCLC, small cell lung cancer; SEGA, subependymal giant cell astrocytoma; SLE, systemic lupus erythematosus; TNBC, triple-negative breast cancer. <sup>a</sup>Chemical structure shown in Fig. 2. <sup>b</sup>Chemical structure shown in Fig. 3.

## Natural product molecular glues

Since the clinical approval of CsA in the 1980s<sup>9</sup>, 12 other molecular glues have been approved (Table 1). These drugs have two main mechanisms of action: inhibition or degradation of the target protein. The early therapeutic glues CsA and FK506 have immunosuppressive properties and bind immunophilins but were clinically approved well before their complete mechanistic elucidation<sup>10–14</sup>. In 1991, Schreiber and Crabtree discovered that both CsA and FK506 bind to the same secondary binding partner, the protein phosphatase calcineurin, and sequester it from native phosphorylation pathways, causing immunosuppressive effects<sup>7,15</sup>.

The discovery of this mechanism kicked off the hunt for more natural product molecular glues. One such drug, rapamycin, functions by inducing a ternary complex between the immunophilin FKBP12 and the mammalian target of rapamycin (mTOR), which regulates pathways responsible for age progression, immune response and autophagy<sup>12,14,16–28</sup>. Rapamycin inhibits mTOR function and is a promising therapeutic candidate to address mTOR-associated diseases<sup>16,21</sup>. It was clinically approved in 1999 as an anti-kidney rejection treatment and has been approved for three other indications. A series of synthetic analogues, named rapalogs (Fig. 2), improved the structure

of rapamycin to make it more stable and better tolerated<sup>18</sup>. The rapalogs show different selectivity, with everolimus selectively inhibiting the mTORC1 pathway<sup>17</sup> (Fig. 1c). Additional synthetic expansion of rapamycin-like macrocycles also produced rapadocin, which does not bind mTOR or calcineurin but instead inhibits the equilibrative nucleoside transporter ENT1 (refs. 29,30).

Many of the foundational natural product molecular glues are inhibitors, but the natural product paclitaxel (taxol; Fig. 2), discovered in 1971 and approved in 1992, is a molecular glue protein–protein stabilizer<sup>31,32</sup>. Paclitaxel inhibits cell division by binding between β-tubulin and α-tubulin to stabilize microtubules and induce apoptosis<sup>31</sup>. The natural products epothilone and discodermolide and the synthetic molecule synstabilin A are mechanistically similar but overcome paclitaxel resistance and increase efficacy, respectively<sup>33–35</sup>. The fungal natural product fusicoccin A stabilizes protein–protein interactions of the scaffold protein 14-3-3 with several partners, most notably with the proton pump H<sup>+</sup>-ATPase, which leads to its increased activation<sup>36,37</sup>. Additionally, the polyketides asukamycin and manumycin A appear to activate the tumour suppressor p53 through multi-covalent interactions with the E3 ligase UBR7 and p53 itself, leading to antiproliferative effects<sup>38</sup>.

## Synthetic non-degradative molecular glues

Although synthetic molecular glues that function as degraders have received much attention recently, glues can also function through inhibition or stabilization mechanisms. Anagrelide (Fig. 2), a small-molecule inhibitor of blood platelet aggregation, was clinically approved in 1997 for patients with platelet diseases such as thrombocytosis<sup>39</sup>. Anagrelide stabilizes the natural interaction between the cAMP phosphodiesterase PDE3A and the RNase SLFN12, facilitating both inhibition of PDE3A and stabilization of SLFN12 (refs. 40,41). Anagrelide belongs to a family of small molecules termed velcrins, which include the mechanistically identical compound BAY266605. It advanced to clinical trials but was terminated due to the lack of a therapeutic window<sup>42</sup>. Trametinib (GSK112012) was clinically approved for cancer indications and stabilizes interactions of MEK1 and MEK2 kinases with the kinase suppressor of RAS (KSR)<sup>43,44</sup>. Optimization of trametinib yielded trametigluce, which exhibited more potent binding to KSR, MEK1 and MEK2 (ref. 45). Other examples include RMC-6236, which inhibits RAS by recruiting it to cyclophilin A (CypA); CC-0651, which inhibits the E2 enzyme UBE2R1 by stabilizing its interaction with ubiquitin; and Compound 24, which prevents parasite migration by stabilizing binding of the glidesome protein TRAP to the *Plasmodium falciparum* aldolase enzyme<sup>46–48</sup>. The discoveries with fusicoccin A described above have prompted ongoing interest in stabilizing interactions of 14-3-3 with its disease-implicated binding partners, and 14-3-3 interactions with PMA2, NF-κB, ERRγ, YAP and TAZ have all been stabilized to varying degrees of selectivity with a mix of covalent and non-covalent approaches<sup>49–52</sup>. In a screen for small-molecule activators of the stimulator of interferon genes (STING), a compound was found to act as a molecular glue by binding at the interface between STING dimers and by promoting STING oligomerization and downstream signalling<sup>53</sup> (Fig. 1d).

## Synthetic molecular glue degraders

The discoveries of the first synthetic molecular glue degraders were serendipitous. Thalidomide, a glutamic acid derivative developed in 1953 as a sleep aid and anti-nausea medicine for morning sickness, was found to cause congenital malformations<sup>54,55</sup>. The drug was pulled from the market; however, further investigation found that it has anti-inflammatory effects and it was reappraised for patients with erythema nodosum leprosum<sup>54,56</sup>. Two analogues of thalidomide (termed immunomodulatory drugs (IMiDs)), lenalidomide and pomalidomide (Fig. 2), were synthesized and clinically approved for the treatment of multiple myeloma<sup>57</sup>. Mechanistic experimentation uncovered that thalidomide directly binds to cereblon (CRBN), the E3 ligase substrate receptor of CRL4, and induces a neo-interaction between CRBN and members of the Ikaros protein family, Ikaros and Aiolos<sup>58–60</sup>. This interaction promotes the ubiquitination and degradation of Ikaros and Aiolos and drives tumour suppressive effects. The transcription factor SALL4 was found to be an additional neosubstrate of thalidomide-bound CRBN and to cause the observed teratogenic effects<sup>61,62</sup>.

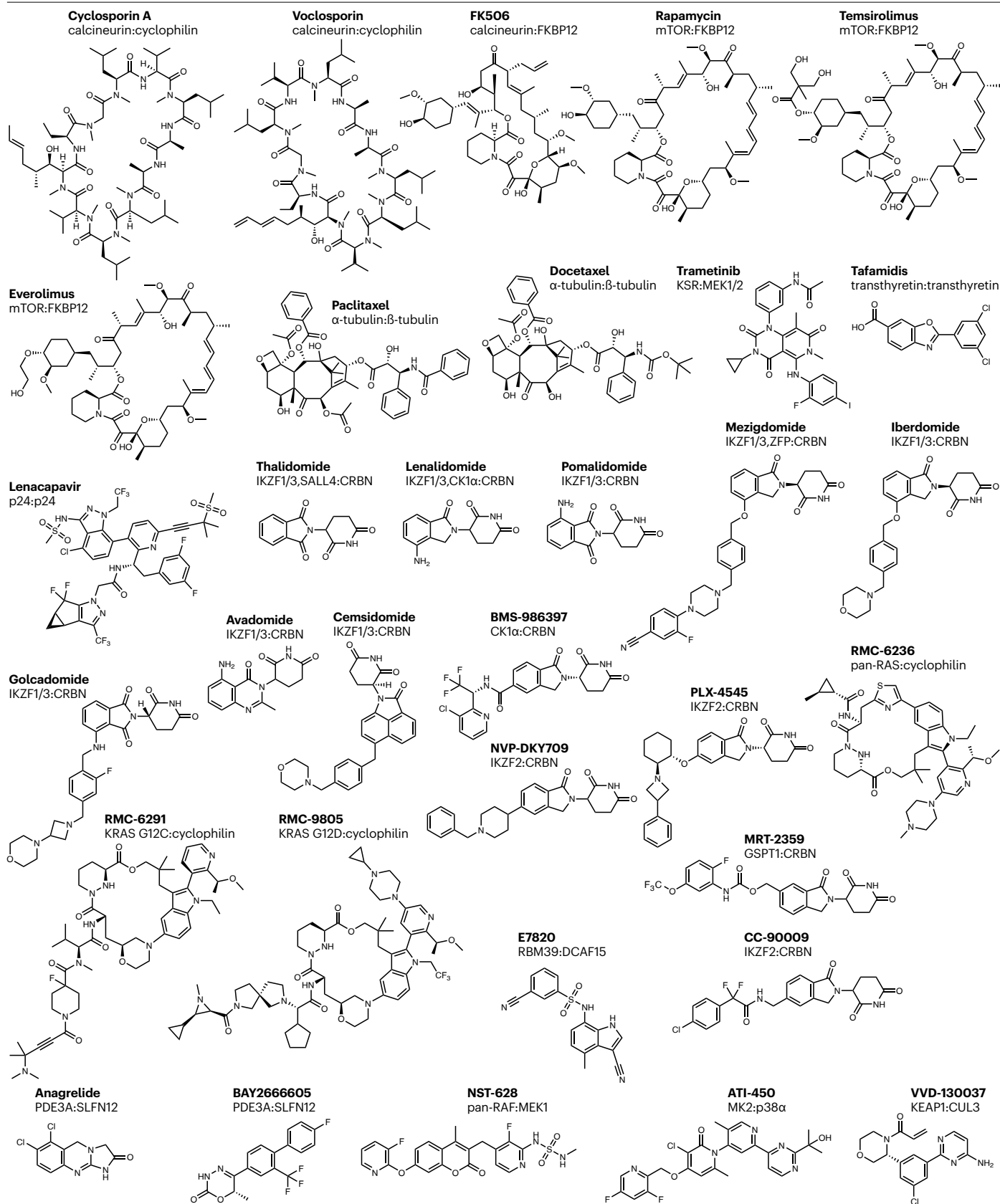
Seminal crystal structures, elucidated by Chamberlain et al.<sup>63</sup> and Fischer et al.<sup>64</sup>, of CRBN and its adaptor DDB1 bound to thalidomide, lenalidomide and pomalidomide established the glutarimide moiety as the main pharmacophore. A conserved binding mode was observed across all three drugs, with binding occurring in a shallow hydrophobic pocket in CRBN containing three essential tryptophan residues: Trp382, Trp388 and Trp400. Structural changes to the phthalimide moiety of the IMiDs affected both the range of neosubstrates degraded and the potency of degradation<sup>65</sup>. The absence of a carbonyl at the

C3 position on the phthalimide of lenalidomide resulted in pronounced degradation of the casein kinase isoform CK1α, which was not seen with thalidomide or pomalidomide, due to elimination of a steric clash with the CRBN backbone and formation of a cooperative ternary complex involving the C-terminal domain of CRBN and a β-hairpin loop containing a critical glycine residue in CK1α<sup>66</sup>. Similarly, ternary complex formation between CRBN, the antiproliferative IMiD derivative CC-885 and neosubstrate GSPT1 is mediated by hydrogen bonds with the CRBN backbone and a critical glycine within a β-hairpin loop on GSPT1 (ref. 67). These hairpin loops, despite having significant sequence divergence, distinctly resemble the structural conformation of the Cys2-His2 (C2H2) zinc finger domains present in Ikaros, Aiolos and ZFP91 that mediate binding to thalidomide–CRBN<sup>66–68</sup>. Subsequently, investigation into the ‘degrome’ of C2H2 zinc finger-containing proteins degraded by IMiD analogues bound to CRBN resulted in the discovery of 11 distinct zinc finger motifs, or ‘degrons’, that lacked conserved sequences<sup>69</sup>. Modifications to a thalidomide analogue altered the C2H2 zinc finger-containing degrome, permitting therapeutic modulation of select sets of these transcription factors<sup>69,70</sup>.

A new generation of IMiDs, including the clinical candidates iberdomide and mezigdomide (Fig. 2), exhibit heightened degradative, antitumour and immunostimulatory activity and contain novel chemical moieties off the phthalimide C4 position<sup>71</sup>. These bulkier substituents enable CRBN to transition from an open, inactive conformer to a closed, active conformer, likely by stabilizing interactions within the thalidomide-binding domain and the LON domain more effectively than the first-generation drug pomalidomide<sup>72</sup>. Given that Ikaros binding and subsequent ubiquitination could only occur with the closed conformer, this groundbreaking study revealed the importance of allosteric effects for rational design of IMiDs and provides the tools to develop more potent and selective degraders<sup>72</sup>. The next-generation clinical candidate avadomide (Fig. 2) contains a substituted oxoquinazoline core in place of the phthalimide and achieves more potent and faster degradation than lenalidomide in preclinical cancer models<sup>73,74</sup>. Pomalidomide analogues have also been synthesized with modifications at the C5 position of the phthalimide ring that lack or mask H-bond donation and show reduced off-target zinc finger protein degradation when used in bifunctional molecules<sup>75</sup>. Clinical candidate DKY709 contains a benzylpiperidinyl substituent at the C5 position and achieves selective degradation of Helios<sup>76</sup>. The thalidomide derivatives dWIZ-1 and dWIZ-2 were developed to degrade the transcription factor WIZ, which represses fetal haemoglobin, for sickle cell disease but they have not progressed to clinical trials<sup>77</sup>. These advances, enabled by structural elucidation and an understanding of the structure–activity relationship, have led to a new generation of more potent clinical candidates and expansion of CRBN neosubstrates.

Although the IMiDs are the most widely derivatized family of synthetic molecular glues, other small-molecule drugs have degradative glue mechanisms and can also utilize other E3 ligases to induce target degradation. The aryl sulfonamide indisulam induces degradation of the RNA-binding protein RBM39 through complexation to the E3 substrate receptor DCAF15, resulting in anticancer activity<sup>78–80</sup>. The structurally analogous aryl sulfonamides tasisulam, E7820 and chloroquinoline sulfonamide were also found to induce complexation of RBM39 and DCAF15, although only E7820 is in the clinic, where it is being tested in acute myeloid leukaemia and other myelodysplastic syndromes<sup>81</sup>.

