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PROXIMITY-INDUCED DEGRADERS (TDPs) - BROAD CATEGORY THAT INCLUDES PROTACs

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ABSTRACT:

Target Protein Degraders (TPDs) have emerged as a transformative therapeutic approach that enables the selective elimination of disease-associated proteins by exploiting the cell's endogenous protein degradation machinery. Unlike conventional small-molecule inhibitorthat temporarily suppress protein activity, TPDs promote the complete removal of target proteins, offering a novel strategy to address both druggable and previously undruggable targets. Among the various TPD technologies, Proteolysis-Targeting Chimeras (PROTACs) have gained significant attention due to their ability to recruit E3 ubiquitin ligases and induce ubiquitin-mediated proteasomal degradation of specific proteins.

The application of TPDs has shown remarkable potential in the treatment of cancer, where aberrant protein expression and signaling pathways contribute to tumor initiation, progression, and therapeutic resistance. By selectively degrading oncogenic proteins, TPDs provide opportunities to overcome limitations associated with traditional inhibitors. In addition, TPDs have emerged as promising candidates for neurodegenerative disorders, including Alzheimer's scharacterized by the accumulation of toxic and misfolded proteins. The targeted removal of these pathogenic proteins may offer disease-modifying benefits beyond symptomatic treatment.

Keywords:

Targeted Protein Degraders (TPDs), PROTACs, Targeted Protein Degradation, Cancer Therapy, Neurodegenerative Diseases, Ubiquitin–Proteasome System, E3 Ligases, Drug Discovery.

INTRODUCTION:

Although chemotherapy remains a cornerstone of cancer treatment, its clinical effectiveness is frequently constrained by limited response rates, systemic toxicity, and the development of resistance. Advances in targeted therapy have led to the emergence of small-molecule inhibitors (SMIs), which selectively interact with specific proteins of interest (POIs) involved in tumor progression. A notable example is chronic myeloid leukemia (CML), where the introduction of tyrosine kinase inhibitors has transformed the disease from one primarily managed with chemotherapy into a chronic, controllable condition, increasing the 10-year survival rate to approximately 83.3%. Despite these successes, SMIs continue to face important limitations. Their imperfect target selectivity can produce undesirable off-target effects by interfering with normal cellular proteins. In addition, therapeutic resistance commonly arises through mechanisms including target mutations, overexpression of the target protein, or activation of compensatory signaling pathways. Furthermore, many disease-associated proteins lack suitable ligand-binding pockets, rendering them inaccessible to conventional small-molecule therapeutics and often classifying them as "undruggable."

TPD is a new therapeutic approach that can overcome limitations of current treatments. It's like a targeted cleanup crew for disease-causing proteins . Instead of merely inhibiting protein function, TPD harnesses endogenous cellular degradation pathways, particularly the ubiquitin–proteasome system (UPS) and lysosomal degradation machinery, to selectively eliminate pathogenic proteins. This event-driven mechanism offers several potential advantages, including improved target specificity, the ability to overcome acquired drug resistance, and access to proteins that cannot be effectively targeted by traditional inhibitors. Consequently, TPD has opened new therapeutic opportunities for a broad range of previously inaccessible disease-related proteins.

The concept of TPD was first proposed in 1999 through a patent filed by Proteinix, marking the beginning of a field whose molecular mechanisms were initially not well understood. As research progressed, efforts shifted toward elucidating the biological basis of targeted degradation, leading to the development of proteolysis-targeting chimeras (PROTACs) and a deeper mechanistic understanding of molecular glues (MGs). These discoveries established the foundation for rapid expansion of the field, resulting in the design of numerous PROTACs and molecular glues, several of which have advanced into clinical evaluation. More recently, lysosome-mediated degradation strategies have further broadened the scope of TPD by enabling the selective degradation of extracellular, membrane-bound, and other proteins that are not readily accessible through proteasomal pathways. In this review, we discuss the evolution of targeted protein degradation technologies, with particular emphasis on PROTACs and molecular glues, highlighting recent advances, optimization strategies, and their potential to reshape the future of precision cancer therapy.