Molecular glue degraders do not need to directly induce an interaction between the E3 effector and substrate to promote degradation.



**Fig. 2 | Molecular glues in or approaching the clinic.** Chemical structures of molecular glues that are clinically approved or in clinical trials, together with the names of the proteins that are recruited by each compound.

The molecular glue degrader VVD-065 (Vividion Therapeutics) has a unique allosteric mechanism of action targeting NRF2, a transcription factor that promotes defence against cellular stress but whose constitutive activation is common in lung and upper aerodigestive cancers<sup>82</sup>. The compound covalently engages Cys151 on the substrate adaptor KEAP1 and stabilizes the interaction between KEAP1 and the E3 ligase CUL3, culminating in increased NRF2 degradation. Phase I clinical trials evaluating a closely related glue VVD-130037 (Fig. 2) in NRF2-dependent solid tumours are ongoing (NCT05954312).

Similarly to early glues, the clinical candidate UM171, which is in phase II–III trials for haematological malignancies and functions by promoting the expansion of haematopoietic stem cells and degrading the transcriptional corepressor CoREST and the histone demethylase LSD1, progressed well into clinical candidacy before the mechanism was fully elucidated in a pair of recent papers. Structural studies revealed that UM171-mediated degradation of CoREST is facilitated by a unique asymmetric and cooperative binding of the CRL3 substrate receptor KBTBD4 with the histone deacetylase HDAC1, a component of the CoREST complex<sup>83</sup>. Inositol hexakisphosphate, an endogenous cofactor, acts as a second molecular glue at the interface and is necessary for quaternary complexation. Additionally, profiling of the mutational landscape of KBTBD4 identified hotspot mutations that insert a bulky side chain into the active site of HDAC1, resulting in aberrant degradation of CoREST and demonstrating convergence with the mechanism of UM171 (ref. 84). Altogether, these discoveries provide an elegant glue mechanism of action for UM171 that utilizes a distinct class of E3 ligases with a novel binding mode and provides a potential springboard for the rational design of degraders that mimic cancer mutations to drive desired interactions.

Several preclinical glue degraders have recently been discovered. Computational correlation of small-molecule cytotoxicity with E3 ligase expression found that the cyclin-dependent kinase (CDK) inhibitor CR8 induces an interaction between CDK12 and the DDB1 E3 ligase adaptor, resulting in degradation of the CDK12 binding partner cyclin K<sup>85,86</sup>. Contemporaneously, two studies found compounds HQ461 and dCeMM2–dCeMM4 with similar cyclin K degradative mechanisms<sup>87,88</sup>. The cyclin K degrader NCT02 was also found to act by the same mechanism<sup>89</sup>. Interestingly, compound dCeMM1 induced proximity between DCAF15 and neosubstrates RBM39 and RBM23 to facilitate their degradation in a similar manner to the aryl sulfonamides<sup>88</sup>. Other examples of preclinical glue degraders include NRX-252114, which stabilizes the interaction between mutant transcription factor  $\beta$ -catenin and its native E3 ligase  $\beta$ -TRCP<sup>90</sup>.

Proteasomal degradation via a molecular glue mechanism can also be induced beyond ternary complexes containing a proteasomal system component. The compound BI-3802 was originally discovered in a screen for inhibitors of the transcription repressor BCL6, which is overexpressed in cancer. BI-3802 was found to bridge two BCL6 proteins between their BTB domains, resulting in higher-order polymerization<sup>91,92</sup>. Genetic screening revealed that polymerized BCL6 is then ubiquitinated for proteasomal degradation by the non-cullin E3 ligase SIAH1.

## Rational design of molecular glues

The molecular glues BI-3802 and CR8 provided the first evidence that small changes in inhibitor structure can transition the mechanism

of action from inhibitor to degrader<sup>85,91</sup>. Shortly thereafter, a transplantable fumerate ‘handle’ appended to a CDK4 and CDK6 inhibitor was shown to induce the degradation of proteins, including CDK4 and CDK6, the chromatin protein BRD4, and the tyrosine kinase BTK, facilitated by covalent binding of the handle to the E3 ligase RNF126 (ref. 93). Appending reactive electrophilic handles onto the BRD4 inhibitor JQ1 culminated in the selective and potent degraders MMH1 and MMH2, which utilize acrylamide and vinyl sulfonamide handles, respectively, to induce proximity of BRD4 to the E3 ligase substrate receptor DCAF16 (refs. 94,95). Furthermore, a vinylsulfonyl piperazine handle was appended onto various protein inhibitors to facilitate DCAF16-mediated degradation<sup>96</sup>. These examples highlight an exciting new direction for the field, albeit at a preclinical stage.

Revolution Medicines has disclosed at conferences several structure-guided, rationally designed molecular glue inhibitors targeting the active, GTP-bound (ON) state of KRAS, including G12C and G12D mutants, through ternary complex formation with CypA. Phase I–II candidate RMC-6291 has a cyclized scaffold derived from the natural product binder of CypA, sangliferin, decorated with an electrophilic protrusion to modify the surface of CypA and create a high-affinity and selective interface with KRAS(ON) G12C<sup>97</sup>. A similar design strategy yielded the mechanistically similar phase I compound RMC-9805, a covalent inhibitor of KRAS(ON) G12D<sup>98</sup>. A pan-KRAS molecular glue inhibitor RMC-6236 has shown promise in phase III clinical trials targeting tumours driven by other undruggable KRAS mutants<sup>47</sup>. Interestingly, specific targeting of GTP-bound KRAS(ON) with these glues circumvents the typical resistance mechanisms to KRAS(OFF) covalent inhibitors (such as sotorasib and adagrasib) that activate upstream signalling and increase KRAS-GTP loading, resulting in more potent or long-lasting therapies<sup>99,100</sup>. These rational, structure-guided design efforts to target previously undruggable KRAS(ON) mutants exemplify the efficacy of molecular glue strategies to remodel protein interfaces without the need for a KRAS-specific binding ligand. Clinical evaluation is ongoing, but preliminary data suggest promising efficacy of KRAS inhibition.

Rational design of molecular glues has been more challenging than occupancy-driven approaches because it requires a deep structural understanding of protein–protein interfaces, which can be highly transient, have low affinity or lack resolved structures. Additionally, information on minimal degron structures or low-affinity interactome partners is lacking beyond well-characterized complexes, such as between the IMiDs and zinc finger domains, but will be necessary for expansion to other neosubstrates as therapeutic targets<sup>101</sup>. The emergence of covalent and non-covalent ligand modification strategies for molecular glue rational design might help identify initial interactions of interest, whereafter structure-based drug design can be employed. Fortunately, these approaches have already expanded the druggable proteome and the outlook is promising.

## Bifunctional induced proximity drugs

Bifunctional compounds consist of two separate targeting ligands that induce proximity between cellular bodies of interest. Small-molecule approaches include chemically induced dimerizers (CIDs) for protein inhibition, PROTACs for protein degradation, autophagy-targeting chimeras for organelle or protein degradation, targeted post-translational

## Box 1 | Bifunctional approaches to targeted RNA degradation

Therapeutic intervention prior to protein translation would help expand the druggable proteome. Although this has been achieved in the clinic by using antisense oligonucleotides or small interfering RNA therapies, these strategies are hindered by significant uptake, half-life and toxicity problems<sup>278</sup>. Development of a small-molecule strategy for targeted RNA silencing through RNA degradation would not only address these challenges but would be able to tackle non-coding RNAs to expand the druggable space<sup>279,280</sup>. Advantageously, RNA degradation through induced proximity could utilize RNA ligands that are biologically inert or have partial occupancy<sup>281</sup>.

### Ribonuclease-independent cleavage

Early efforts at targeted RNA degradation involved appending bleomycin, a clinically approved drug capable of inducing phosphodiester bond cleavage, to RNA-targeting ligands<sup>282,283</sup>. This approach has been employed to degrade the r(CUG) repeat expansions found in myotonic dystrophies and pri-miRNA-96 in triple-negative breast cancer; however, there is no clinical applicability, possibly due to polypharmacological effects from bleomycin treatment alone<sup>284–287</sup>.

Inducing proximity between an RNA target and an imidazole warhead, mimicking the conserved RNA-degrading moiety in ribonucleases, is sufficient for magnesium-dependent RNA degradation<sup>288</sup>. Elaborating on this approach, the compounds pyridostatin and MTDB, which target G-quadruplexes and betacoronaviral pseudoknots, respectively, were linked to imidazole and shown to cleave their RNA targets *in vitro*<sup>289</sup>. Although these molecules (termed proximity-induced nucleic acid degraders) showed efficacy in SARS-CoV-2 disease models, they have not advanced past the proof-of-concept stage. Further analysis of mechanistic selectivity would be needed prior to platform expansion.

### Ribonuclease recruitment

Small-molecule-mediated targeted RNA degradation by recruiting and activating ribonuclease L (RNase L) to degrade a target RNA was pioneered by the Disney lab through development of the ribonuclease-targeting chimera (RIBOTAC) platform<sup>290</sup>. Early efforts to degrade miRNA-96 and miRNA-210 induced breast cancer apoptosis in cellular models but the RNase-recruiting element, a polyA oligonucleotide, was hindered by innate immunity activation and cellular permeability challenges<sup>291</sup>. Optimization of this moiety into a small heterocyclic ligand resulted in RNase L-dependent cleavage of oncogenic miRNA-21 in a mouse cancer model<sup>290</sup> as well as degradation of pathological expanded RNA repeats in models of amyotrophic lateral sclerosis<sup>292,293</sup>. Furthermore, RNA-binding chemotypes for miR-155, *JUN* and *MYC* RNAs predicted to be biologically inert were capable of lowering relevant protein expression levels when converted into RIBOTACs<sup>294</sup>. Although clinical relevance has been demonstrated in relevant preclinical models, some RIBOTACs are not as efficacious as established proteolysis-targeting chimera (PROTAC) degraders in similar models, and maximal RNA degradation is usually no higher than 60%, suggesting a need for further optimization before clinical application.

The main hindrance to clinical progression of chimeric RNA modulators is ligand discovery, both for effectors and for target RNAs, but several companies have been launched in the past decade that are using structure-guided bioinformatics, modelling and RNA-targeted screening libraries to discover selective RNA-binding ligands with a view to developing RNA degraders. Arrakis Therapeutics and Expansion Therapeutics have programmes in expansion repeat diseases, Skyhawk Therapeutics has a clinical candidate for Huntington's disease, and Ribometrix has an oncology programme targeting c-MYC RNA.

modifiers, targeted transcriptional regulators, and targeted RNA degraders (Box 1). Biologic approaches include nanobody conjugates and bispecific antibodies, which function through dual specific arms to induce proximity between two membrane-bound or extracellular proteins. Although bispecific antibodies are proximity-inducing drugs, they are extensively reviewed elsewhere. We briefly summarize their clinical outlook in Box 2 and elaborate in the text on recent antibody-based technologies that have afforded advancements in targeted degradation or post-translational modification.

### Chemically induced dimerizers

The first foray into the synthesis of molecular glues was the creation of homobifunctional and heterobifunctional CIDs. In 1993, a homodimer of the compound FK506, termed FK1012, was shown to induce dimerization and inhibition of FKBP12 (ref. 102). Over 20 years later, a similar compound, named rimiducid, was granted orphan drug status as a pharmacological on/off-switch for chimeric antigen receptor (CAR) T cell therapy that acts through chemically inducing the dimerization of an FKBP–caspase 9 fusion protein in CAR T cells to induce rapid apoptosis<sup>103</sup>. These early bifunctional compounds paved the way for the development of other bifunctional modalities, including PROTACs.

### Proteolysis-targeting chimeras

Since their introduction as peptide-based bifunctional molecules in 2001, PROTACs have expanded into a vast field and promising therapeutic modality<sup>104–106</sup> (Fig. 3). However, the first generation of permeable small-molecule PROTACs had detrimental off-target effects and unfavourable molecular properties that prevented the development of a generalizable technology and clinical application<sup>107</sup>.

The modern generation of small-molecule PROTACs has achieved high potency and target-specific, mechanism-based degradation, which enables clinical translation. These PROTACs were enabled by the discovery of IMiDs and optimization of small-molecule ligands for the VHL E3 ligase complex. In seminal papers published concurrently in 2015, the Crews and Ciulli groups optimized a VHL-binding peptide into a series of small-molecule ligands based around a critical hydroxyproline moiety, resulting in improved binding and favourable physicochemical properties<sup>108–111</sup>. The Crews lab used these improved ligands to design PROTACs that degraded the hormone receptor ERKα and RIPK2 kinase<sup>112</sup>, whereas the Ciulli lab developed PROTACs such as MZ1, specific for the BRD4 BET bromodomain<sup>113</sup>. The Bradner and Crews labs also developed pan-BET PROTACs (dBET1 (ref. 114) and ARV-825 (ref. 115), respectively) that elicited more potent antiproliferative effects than small-molecule BET inhibition alone<sup>116</sup>. These discoveries

set the groundwork for a modular and efficacious strategy for targeted protein degradation, and PROTACs have now been developed against many protein targets implicated in cancer<sup>117–121</sup>, immune disease<sup>122–126</sup>, neurodegenerative disease<sup>127–129</sup>, cardiovascular disease<sup>130,131</sup> and microbial disease<sup>132</sup>.

## Clinical outlook of PROTACs

PROTACs enable catalytic, sub-stoichiometric degradation of targets, allowing for prolonged activity and more complete biological efficacy compared with occupancy-driven approaches. However, they have typically suffered from challenges in the clinic due to their beyond the ‘Rule of Five’ properties (the drug-like properties determined by Lipinski), including high molecular weight, lipophilicity, hydrogen bond acceptors and donors, and metabolic instability. To overcome these challenges, several optimization efforts have emerged: first, rigidification of the linker to improve metabolic stability and reduce rotatable bonds; second, inclusion of intramolecular hydrogen bonds to enhance chameleonicity, decrease total molecular size and thus improve cellular permeability; and third, selection of target and

E3 ligase ligands for smaller polar surface areas and lower molecular weight, resulting in most clinical efforts preferring ligands for CRBN over VHL<sup>133,134</sup>. While further exploration of the rules dictating favourable drug-like properties of PROTACs is ongoing, these key optimizations have prompted more than 30 PROTACs to enter clinical trials against both haematological malignancies and solid tumours (Table 1).

In 2019, the first PROTAC entered the clinic. ARV-471 (Arvinas) (NCT05654623) is a CRBN-based PROTAC that targets the oestrogen receptor<sup>135</sup> and has now achieved FDA approval for breast cancer<sup>136</sup>. Several other PROTACs now have clinical trials ongoing, mainly for oncology indications (Fig. 3 and Table 1). For example, several PROTACs that degrade androgen receptor to treat prostate cancer have entered clinical trials, the most advanced being ARV-766 (Arvinas) (NCT05067140) and BMS-986365 (BMS) (NCT06764485). Besides ARV-471, two other PROTACs directed against the oestrogen receptor have entered the clinic for the treatment of breast cancer. Additional PROTACs that demonstrated preclinical promise and have advanced to phase I trials include several that target BTK and one that targets TRK kinase isoforms. Furthermore, PROTACs that target

## Box 2 | Bispecific antibodies as induced proximity therapeutic modalities

The application of bispecific antibodies for extracellular targeted protein degradation is an exciting new potential therapeutic approach. However, these constructs have been increasingly used for induced proximity-based pharmacology over decades. Bispecific antibodies have been reviewed in detail, so we will briefly discuss their clinical outlook<sup>295–299</sup>.

### Bispecific antibodies redirect immune effector cells in trans

Bispecific antibodies can redirect T cell functions through induced proximity between immune effector T cells and tumour cells, which generates tumour-specific cytotoxicity<sup>300,301</sup>. Efficacy can be affected by the affinity of the antibody for both targets, the expression level of the receptors, antigen mobility within the membrane, and the structure of the immunological synapse formed between the contacting cells<sup>296</sup>. The FDA has approved 11 bispecific antibodies, 8 of which are for cancer, with hundreds more in clinical trials<sup>302,303</sup>. Most clinical stage constructs target CD3ε for T cell engagement. Haematological malignancies are usually targeted through CD20, CD33, CD123 or B cell maturation antigen, whereas solid tumours are targeted through tumour-specific receptors such as prostate-specific membrane antigen or HER2 (refs. 304,305). Targeting subclasses of T cells, such as Vγ9Vδ2 T cells through their γδ T cell receptor, has also shown promising preclinical efficacy and reduced off-target T cell activation but has not been investigated in a clinical setting<sup>306</sup>.

T cell redirection combined with immune-checkpoint blockade in a single construct is more effective than combination therapy of the individual antibodies, suggesting that induced proximity of T cells to the tumour is critical to the mechanism of action<sup>307–312</sup>. Dual immune-checkpoint blockade constructs, typically utilizing arms for tumour checkpoint protein PDL1 and immune checkpoints such as PD1, LAG3 and TIGIT, have recently demonstrated enhanced immune activation and tumour suppression in preclinical models<sup>313,314</sup>.

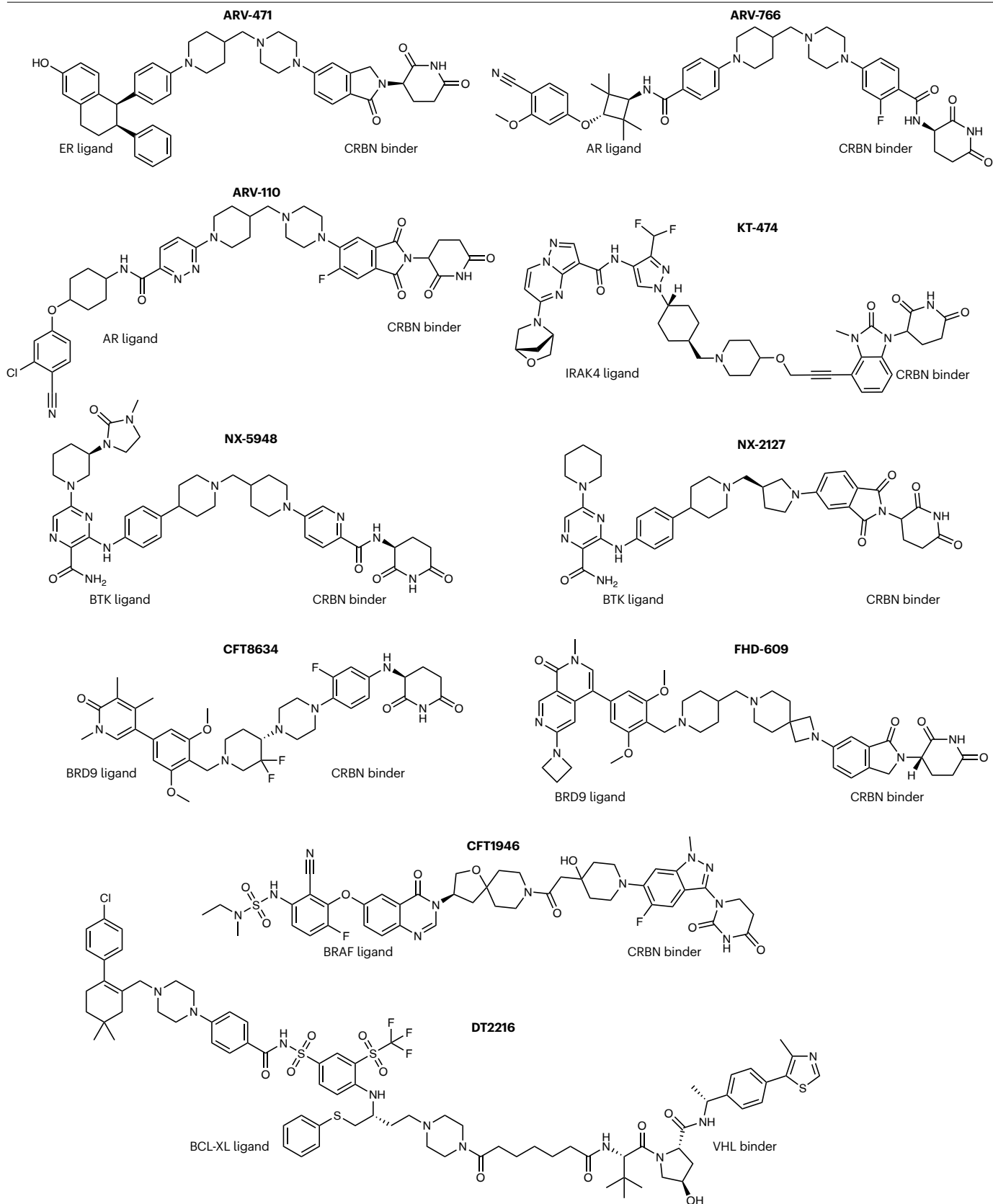
### Bispecific antibodies induce proximity in cis

Cell surface receptors can be bridged in *cis* to activate downstream pathways. The clinically approved bispecific antibody emicizumab dimerizes cell signalling proteins FIXa and FX to prevent bleeding in patients with haemophilia A<sup>315–317</sup>. Next-generation bispecific antibodies NXT007 and Mim8 have shown increased potency over emicizumab in preclinical studies<sup>318–320</sup>.

Bispecific antibodies can achieve selective receptor targeting on a subset of a larger cell population. For example, the negative regulator of phagocytosis CD47 mediates immune evasion of tumour cells but is widely expressed and difficult to target<sup>321,322</sup>. An antibody with a low-affinity arm for CD47 and a high-affinity arm for the tumour antigen CD19 selectively inhibited the CD47 signal only on CD19<sup>+</sup> tumour cells and prompted their selective phagocytosis<sup>323,324</sup>.

Bispecific antibodies can also target multiple inhibitory pathways specifically within a tumour cell or cell of interest. Dual immune-checkpoint blockade antibodies targeting PD1 and CTLA4 are being evaluated for efficacy in clinical trials<sup>325–328</sup>. Other tumour-associated receptors, such as TGFβ, can be targeted with PDL1 bispecific antibodies and simultaneously activate cytotoxic lymphocytes and decrease suppressive cells<sup>329–331</sup>. Dual pathway inhibition of HER2 and HER3 can treat cancers with HER3-driven resistance to HER2-focused immunotherapy<sup>332</sup>. Amivantamab, which is clinically approved, simultaneously inhibits cMET and EGFR, resulting in antibody-dependent cellular cytotoxicity<sup>333</sup>. These are just a few examples of dual pathway inhibition using bispecific antibodies.

Overall, bispecific antibodies are a well-studied class of therapies that has seen immense growth in the past decade. Although serious concerns remain about cytokine release syndrome from immune activation, emerging approaches improve specificity for tumour cells or utilize subsets of immune effector cells<sup>334</sup>. Emerging therapies are more efficacious against haematological malignancies and therefore efforts to advance solid tumour penetration are needed. Diverse tumour-associated antigens are being investigated in clinical trials to improve positive patient responses.



**Fig. 3 | Proteolysis-targeting chimeras in the clinic.** Chemical structures of the proteolysis-targeting chimeras in clinical trials that have disclosed chemical structures. The ligand for the degraded target is on the left of each structure

and the E3 ligase ligand is on the right. AR, androgen receptor; CRBN, cereblon; ER, oestrogen receptor.

oncogenic mutants are in phase I trials, including two that target KRAS G12D (Astellas) and others that target BRAF V600E and EGFR L858R (C4 Therapeutics). Unfortunately, several other programmes have been discontinued, including BRD9-targeting PROTACs from Foghorn and C4, which lacked efficacy, and STAT3-directed and MDM2-directed PROTACs from Kymera, which were deprioritized.

Several PROTACs are being evaluated for clinical efficacy in non-cancer indications. GT20029 (Kintor) (NCT05428449) is administered topically to treat acne vulgaris and androgenetic alopecia. It functions by degrading the androgen receptor and is the only PROTAC in the clinic not dosed orally or intravenously<sup>137</sup>. ARV-102 (Arvinas) leads to degradation of the kinase LRRK2 that is linked to neurodegenerative diseases such as Parkinson's disease and is the first PROTAC to pass the blood–brain barrier. A PROTAC KT-474 that degrades the IRAK4 kinase (Kymera/Sanofi) is demonstrating anti-inflammatory efficacy in phase I clinical trials and is now in phase II trials for immunoinflammatory diseases, particularly atopic dermatitis and hidradenitis suppurativa<sup>138</sup>. A STAT6-directed PROTAC KT-621 (Kymera) is also in phase I clinical trials for allergic diseases<sup>139</sup>.

Although the preliminary clinical results are promising for therapeutic applicability of the PROTAC technology, there are limitations in delivering PROTACs to specific tissues of interest without significant off-target interactions. One way to overcome this challenge is to link a tissue-directing moiety, such as an antibody or a folate cage, to the PROTAC. An antibody-linked PROTAC would function similarly to an antibody–drug conjugate by releasing the drug at the desired pathogenic tissue, thus providing specificity. Analogously, folate would bind to the folate receptor FOLR1, which is overexpressed on tumour cell surfaces. Once in the cytosol, the folate cage is removed by hydrolysis and the PROTAC is released in an active form. An anti-HER2–GSPT1 degrader conjugate and an anti-CD33–GSPT1 degrader conjugate are in phase I clinical trials (Orum Therapeutics and BMS). Although these conjugates are only employed with molecular glue degraders, they could theoretically be implemented with PROTACs as well.

The majority of PROTACs in the clinic use existing inhibitors as the targeting moiety, and conversion of inhibitors to degraders has several benefits. Firstly, PROTACs have outperformed parent or other standard-of-care inhibitors in head-to-head studies, and this is an active area of evaluation in clinical trials. Additionally, PROTACs are showing success in the clinic in populations that are resistant to inhibitors. Much of this success is due to the sub-stoichiometric, catalytic and durable degradation of the target protein observed with PROTACs compared to the original inhibitor. PROTACs will eliminate the scaffolding function as well as the enzyme function of certain kinase targets compared to active-site inhibitors, and they can engage and degrade not only wild-type but also mutant forms of oncogenic targets. Finally, pan-inhibitors with limited selectivity between protein family members can become converted into isoform-specific degraders, likely due to the necessity for optimal ternary complex formation. This has been robustly demonstrated in kinases: a pan-CDK binding ligand conjugated to thalidomide (THAL-SNS-032) induced CDK9-specific degradation, and dual CDK4 and CDK6 or CDK12 and CDK13 inhibitors were converted into isoform-specific CRBN-recruiting degraders<sup>140–142</sup>. Other examples include isoform-specific PROTAC degraders of p38, the kinase SGK and

the phosphodiesterase PDE4 (refs. 143,144). These benefits highlight the utility of PROTACs over traditional occupancy-driven approaches.

Although PROTACs can overcome the resistance to small-molecule inhibitors that occurs in treating cancer indications, PROTACs and glue degraders have also been subject to acquired resistance mechanisms. Patients receiving IMiD treatment for myeloma develop resistance over time, depending on their genetic background and treatment regimen, and eventually relapse – primarily due to loss-of-function (LOF) mutations in CRBN<sup>145</sup>. The neosubstrate targets can also confer resistance through mutations in domains necessary for ternary complex formation, although this is less evident in a clinical context<sup>146</sup>. Cultured cells can become rapidly desensitized to consistent PROTAC treatment as soon as 2 weeks post-treatment, with LOF mutations or decreased expression observed primarily in components of the cullin–RING ubiquitin ligase (CRL) machinery required for degradation, including substrate receptors, cullin proteins or CRL regulators such as the COP9 signalosome or the substrate receptor exchange protein CAND1 (refs. 146–151). Notably, the drug efflux protein MRD1, which confers resistance to many cancer therapies, was found to desensitize cells to PROTACs through adaptive and innate mechanisms with increases in cellular MRD1 levels detectable as early as 7 days post-treatment<sup>152</sup>. Although clones resistant to degraders are not observed in every cell line, the rapidity of cellular adaptation necessitates consideration of alternative strategies. Expansion to other, more essential E3 ligase components might circumvent LOF mutations and extracellular protein degraders could overcome drug efflux challenges, both of which are discussed below.

## Expanding PROTAC design

PROTAC development has been enabled by the existence of ligands for E3 ligases such as CRBN and VHL but only a small fraction of E3 ligases have been utilized for PROTAC applications. Expanding the arsenal of E3 ligases recruited by PROTACs, as well as the components of the degradation machinery that are recruited, promises to expand the scope of neosubstrate targets, expand the therapeutic window, overcome resistance mechanisms and achieve tissue-specific degradation<sup>153</sup>.

The molecular chaperone HSP90, which catalyses protein-folding and accelerates misfolded protein degradation by interacting with many E3 ligases, was co-opted for degradation of CDK4 and CDK6 through the use of BIIB021, an HSP90 inhibitor, in a bifunctional compound termed HSP90-mediated targeting chimeras<sup>154</sup>. A similar unpublished approach (T-PEACH) involves linkage of a different HSP90 ligand to JQ1 and led to degradation of BRD4 and antiproliferative effects<sup>155</sup>. Chaperone-mediated degradation technology is being used by Ranok Therapeutics to develop clinical candidates.

The inhibitor of apoptosis proteins (IAPs) are liganded by the bestatin class of aminopeptidase inhibitors and have been used for degradation of targets involved in cancer (such as oestrogen receptor, BCR-ABL and BRD4)<sup>156–160</sup>, immune diseases (such as H-PGDS, IRAK4 and RIPK2)<sup>161–163</sup>, and neurodegenerative diseases (such as mutant huntingtin protein (mHTT) and PDE4)<sup>164,165</sup> but are hindered by low catalytic efficiency due to auto-ubiquitination and subsequent degradation<sup>166</sup>. In another approach, an atypical substrate adaptor for CRL4 complexes, the aryl hydrocarbon receptor, was exploited for targeted protein degradation using  $\beta$ -naphthoflavone flavonoid or the ITE metabolite as ligands<sup>167,168</sup>.

Similarly, KEAP1, an adaptor subunit of the CRL3 complex, was exploited for degraders using peptides and the compounds bardoxolone, KI696 and piperlongumine as ligands but has a limited target scope<sup>169–173</sup>. The DCAF15-dependent aryl sulfonamide E7820 was able to promote BRD4 degradation but required high concentrations<sup>174</sup>. Interestingly, a bifunctional agent consisting of the DCAF15-dependent glue indisulam linked to a JQ1 derivative degraded BRD2 and BRD4 independently of DCAF15. The compound prompted formation of an intramolecular BRD4 complex with enhanced affinity for DCAF16, which led to target degradation<sup>175</sup>. These results indicate that the mechanism of action for each new ligase-recruiting ligand must be thoroughly investigated.

Covalent ligand screening combined with cysteine-reactive chemoproteomics has been used to identify several covalent recruiters of E3 ligases and associated components of ligase complexes for targeted protein degradation applications. The Cravatt group identified electrophilic fragments that facilitated target degradation through covalent binding to DCAF16 (ref. 176) or DCAF11 (ref. 177). The Nomura lab, in collaboration with Novartis, discovered covalent ligands for the E3 ligases RNF4, RNF114 and FEM1B<sup>178–182</sup>, the substrate adaptor proteins DDB1 and SKP1 (refs. 183,184), and E2 ubiquitin-conjugating enzyme UBE2D2 (ref. 185) for degradation of various neosubstrate proteins.

Cellular resistance mechanisms to PROTACs frequently impair or eliminate non-essential components of the ubiquitin proteasome system that contribute to degradation<sup>147,148</sup>. Most PROTACs focus on RING E3 ligases or substrate receptor proteins for E3 ligase complexes but none of these components seem singularly essential for cell survival due to significant cellular redundancy<sup>186–189</sup>. Focusing on targeted protein degradation, recruiting core components of the ubiquitin proteasome system might aid in abrogating the development of resistance mechanisms to degraders.

A study investigated whether substrates could be degraded through direct recruitment to the 26S proteasome<sup>190</sup>. A ligand for the unfoldase pore in the PSMD2 subunit of the proteasome was discovered through mRNA display. A series of heterobifunctional degraders, termed chemical inducers of degradation, were synthesized and optimized for potent degradation of BRD4. Although these degraders are less potent than those in the clinic, this study provides a promising proof of concept of direct recruitment of the proteasome for targeted protein degradation.

A mitochondrial-specific PROTAC, termed MtPTAC, was developed utilizing the AAA<sup>+</sup> mitochondrial serine protease subunit ClpP as a means of proteolytic degradation for target proteins localized in the mitochondria<sup>191</sup>. Administration of the MtPTAC targeting human mitochondrial RNA polymerase (POLRMT) resulted in decreased levels of POLRMT and tumour regression. More broadly, the authors demonstrated that the system of hydrolytic proteases can be utilized for targeted protein degradation. Utilizing a similar ClpC:ClpP protease complex in bacteria, BacPROTACs were introduced to induce bacteria-specific target protein degradation using peptidyl pArg mimics and the cyclic peptide cyclomarin as protease ligands<sup>192</sup>. Homo-BacPROTACs direct ClpC1 and ClpC2 self-degradation and exhibit killing activity in *Mycobacterium tuberculosis*<sup>193</sup>. This strategy could improve antibiotic therapy because it does not target any host proteins and might be broadly applicable.

## Targeted protein degradation beyond intracellular targets

Despite the success of PROTACs in tackling undruggable targets, proteasome-based approaches are mostly limited to intracellular

cytosolic, membrane and nuclear targets. Lysosomal pathways might be preferred for misfolded proteins, aggregates, organelles, and cell-surface and extracellular proteins because these targets might not be accessible by cytosolic or nuclear E3 ubiquitin ligases or might be resistant to proteasome-mediated degradation<sup>194,195</sup>. Herein, we discuss the expansion of targeted protein degradation to the two major lysosomal degradation pathways: the autophagosome–lysosomal and the endosome–lysosomal pathways. The modalities being developed include bifunctional small molecules, antibody–small-molecule or antibody–peptide bioconjugates, and bispecific antibodies.

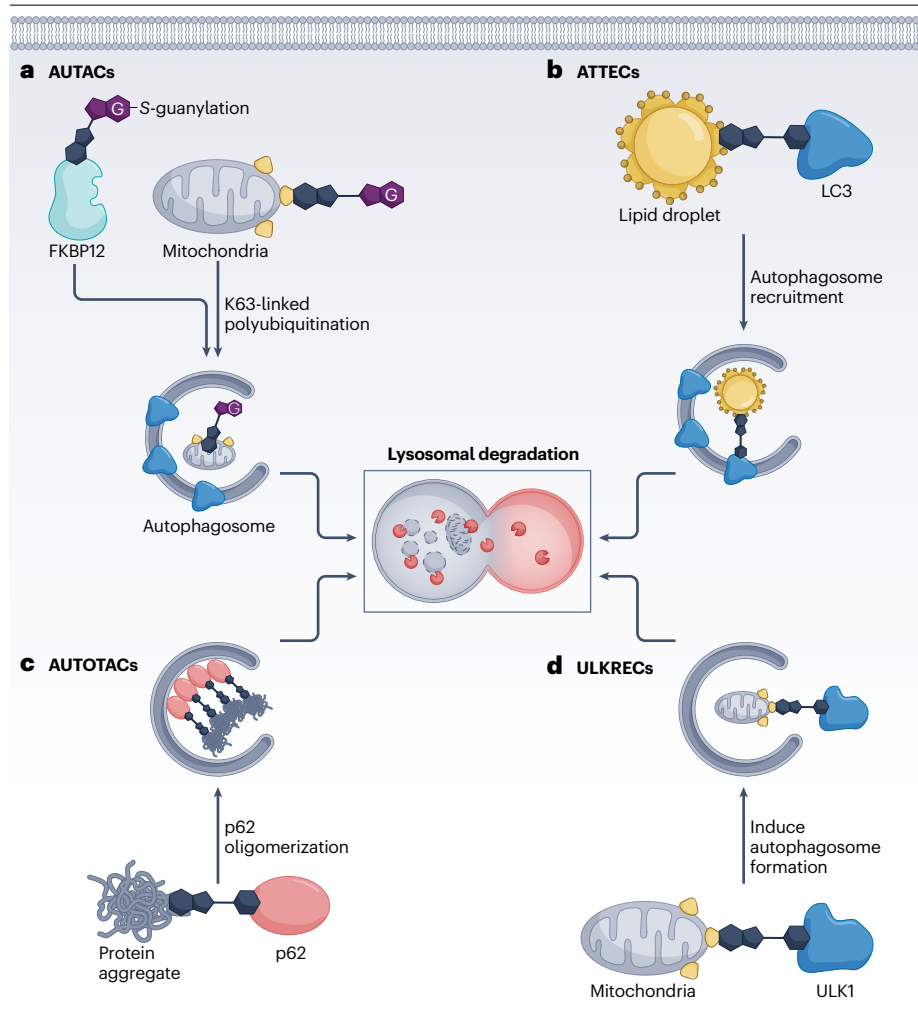
## Autophagosome–lysosomal approaches to targeted protein degradation

Autophagy-targeting chimeras utilize S-guanylation as an autophagy-dependent and ubiquitin-dependent degradation tag<sup>196</sup>. Chimeric molecules that fused a modified guanine tag, *p*-fluorobenzylguanine (FBnG), to a target-specific ligand via a linker could induce K63-linked polyubiquitination and autophagic degradation of the target soluble proteins FKBP12 and methionine aminopeptidase MetAP2. The approach was also applied to induce autophagic degradation of mitochondria (Fig. 4a). A second generation of autophagy-targeting chimeras was established through structure–activity relationship studies in which sub-micromolar potency was achieved by replacing the cysteine residue connected to the guanine tag with benzene, pyrazole, or triazole and adjusting linker length<sup>197</sup>.

Autophagosome-tethering compounds were first introduced using a chimeric molecule that was purported to interact with both the LC3 autophagosome protein and mHTT, thereby targeting mHTT for autophagy<sup>198</sup>. Further work expanded this platform to degrade soluble oncoproteins<sup>199,200</sup> and non-proteinaceous biomolecules, including lipid droplets<sup>201</sup> and mitochondria<sup>202</sup> (Fig. 4b). Although these studies demonstrated evidence of autophagy dependence and ternary complex formation, recent work has challenged the mechanism. Arylidene-indolinones, like those used in autophagosome-tethering compounds, were reported to covalently bind the E3 substrate receptor DCAF11 and appear to degrade targets through a proteasome-dependent mechanism rather than autophagy<sup>203</sup>. These results suggest that more structural information about the ternary complexes induced by these compounds is required to fully understand the mechanism of action and how to expand the strategy to encompass different types of target.

Another autophagy-targeting chimera platform, termed AUTO-TAC, utilizes ligands that mimic the N-terminal arginine degron to bind to the autophagy receptor p62, inducing a conformational change in p62 that causes its oligomerization and leads to autophagy of p62–cargo complexes<sup>204</sup>. Several p62-dependent bifunctional compounds were designed to induce degradation of various targets, such as oestrogen receptor, MetAP2 or androgen receptor, resulting in attenuated cancer cell growth and disease progression (Fig. 4c). Additional AUTOTACs using ligands that bind hydrophobic motifs or neurodegenerative aggregates resulted in clearance of proteasome-resistant aggregates and were efficacious in Alzheimer's and Parkinson's disease models<sup>204</sup>.

A study described in a preprint<sup>205</sup> reported recruiting the autophagosome-related kinase ULK1 to mitochondria using chimeric molecules termed ULK1-recruiting chimeras. In these chimeras, a ligand that recruits and activates ULK1 was linked to a ligand that targets the translocator protein on the outer mitochondrial membrane<sup>205</sup> (Fig. 4d).



**Fig. 4 | Autophagosome–lysosomal-dependent targeted protein degradation modalities.** **a**, Autophagy-targeting chimeras (AUTACs) are comprised of an S-guanylation tag attached via a linker to a specific ligand for a target of interest such as a protein or organelle. S-guanylation of targets, such as FKBP12 or mitochondria, leads to their K63-linked ubiquitination, recruitment to the autophagosome and degradation in the lysosome. **b**, Autophagosome-tethering compounds (ATTECs) tether a target, such as a lipid droplet, to the LC3 autophagosome protein, leading to target degradation by prompting autophagosome recruitment at the site of the target. **c**, AUTOTACs bind the autophagy receptor p62 and recruit targets such as neurodegenerative protein aggregates, leading to p62 oligomerization, autophagy and aggregate clearance. **d**, ULK1-recruiting chimeras (ULKRECs) recruit the autophagosome-related kinase ULK1 to induce autophagosome formation around the target, namely a mitochondrial membrane protein, leading to mitochondrial degradation.

The ULK1-recruiting chimeras promoted autophagosome formation around the mitochondria and induced mitochondrial degradation via autophagy (mitophagy). They also rescued mitophagy defects in fibroblasts from patients with Parkinson’s disease.

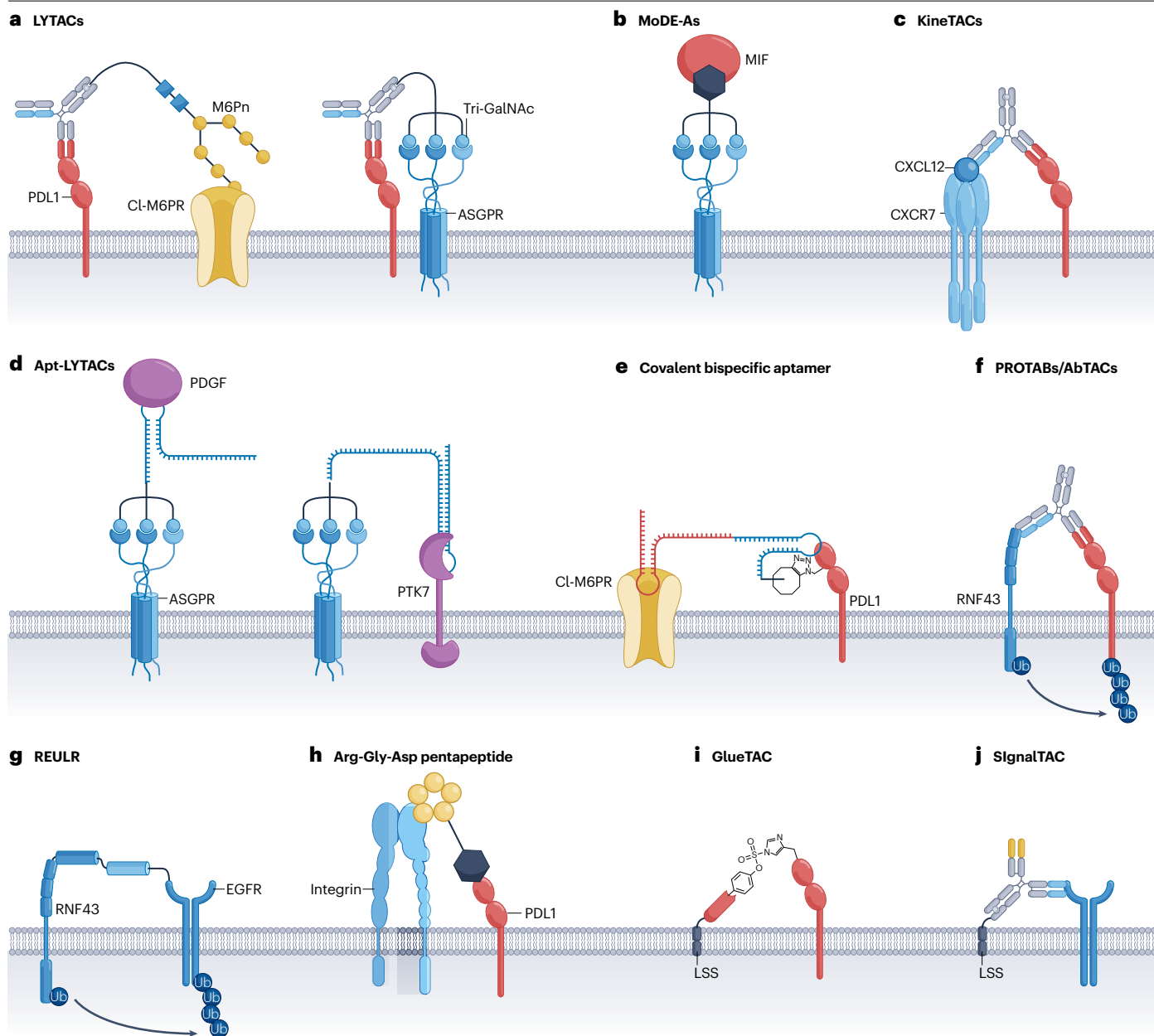
There is significant interest in developing autophagy-targeting bifunctional compounds for clinical applications, with several companies pursuing these drugs but no candidates have yet progressed to clinical trials.

**Endosome–lysosomal approaches to targeted protein degradation**

PROTACs largely target intracellular proteins, although some intracellular domains from membrane receptor families have been successfully targeted with small-molecule PROTAC approaches<sup>124,169,206–216</sup>. To address this significant shortcoming, researchers have turned to utilizing the endosome–lysosomal pathway for targeting transmembrane receptors or secreted proteins (Fig. 5).

Lysosome-targeting chimeras (LYTACs), introduced by Banik et al., utilize the widely expressed and efficient lysosomal-trafficking mannose-6-phosphate (M6P) receptor CI-M6PR to shuttle target proteins into the endosome<sup>217</sup>. Glycopolypeptide agonists of CI-M6PR conjugated to protein-targeting antibodies increased internalization

and subsequent degradation of clinically relevant cell surface proteins such as EGFR, CD71 and PDL1 (Fig. 5a). LYTAC degrader activity was attributed to several cellular pathways: targeting of the cell-surface receptor M6PR, a recycling retromer complex that returns LYTAC-CI-M6PR to the plasma membrane, natural M6P biosynthetic pathways, and Cul3-mediated ubiquitination of sequestosome 1 (ref. 218). A second generation of lysosomal-targeting compounds was developed utilizing tri-antennary N-acetylgalactosamine (tri-GalNAc) motifs to target the liver-specific lysosomal-trafficking asialoglycoprotein receptor ASGPR for internalization of target proteins in specific cell types<sup>219</sup> (Fig. 5a). Interestingly, antibody engineering to adjust the conjugation site and antibody-to-ligand ratio can optimize target degradation and pharmacokinetic properties. Tri-GalNAc small-molecule conjugates (termed MoDE-As) were used to degrade an extracellular antibody and the cytokine MIF<sup>220</sup> (Fig. 5b). Efficacy of internalization increases as the size of the degrader-target complex decreases, suggesting that therapeutic optimization will need to consider the size of both the conjugate and desired protein target<sup>221</sup>. These results present LYTACs as a powerful platform for targeted protein degradation of secreted and transmembrane proteins and provide valuable mechanistic insights to allow for better translation into the clinic. A few LYTACs have entered phase I clinical trials. A MoDE degrader



**Fig. 5 | Endosome-lysosomal-dependent targeted protein degradation modalities.** **a**, Lysosome-targeting chimeras (LYTACs) utilize mannose-6-phosphate multimers (M6Pn) or tri-antennary N-acetylgalactosamine (tri-GalNAc) to ligand the M6Pn receptor CI-M6PR or the asialoglycoprotein receptor ASGPR, respectively. These ligands are conjugated to an antibody recognizing the target (PDL1), leading to target degradation. **b**, MoDE-As utilize tri-GalNAc to ligand ASGPR linked to a small molecule to mediate degradation of the target (MIF). **c**, KineTACs are bispecific engineered antibodies that contain an arm pair targeting cytokine (CXCL12) or cytokine receptor (CXCR7) and a PDL1-targeting arm for PDL1 degradation. **d**, Aptamer-based LYTACs (Apt-LYTACs) incorporate tri-GalNAc to ligand ASGPR linked to aptamers for the target proteins PDGF (extracellular) or PTK7 (transmembrane) to facilitate target degradation. **e**, A bispecific aptamer linking CI-M6PR and PDL1 utilizes strain-promoted azide

alkyne cycloaddition-mediated covalent linkage to the target to induce PDL1 degradation. **f**, In the proteolysis-targeting antibodies (PROTABs)/antibody-targeting chimaera (AbTAC) platform, bispecific antibodies link the membrane-bound E3 ligase RNF43 to PDL1 for ubiquitin (Ub)-dependent target degradation. **g**, The receptor elimination by E3 ubiquitin ligase recruitment (REULR) platform uses heterobifunctional nanobodies that link a transmembrane E3 ligase, such as RNF43, to a target, such as EGFR, for Ub-dependent degradation. **h**, An integrin-targeting pentapeptide linked to a small-molecule targets PDL1 for degradation. **i**, In GlueTACs, lysosomal-sorting sequences (LSS) are linked covalently to PDL1 through use of an unnatural amino acid for PDL1 degradation. **j**, In signal-mediated lysosome-targeting chimeras (SignalTACs), an LSS from CI-M6PR is linked to antibodies for EGFR, resulting in target degradation.

targeting IgG (BHV-1300) developed by Biohaven entered clinical trials in 2024 and was reported to induce target degradation in patients<sup>222</sup>. Biohaven also has two extracellular lysosome-mediated degraders targeting Gd-IgA1 (BHV-1400) and  $\beta$ 1AR auto-antibodies (BHV-1600) in phase I trials and another one targeting IgG in its pipeline. Other companies have LYTACs utilizing the ASGPR and M6PR receptors at early development stages.

## Expanding the substrate scope of endosomal-dependent degraders

Transmembrane and secreted protein targets have also been degraded via the endosome–lysosomal pathway using recombinant bispecific antibodies, termed KineTACs, that induce cytokine-mediated internalization of cytokine receptors<sup>223</sup>. As a proof of concept, the chemokine CXCL12, which binds to the receptor CXCR7 and internalizes, was linked to the antigen-binding fragment of the immune-checkpoint blockade PDL1 antibody atezolizumab. This KineTAC induced PDL1 degradation in breast cancer cells at nanomolar concentrations (Fig. 5c). The strategy was expanded with similar potency and efficacy to degrade the transmembrane targets HER2, EGFR, and PD1 and soluble targets VEGF and TNF. The additional cytokines IL-2, vMIP2 and CXCL11 were also co-opted via their respective receptors for degradation of PD1, illustrating promising generalizability.

Aptamer-based systems provide advantages over antibody-based platforms, including much lower molecular weight, a broad range of existing targeting ligands and a modular, rapid synthesis with known binding sites. An aptamer-based LYTAC platform (Apt-LYTAC) was developed in which the ASGPR-binding tri-GalNAc was conjugated to aptamers that targeted either extracellular PDGF or transmembrane PTK7 to induce lysosomal degradation<sup>224</sup> (Fig. 5d). Bispecific aptamers targeting the cell-surface lysosome-shuttling receptor IGFIIR induced endocytosis and lysosomal degradation of membrane proteins Met, PTK7 and HER2 (refs. 225,226). Furthermore, a CI-M6PR-dependent bispecific aptamer that covalently targets PDL1 induced degradation of PDL1 with improved on-target retention and efficiency compared with non-covalent counterparts<sup>227</sup> (Fig. 5e). These results demonstrate that the range of possible receptors could be expanded with an aptamer approach.

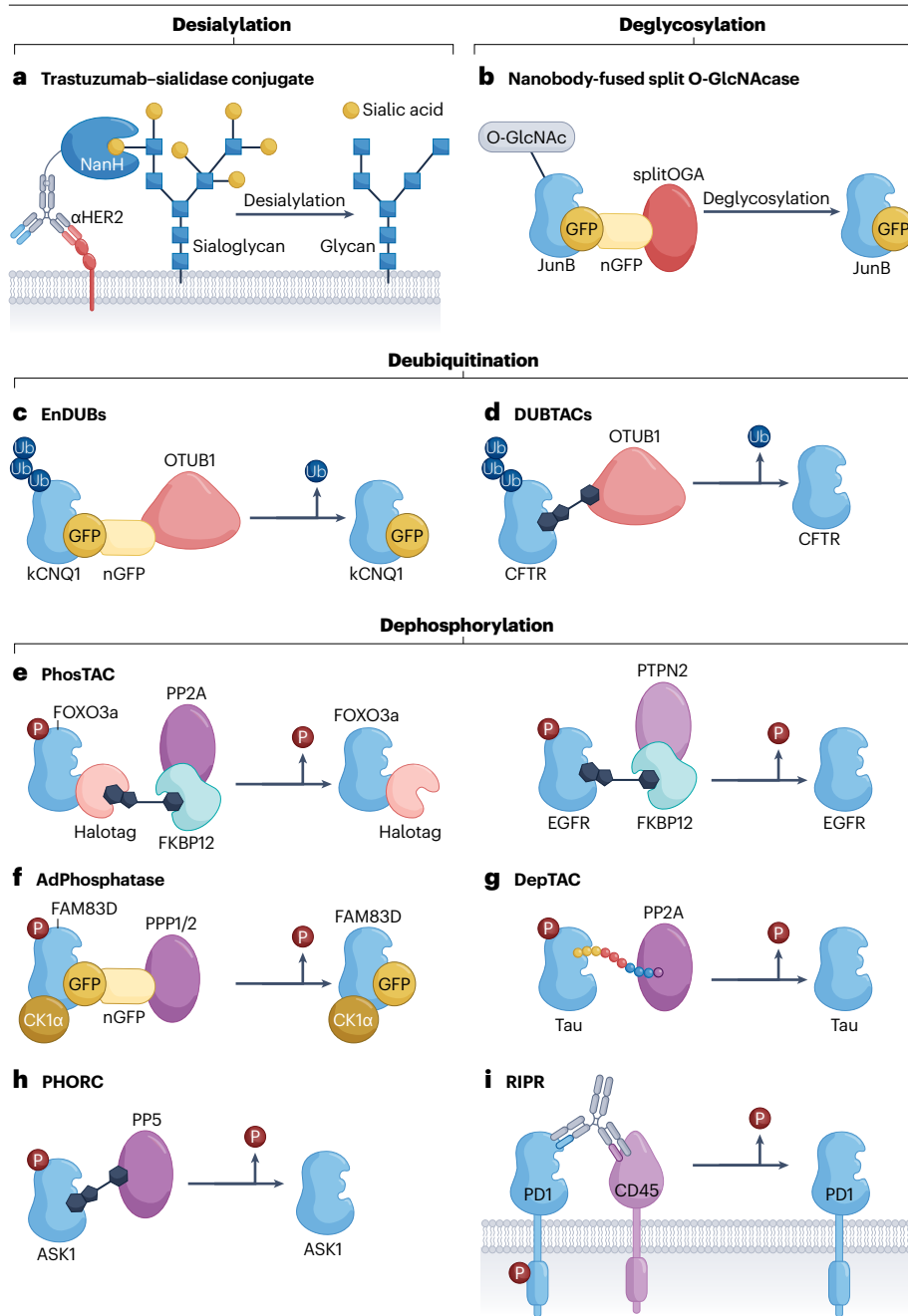
Bispecific antibodies that recruit two proteins into the lysosome simultaneously have also been explored as extracellular degraders. In the antibody-targeting chimaera platform, IgGs that bound one of the transmembrane E3 ligases RNF43 or ZNRF3 were engineered into bispecific antibodies that targeted either PDL1 or EGFR for lysosomal degradation<sup>228</sup> (Fig. 5f). Optimization efforts revealed that degradation efficiency is dependent on binding epitopes, orientation, valency and flexibility<sup>229</sup>. Proteolysis-targeting antibodies similarly induce proximity between protein targets and the extracellular domains of ZNRF3 or RNF43 to induce membrane clearance and proteasome-dependent degradation<sup>230</sup>. RNF43 is enriched in liver and intestinal tissues and exhibits higher expression in colon adenoma cancers, whereas ZNRF3 has lower tissue and cancer specificity. As such, the expansion of bispecific antibody techniques to other transmembrane E3 ligases and membrane-associated targets and their translation to the clinic will benefit from careful selection of ligase–target pairs to enable tissue or cancer-specific treatments.

Expansion to other membrane-associated receptors has been further explored through a variety of strategies. The receptor elimination by E3 ubiquitin ligase recruitment platform utilized highly specific heterobifunctional nanobodies against five transmembrane

RING-type E3 ubiquitin ligases (RNF128, RNF130, RNF167, RNF43 and ZNRF3), which were conjugated to target nanobodies and induced membrane clearance of EGFR, EPOR and PD1 targets at varying levels depending on the E3 (ref. 231) (Fig. 5g). Nanobodies were also designed as homobifunctional molecules to achieve clearance of E3 ligases from the membrane and downregulation of their signalling pathways. A short 5-amino acid gluten peptide that targets the active site of the transglutaminase TG2 conjugated with small molecules or peptides was able to internalize and degrade secreted, cell surface and transmembrane proteins<sup>232</sup>. Additionally, a cyclic Arg-Gly-Asp-containing pentapeptide was used to bind selectively to  $\alpha_v\beta_3$  integrin and linked to a small-molecule binder to induce the degradation of PDL1 (ref. 233) (Fig. 5h). Additionally, a poly-thymine dendronized DNA, which engages scavenger receptors (anionic ligand-binding receptors on the cell surface), was used to degrade EGFR and NCL<sup>234</sup>. All these strategies offer new opportunities for targeted degradation of extracellular and membrane proteins across different tissues and tumour types; for example, RNF128 is enriched in intestinal and liver tissues, RNF130 is enriched in innate immune cells, TG2 is enriched in cervical tissue,  $\alpha_v\beta_3$  integrin is associated with kidney and skin cancers, and many scavenger receptors exhibit differential expression profiles.

Lysosomal-sorting sequences (LSSs) in the cytosolic domains of lysosome-targeting receptors are leucine-based or tyrosine-based peptides that modulate clathrin-mediated endocytosis and lysosomal trafficking and can also be used as an internalization strategy. An LSS and cell-penetrating peptide conjugated to a covalent nanobody for PDL1 (in an agent termed GlueTAC) increased membrane clearance of PDL1 by more than 150% compared with a non-covalent analogue, and restored T cell activity and decreased tumour volume in mouse xenografts<sup>235</sup> (Fig. 5i). In another study, signal-mediated lysosome-targeting chimeras were developed by fusing the LSS motif from CI-M6PR to the C-termini of antibody binders for the membrane proteins EGFR, HER2, PDL1, CD20 and CD71, resulting in target degradation<sup>236</sup> (Fig. 5j). HER2 signal-mediated lysosome-targeting chimeras also attenuated signalling, resulting in suppressed cell proliferation and tumour growth. Taken together, these results suggest that peptide signalling motifs for lysosomal localization can be conjugated to several different target binders to induce uptake and degradation of membrane-associated proteins. This strategy for degradation of targets regardless of the cell type and differential expression of receptors could be useful in treating cells with immune escape or other resistance mechanisms, and the LSS motifs are genetically encoded, allowing for easier production and purification of uniform conjugates.

All these strategies towards control of cell surface or extracellular proteins have focused on eliminating undesired target proteins rather than restoring deficiencies in critical cell surface proteins. The Ber-tozzi lab attempted to hijack the native process of plasma membrane exchange between cells, called trogocytosis, by synthesizing chimeric ‘TrogoTACs’ consisting of a small-molecule ligand to the B cell receptor CD22 conjugated to an antibody for the target protein of interest<sup>237</sup>. Proof-of-concept experiments demonstrated CD22-dependent cell surface protein transfer of the therapeutically relevant target proteins CD30, IL2R $\alpha$ , PD1, EGFR and HER2 from their target cell to B cells. Additionally, TrogoTAC-mediated transfer of MHC class I to CD22-positive HeLa cells resulted in a functionally relevant immune response and increased cytotoxicity. This platform offers an exciting new paradigm of gain-of-function induced proximity.



**Fig. 6 | Targeted post-translational modification erasers.** **a**, The anti-HER2 antibody trastuzumab fused to the sialidase NanH facilitates removal of sialic acid from sialoglycans on the tumour cell surface. **b**, A domain of O-GlcNAcase (splitOGA) is fused to a nanobody and induces deglycosylation of a GFP-tagged target, JunB. **c**, In engineered deubiquitinases (EnDUBs), the catalytic domain of the deubiquitinase OTUB1 is fused to a nanobody recognizing GFP (nGFP), leading to deubiquitination of the GFP-tagged ion channel kCNQ1. **d**, Deubiquitinase-targeting chimeras (DUBTACs) are heterobifunctional small molecules that recruit the deubiquitinase OTUB1 to a target protein, for example, leading to deubiquitination of CFTR. **e**, The phosphorylation-targeting chimera (PhosTAC) platform employs FKBP12 fusions of the phosphatases PP2A or PTPN2 to target a tagged protein (HaloTag-FOXO3a) or an untagged protein (EGFR), together with a HaloTag linked to an FKBP12-targeting ligand for dephosphorylation. **f**, AdPhosphatase uses the phosphatases PPP1 and PPP2 fused to nGFP to dephosphorylate GFP-tagged targets such as FAM83D, which is phosphorylated by CK1α kinase. **g**, The dephosphorylation-targeting chimera (DepTAC) platform uses bispecific peptides to recruit the phosphatase PP2A to the phosphoprotein Tau to prompt dephosphorylation and degradation of Tau. **h**, Phosphatase recruitment chimeras (PHORCs) link small-molecule ligands for the phosphatase PP5 and the phosphoprotein ASK1, leading to ASK1 dephosphorylation. **i**, In receptor inhibition by phosphatase recruitment (RIPR), bispecific antibodies bind the membrane-localized phosphatase CD45 and the target PD1 receptor to effect dephosphorylation of PD1. O-GlcNAc, O-linked N-acetylglucosamine; Ub, ubiquitination.

## Bifunctional modulators of post-translational modifications

Protein quality control through autophagic or proteasomal degradation is just one pathway out of a vast set that control protein fate and function. Post-translational modifications, such as acetylation, phosphorylation, ubiquitination and glycosylation, can affect a protein's stability, interactome and localization, influencing cell signalling, gene expression, and a plethora of other cellular processes. The concepts of induced proximity established in the work discussed above have allowed the development of approaches to manipulate

post-translational modifications through bifunctional biological conjugates or small molecules (Fig. 6).

### Desialylation

Sialylation, a modification in which sialic acids are appended to the termini of lipoproteins and glycans, functions to induce cell signalling, promote anti-adhesion, and downregulate immune and anti-inflammatory responses. As such, sialoglycans on the cell surface serve as glycoimmune checkpoints by inhibiting immune cell activation<sup>238</sup>. Targeted desialylation can be both a powerful therapeutic

approach to direct immune responses to tumours and a tool to uncover signalling pathways underlying the antitumour immune response. The HER2 antibody trastuzumab conjugated to a sialidase achieved significant, selective and long-lived desialylation of HER2, resulting in natural killer cell-mediated antibody-dependent cellular cytotoxicity of HER2<sup>+</sup> cells<sup>239</sup> (Fig. 6a). This effect was highly dependent on expression of functional Siglec-E receptor on tumour-infiltrating myeloid cells within the tumour microenvironment<sup>240</sup>. Palleon Pharmaceuticals has a non-targeted sialidase (E-602) in phase II clinical trials but is also developing targeted sialidases, including one that targets the tumour-associated antigen B7H3 for tumour-selective desialylation.

## Deubiquitination for targeted protein stabilization

Targeted protein stabilization through deubiquitinase-targeting chimeras (DUBTACs) could remove ubiquitin from protein targets and specifically abrogate proteolytic degradation. Two main strategies have been developed towards this goal.

Engineered deubiquitinases were designed in which the catalytic domain of the deubiquitinase OTUD1 was fused to a nanobody that recognized YFP-tagged or GFP-tagged proteins and targeted them for deubiquitination<sup>241</sup> (Fig. 6c). These constructs provide tools to study the substrate scope of deubiquitinases and were applied to establish a proof of concept for their therapeutic relevance in rescuing the expression of ion channels in long QT syndrome and cystic fibrosis disease models<sup>241</sup>.

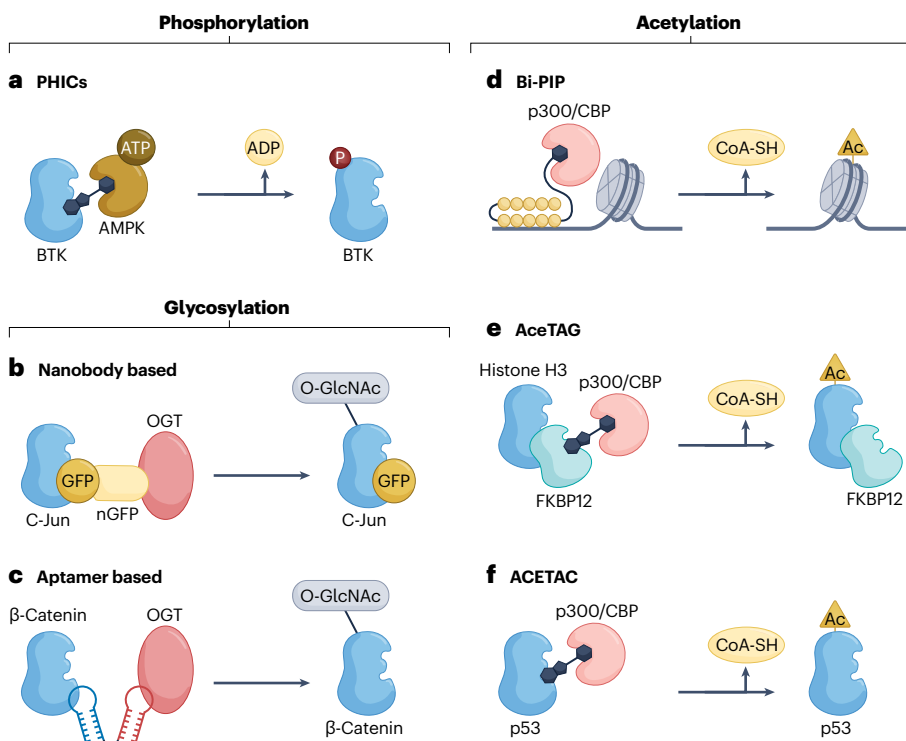
DUBTACs are heterobifunctional molecules comprised of a covalent ligand for the deubiquitinase OTUB1 linked to a molecule recognizing a specific target. Chimeric molecules recognizing the cystic fibrosis transmembrane conductance regulator CFTR led to its deubiquitination and stabilization, resulting in improved cell surface CFTR-dependent transepithelial conductance<sup>242</sup> (Fig. 6d). Similarly,

OTUB1-recognizing chimeras stabilized the tumour suppressor WEE1 and transcription factors FOXO, IRF and p53 (refs. 242,243).

DUBTACs have shown similar geometric restraints for ternary complex formation as PROTACs, suggesting that optimal pairing of deubiquitinases and linkers might be necessary for maximal stabilization<sup>242</sup>. Additionally, identifying which targets would benefit from stabilization in a diseased state with minimal off-targets in healthy cells will require meticulous selection criteria. Further, DUBTACs might not be able to control non-degradative ubiquitin-dependent processes. Agents for targeted protein deubiquitination are not yet in clinical trials although several companies are focused on developing them.

## Phosphorylation modulation

Phosphorylation of proteins has a myriad of effects in cells, including remodelling protein structure, inducing signalling cascades and modulating interactomes, including interactions between proteins and RNA or DNA. The first phosphorylation-inducing chimeric small molecules (PHICs) were designed using activators for the kinases AMPK or PKC fused to ligands of the targets BRD4 and BTK. Induced proximity of AMPK or PKC led to phosphorylation of BRD4 and BTK as neosubstrates<sup>244</sup> (Fig. 7a). Further efforts have demonstrated inhibitory neo-phosphorylation of BCR-ABL induced by PHICs and initiation of signalling cascades through tyrosine phosphorylation of EGFR<sup>245,246</sup>. PHICs exhibit hallmarks of bifunctional degraders, including ternary complex formation, catalytic turnover and conformation limitations but have limited scope because they require high-affinity, non-inhibitory kinase ligands. This issue can be circumvented by low-affinity kinase inhibitors, which are released from the inhibitor-binding pocket after initial binding through group-transfer chemistry of a covalent linkage onto a nearby cysteine residue<sup>245</sup>. BTK inhibitor scaffolds showed decreased occupancy in the ATP pocket compared with the original inhibitors and were used



**Fig. 7 | Targeted post-translational modification writers.** **a**, The phosphorylation-inducing chimeric small-molecule (PHIC) platform utilizes heterobifunctional small molecules to induce proximity between the kinase AMPK and protein target BTK to facilitate ATP-dependent BTK phosphorylation. **b**, A GFP nanobody (nGFP)-fused O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT) effects glycosylation of the GFP-tagged target cJun. **c**, A bispecific aptamer with dual affinity for OGT and the target  $\beta$ -catenin facilitates glycosylation of  $\beta$ -catenin. **d**, Bi-PIP molecules target histones for acetylation (Ac) using heterobifunctional constructs consisting of a polyamide DNA-binding scaffold linked to a small-molecule ligand for the p300/CBP histone acetyltransferase. **e**, The AceTAG system uses a heterobifunctional small molecule consisting of a p300/CBP ligand and FKBP12 ligand to facilitate lysine acetylation of target proteins such as histone H3. **f**, The acetylation-targeting chimera (ACETAC) system is a tag-free, small-molecule heterobifunctional approach wherein a ligand for p300/CBP is linked to a ligand for a mutant p53 target to facilitate acetylation.

in PHICs that increased phosphorylation of BRD4. This group-transfer chemistry can be explored in other induced proximity approaches to convert low-affinity inhibitors into bioactive non-inhibitory effectors.

Targeted dephosphorylation in the form of phosphorylation-targeting chimeras (PhosTACs) has also been investigated for attenuation of signalling pathways, activation of gene transcription or protein downregulation. Proof-of-concept chimeras using genetic systems with a serine–threonine phosphatase PP2A–FKBP12(F36V) fusion, Halo-tagged phosphoprotein substrates, and PhosTACs consisting of a HaloTag ligand linked to an FKBP12(F36V) ligand demonstrated dephosphorylation of FOXO3a and tau, resulting in activation of FOXO3a-responsive genes and tau downregulation, respectively<sup>247,248</sup> (Fig. 6e). Additionally, a PhosTAC that induced tyrosine dephosphorylation of EGFR by the phosphatase PTPN2 promoted apoptosis in cancer cells and led to different signalling effects compared with an EGFR-targeted tyrosine kinase inhibitor, highlighting differential modulatory properties of PhosTACs<sup>249</sup> (Fig. 6e). Another tag-based targeted dephosphorylation system, AdPhosphatase, used the PPP1 or PPP2A catalytic domains conjugated to an antigen-stabilized nanobody to dephosphorylate tagged protein targets FAM83D and ULK1, resulting in a system where off-target effects are controlled for by degradation of unbound phosphatase fusion proteins<sup>250</sup> (Fig. 6f). This approach elucidated a previously unknown role of CK1 $\alpha$ -mediated phosphorylation of FAM83D in FAM83D proteolysis and confirmed that different phosphatases are needed for specific phosphoprotein targets. Overall, these tools have informed translational design but are not themselves easily translated to the clinic.

As a parallel to initial PROTAC efforts, early dephosphorylation-targeting chimeras containing peptidyl ligands for phosphatases or phosphoproteins achieved modest dephosphorylation of AKT and tau, preventing tau accumulation<sup>251,252</sup> (Fig. 6g). However, these compounds required long treatment times, high concentrations or cell-penetrating peptides to improve potency.

The phosphatase recruitment chimaera system is the first small-molecule tag-free dephosphorylation approach. Compounds containing binders and activators of phosphatase PP5 linked to the ASK1 inhibitor TCASK10 resulted in PP5-dependent dephosphorylation of ASK1 and gastric cancer cell death<sup>253</sup> (Fig. 6h).

The receptor inhibition by phosphatase recruitment approach, in which bispecific antibodies bridge receptors containing tyrosine phosphorylation motifs to the cell-surface phosphatase CD45, resulted in target dephosphorylation and attenuation of receptor signalling<sup>254</sup> (Fig. 6i). Notably, chimaera-induced inhibition was more effective than monovalent antibody treatment alone.

Evidence suggests that phosphorylation changes could result in distinct functional consequences from kinase inhibition or activation, but the outcomes need to be established for each target. Clinical applications of targeted phosphorylation are being explored by Photys Therapeutics<sup>255</sup>.

## Glycosylation modulation

Protein glycosylation is implicated in many cellular processes, and its dysregulation can lead to cancer, diabetes and neurodegeneration, but the effects of glycosylation on specific sites within glycoproteins remain underexplored. Generalizable strategies for O-linked N-acetylglucosamine (O-GlcNAc) glycosylation and deglycosylation of target proteins have been developed to overcome limitations of other non-specific or restricted strategies. Engineered nanobody O-GlcNAc transferase (OGT) constructs were able to increase O-GlcNAcylation

of tagged JunB, cJun and Nup62 proteins with high specificity and conformity to the expected native glycosylation sites<sup>256</sup> (Fig. 7b). Similarly, the deglycosylating enzyme O-GlcNAcase (OGA) was split with one domain fused to a nanobody while the catalytic domain remained unfused such that catalytic deglycosylation would only occur upon nanobody-directed localization to a tagged target protein<sup>257</sup> (Fig. 6b). Notably, targeted deglycosylation of transcription factors cJun and cFos accelerated cJun degradation and deconvoluted some previously observed effects of deglycosylation on transcriptional activity of the cJun–cFos complex. Another platform, O-GlcNAcylation-targeting chimeras, utilized heterobifunctional small molecules to induce proximity between FKBP12(F36V)-tagged OGT and tagged substrates, resulting in O-GlcNAcylation of BRD4, CK2 $\alpha$  and EZH2 (ref. 258). These strategies provide useful tools to study O-GlcNAcylation effects in different cellular contexts, but further investigation of accessibility and specificity of glycosite targeting with this approach is necessary.

More recently, a dual-specificity aptamer targeting  $\beta$ -catenin for deglycosylation by OGT uncovered a role of  $\beta$ -catenin glycosylation in inhibiting and promoting protein–protein interactions and activating downstream transcription<sup>259</sup> (Fig. 7c). Specific glycosites and phenotypic effects were not investigated, making this modality more of a valuable chemical biology tool than a therapeutic modality.

## Acetylation modulation

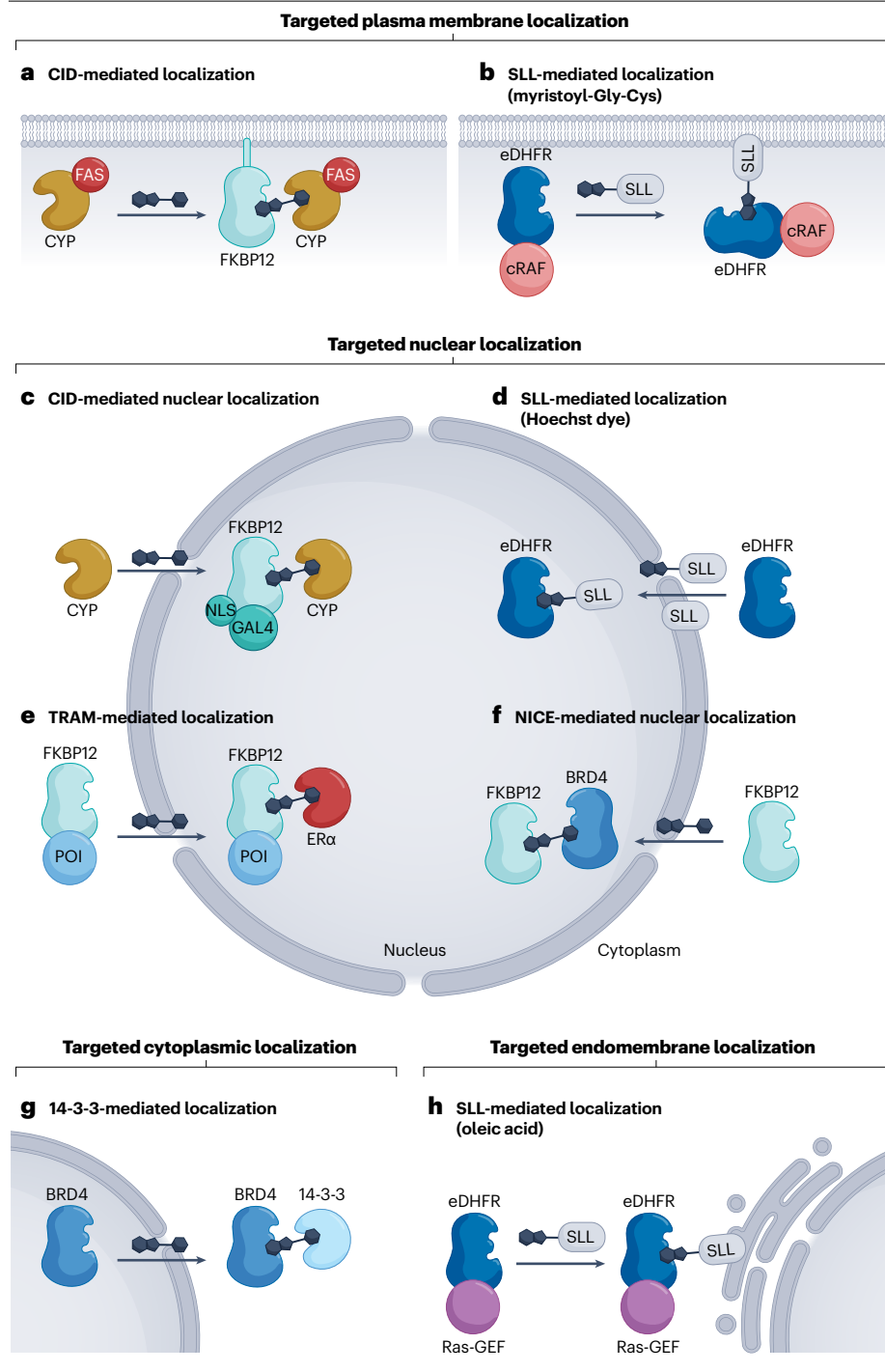
Protein acetylation on lysine residues is a widely occurring post-translational modification that affects disease states, protein stability and gene expression<sup>260</sup>. Bifunctional molecules termed Bi-PIPs, consisting of ligands for the histone acetyltransferase p300/CBP and polyamide DNA-binding scaffolds, were designed for targeted histone acetylation and resultant induced transcription of target genes<sup>261</sup> (Fig. 7d). Although increased histone acetylation within cells was confirmed, it was not well characterized and concerns exist about the pharmacokinetics of polyamides.

Another approach for inducing acetylation, AceTAG, targeted FKBP12-tagged proteins to p300/CBP or the lysine acetyltransferase PCAF/GCN5, resulting in selective acetylation at functionally relevant lysine residues on targets such as RelA and p53 (refs. 262,263) (Fig. 7e). Although no phenotypic effects were observed, p53-targeted acetylation events elicited acetyltransferase-specific differential transcriptional changes, and acetylation of hotspot p53 mutants resulted in p53 stabilization and increased activity. These tool compounds can be expanded to profile the effects of differential acetylation on targets of interest.

In the acetylation-targeting chimera (ACETAC) approach, a small-molecule bifunctional compound targeting p300/CBP was reported to acetylate a p53 mutant at Lys382 (ref. 264) (Fig. 7f). The chimaera arrested cell growth more potently than p53 inhibition alone, suggesting a therapeutic advantage for targeted acetylation in this context. These ACETAC compounds only utilize one acetyltransferase that, although promiscuous, does not have a fully defined substrate scope. Additionally, it is unclear how many protein targets can be functionally annotated using this ACETAC strategy or how specific acetylation will be.

## Bifunctional modulators of protein function

Several bifunctional modalities for modulating protein function independently of altering modifications have shown promising proof of concept, including approaches for targeted subcellular localization, targeted transcriptional activation and tissue-specific cell death.



**Fig. 8 | Examples of targeted protein localization.**

**a**, A cyclophilin (CYP) domain fused to the FAS death receptor localizes to the plasma membrane at a membrane-tethered FKBP12 protein through induced proximity using chemically induced dimerizer (CID) compounds that bind FKBP12 and CYP. **b**, The self-localizing ligand (SLL) myristoyl-Gly-Cys linked to an *Escherichia coli* dihydrofolate reductase (eDHFR) small-molecule ligand induces localization of eDHFR-tagged cRAF to the plasma membrane. **c**, A CID system (similar to part **a**) localizes CYP to the nucleus through proximity with a FKBP12–GAL4 fusion protein containing a nuclear localization signal (NLS). **d**, A SLL for the nucleus, Hoechst dye, linked to an eDHFR ligand effects nuclear localization of eDHFR. **e**, Targeted relocation-activating molecules (TRAMs) consist of an FKBP12 ligand conjugated to a small-molecule oestrogen receptor- $\alpha$  (ER $\alpha$ ) binder and function to localize a cytoplasmic FKBP12-tagged protein of interest (POI) to the nucleus. **f**, The nuclear import and control of expression (NICE) system utilizes a heterobifunctional small molecule consisting of ligands for FKBP12 and the nuclear protein BRD4 to effect nuclear localization of cytoplasmic FKBP12. **g**, A small-molecule covalent binder of the cytoplasmic 14-3-3 protein linked to a small-molecule BRD4 ligand results in cytoplasmic localization of nuclear BRD4. **h**, The SLL oleic acid linked to an eDHFR ligand localizes cytoplasmic eDHFR-tagged Ras-GEF to endomembranes of the endoplasmic reticulum or Golgi apparatus.

## Targeted protein subcellular localization

Historically, CIDs have been used to selectively localize FKBP12 and CypA fusion proteins using heterobifunctional compounds<sup>103,265,266</sup> (Fig. 8a). Efforts include the relocation of signalling proteins to the cell membrane, the oligomerization of receptors to initiate signalling cascades, the relocation of cytoplasmic proteins to the nucleus and recruitment of transcriptional activating domains to DNA (Fig. 8c).

Several groups have used the rapamycin CID system to achieve similar control over localization<sup>267</sup>. A more recent series of papers detailed the redirection of target proteins to the plasma membrane or other endomembranes using lipopeptide-based heterobifunctional small molecules in attempts to overcome stoichiometric challenges with CID approaches<sup>268–271</sup> (Fig. 8b,h). This system relied on an *Escherichia coli* dihydrofolate reductase (eDHFR) chemogenetic tag and trimethoprim

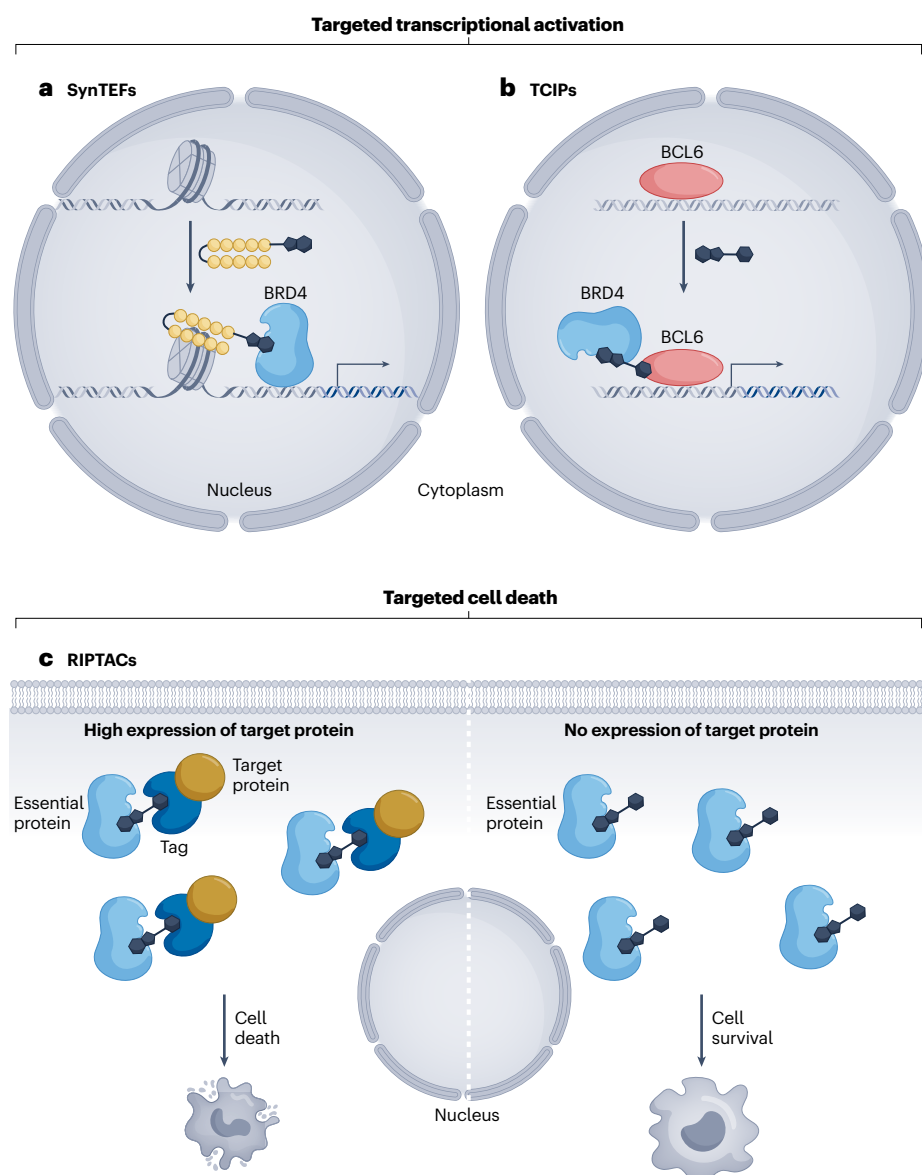
ligand pair that, when linked to a cysteine-tethered myristoyl lipopeptide, rapidly and reversibly localized the tagged protein to the plasma membrane. Nuclear targeting was also achieved by replacing the myristoyl group with the Hoechst DNA intercalator<sup>269</sup> (Fig. 8d). In another approach, targeted relocation-activating molecules that utilize nuclear hormone receptors, such as oestrogen receptor- $\alpha$  and glucocorticoid receptor, can localize various target proteins with chemogenetic tags into the nucleus<sup>272</sup> (Fig. 8e). These tools require expression of tagged proteins but are useful in elucidating which disease models could benefit from targeted localization.

Small-molecule tag-free approaches to targeted localization have now been demonstrated. A system whereby FKBP12 was sequestered into the nucleus using either glucocorticoid receptor or BRD4 as the recruiting protein and a bifunctional ligand that binds both the recruiter and FKBP12 (refs. 272,273) (Fig. 8f) was then used to re-localize several targets, including oncogenic mutant nucleophosmin

and a disease-driving mutant SMARCB1. Alternatively, a covalent fragment-based chimera that binds 14-3-3 sequestered BRD4 in the cytoplasm<sup>49</sup> (Fig. 8g). Protein sequestration appears to be dependent on the strength of the respective import and export signals on the proteins. Deeper evaluation of which targets can be successfully relocated and which shuttling proteins have the strongest localization signals will be required to enable therapeutic advancement of this technology.

## Targeted transcriptional activation

Targeted activation of transcription at desired genes has been achieved using genetic tags and the CID platform described above. Additionally, polyamide ligands for GAA DNA repeats were linked to the BRD4 ligand JQ1 to form molecules termed sequence-specific synthetic transcription elongation factors<sup>274</sup> (Fig. 9a). Sequence-specific synthetic transcription elongation factors activated frataxin gene expression



**Fig. 9 | Targeted cell death and transcriptional activation.** **a**, In sequence-specific synthetic transcription elongation factors (SynTEFs), a polyamide DNA-binding scaffold linked to a ligand for transcriptional activator BRD4 activated gene expression at various loci. **b**, Transcription chemical inducers of proximity (TCIP) compounds consist of chimeric small-molecule ligands for a transcriptional regulator, such as BCL6, and the transcriptional activator BRD4. The compounds recruit BRD4 to BCL6-repressed promoters and activate transcription of downstream genes. **c**, Regulated induced proximity-targeting chimeras (RIPTACs) are small-molecule heterobifunctional compounds containing ligands for a widely expressed essential protein and a tagged target protein with more tissue-specific expression. Induced proximity in cells expressing the tissue-specific target protein results in sequestration and inhibition of the essential protein and causes cell death, while cells lacking expression of the target protein survive.

and rescued mitochondrial function in Friedrich's ataxia disease models, but genetic tag and polyamide-based approaches are not therapeutically tractable.

Transcription chemical inducers of proximity consist of chimeric protein-targeting ligands that bind a transcriptional or epigenetic regulator and a second transcription factor that interacts with the regulatory region of a target gene, functionally activating expression of the target gene<sup>275</sup> (Fig. 9b). Proof of concept was achieved by recruiting BRD4 or oestrogen receptor to BCL6, resulting in activation of BCL6-repressed promoters and functional downstream transcriptional effects. Similarly to other chimeric modalities, only fractional occupancy of BRD4 was necessary to induce robust transcriptional changes. These molecules are more potent antiproliferative agents than degraders or inhibitors of BET proteins or BCL6, suggesting a gain-of-function mechanism and a therapeutic benefit over existing treatments. However, these compounds are limited to cell lines with high BCL6 expression, so further efforts targeting proteins such as TP53 or BCL2 are needed.

## Targeted cell death

Building on the concept of targeted protein inhibition using CIDs, the regulated induced proximity-targeting chimera (RIPTAC) system involves inducing proximity between a specific intracellular target protein and a widely expressed essential effector protein required for cell survival. The interaction abrogates function of the essential protein and leads to cell death only in cells expressing the target protein<sup>276</sup> (Fig. 9c). The proof-of-concept system utilized chemogenetic tags and the effectors BET-domain proteins, PLK1 and CDK proteins to profile differential cell proliferation upon RIPTAC treatment in various cell lines. Non-covalent compounds seemed more effective at linking proteins that usually exist in different subcellular compartments. However, it was not clear whether the antiproliferative effects were due to inhibition of the effector protein, subcellular redirection, aggregation or a combination of these mechanisms. This approach has the potential to induce tumour-specific cell death without directly engaging a disease-causing mutant. A RIPTAC for prostate cancer, HLD-0915 (Halda Therapeutics), is entering phase I clinical trials (NCT06800313)<sup>277</sup>.

## Conclusion

The topic of induced proximity modalities and therapeutics is an exciting and rapidly growing field. The rich history of molecular glues, beginning with auxin, CsA and thalidomide, has paved the way for the discovery of molecular glue inhibitors, degraders, stabilizers and activators. Although many of the molecular mechanisms of action were discovered much later than the commercial approval of the drugs, mechanistic insight has been pivotal for the field to advance beyond serendipitous discovery and has led to the emergence of rational design and the discovery of synthetic and natural product molecular glues. Bifunctional targeted protein degradation approaches initially helped to overcome the limited target scope of existing molecular glue degraders but have since evolved far beyond that to targeting post-translational modifications and controlling cellular processes. Expansion of targeted protein degradation approaches to involve components of the lysosomal pathways has allowed many historically undegradable targets, particularly membrane proteins and protein aggregates, to be cleared from cells.

The principles of designing an ideal induced proximity molecule drug follow the same rules as for designing the ideal small-molecule inhibitor: excellent oral bioavailability, high potency, limited off-target

effects, and favourable pharmacokinetic and pharmacodynamic properties. Of the induced proximity modalities we have discussed, molecular glues most closely meet these criteria. They are smaller, typically form more stable and high-affinity ternary complexes through positive cooperativity, and demonstrate selective interactions with an interaction surface formed by both binding partners and little affinity for each protein individually. In theory, clinical translation of any induced proximity mechanism would benefit from the favourable chemical properties of molecular glues. The stability of glue-induced ternary complexes is favourable for degradation and sequestration but could be problematic when maintenance of native effector functions is necessary. Notably, targeted deubiquitination, post-translational modifications or transcriptional activity approaches that provide rescue of function would suffer from compound-induced cytotoxicity or altered basal functioning and might be more feasible with bifunctional approaches.

Bifunctional molecules benefit from their modularity and frequently employ existing ligands for efficacy. We have discussed the benefits of inhibitor use in PROTACs, but there are several associated challenges. Ultimately, the number of bifunctional molecules that can be developed from inhibitors is still limited by the number of inhibitors themselves. Although an inhibitor can be synergistic with degraders, the use of inhibitors for other non-degradative induced proximity modalities that require functional effectors would obstruct the efficacy of the mechanism. As such, we believe that annotation of silent protein binders that do not disrupt function is imperative for the field. Other promising strategies include ligand-directed release chemistry or modification of existing inhibitors to maintain binding with minimal effect on protein activity. Proteome-wide fragment ligand discovery has already begun, and optimization of these fragments or expansion of the profiled chemical space will be critical to allowing full translation of non-degradative approaches.

It is also important to consider the strengths and weaknesses of biologic-based induced proximity modalities as compounds such as LYTACs approach the clinic. Bispecific antibodies mainly benefit from high specificity and reduced off-target effects. Significant advances in formatting approaches have made bispecific antibody discovery both rapid and efficient. Recently discovered recycling mechanisms of internalized bispecific antibodies also suggest that they can achieve catalytic activity, although that would require significant optimization of each system and cellular context. Additionally, although they are not cell permeable, their utilization in intercellular signalling or cell surface receptor degradation is not strongly affected by this limitation. Their high specificity is an advantage over small-molecule degraders but could be a detriment in post-translational modification-based modalities, as discussed above. Additional challenges associated with biologics are that they are not orally bioavailable, are more expensive to administer, can be highly immunogenic, and are limited to extracellular or cell surface protein targets. A combination of a small molecule and a biologic, as in the case of antibody-degrader conjugates, is a powerful emerging therapy that can achieve a better therapeutic window through delivery of small-molecule degraders to specific cell and tissue types. The next few years will test the application of these combined modalities as protein degraders in the clinic and will inform the next generation of discovery.

As we expand the proximity-inducing modalities, a consideration is not to develop 'overengineered hammers' for which there are no differentiated 'nails' or applications. Just because an induced proximity modality can be developed does not mean that it will necessarily have

a broad application. With each new proximity-inducing technology comes different challenges associated with manipulating the biology of the respective targets. Looking to the future, exciting potential areas of expansion in induced proximity paradigms include the rational design and discovery of non-degradative molecular glues in a target-centric manner, expansion of approaches for targeted transcriptional repression or activation, and the development of transcriptional modulators to confer novel programming.

As new modalities in this field are developed and existing modalities progress, a critical consideration will be whether we can harness these new platforms to create truly distinct medicines against therapeutic targets that could not have been developed with other modalities. We are nonetheless hopeful that proximity-inducing medicines will usher in a new era of next-generation therapeutics against previously intractable targets.

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## Author contributions

E.A.K. and M.M. researched data for the article. All authors contributed substantially to the discussion of the content. All authors wrote the article. All authors reviewed and/or edited the manuscript before submission.

## Competing interests

D.K.N. is a co-founder, shareholder and scientific advisory board member for Frontier Medicines and Zenith Therapeutics, is also on the scientific advisory board of and receives payment and/or holds shares in The Mark Foundation for Cancer Research, Oerth Bio, Photys Therapeutics, Apertor Pharmaceuticals, Oerth Bio, Ten30 Biosciences, and Deciphera Pharmaceuticals, and is an Investment Advisory Partner for a16z Bio, an Advisory Board member for Droia Ventures, and an iPartner for The Column Group.

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