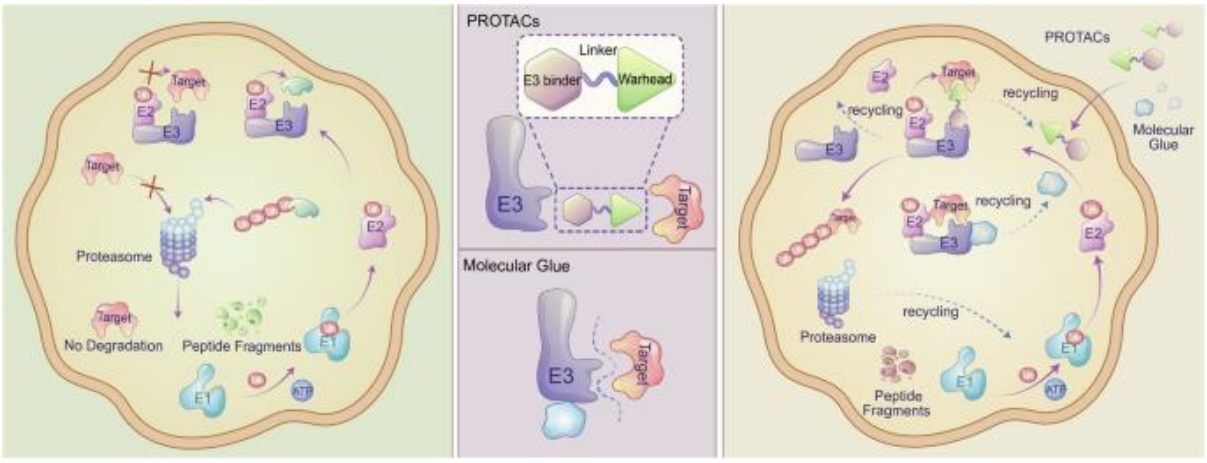
he development of TPD tech has three eras:Foggy Era: Thalidomide & CsA laid groundwork, but mechanisms were unclearDeciphering Era: TPD concept formed, molecular mechanisms understoodGlorious Era: Rapid clinical progress, new pathways (like LYTAC), transforming disease treatments

Different TPD strategies: mechanisms, development, and advancement:

Proteasome-Mediated Protein Degradation:

Proteasome-dependent degradation represents one of the most extensively studied approaches in targeted protein degradation (TPD). Two major technologies, proteolysis-targeting chimeras (PROTACs) and molecular glues (MGs), exploit the ubiquitin–proteasome system (UPS) to achieve selective elimination of disease-associated proteins. The UPS is the primary intracellular pathway responsible for maintaining protein homeostasis by recognizing and removing damaged, misfolded, or unnecessary proteins. In this process, target proteins are covalently modified with ubiquitin through a coordinated cascade of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin ligase (E3) enzymes. Polyubiquitinated proteins are subsequently recognized and degraded by the 26S proteasome, while deubiquitinating enzymes recycle ubiquitin molecules for repeated use

FIG:1



Schematic overview of targeted protein degradation (TPD) modalities:

The left panel illustrates the ubiquitin–proteasome system (UPS), where proteins that are not recognized by E3 ubiquitin ligases remain stable and are not directed toward degradation. Two TPD strategies compared:Molecular Glues (MGs):PROTACs The right panel depicts the degradation mechanism, highlighting how PROTACs repeatedly recruit a target protein and an E3 ligase to form a productive complex that promotes ubiquitination and subsequent proteasomal degradation. It also demonstrates how molecular glues enhance or stabilize protein–protein interactions between an E3 ligase and the target protein, leading to ubiquitination and degradation. Following this process, both PROTACs and molecular glue molecules are released and can participate in additional degradation cycles.

PROTACs are heterobifunctional molecules consisting of three essential components: a ligand that binds the protein of interest (POI), a ligand that recruits an E3 ubiquitin ligase, and a chemical linker connecting the two. By simultaneously engaging both proteins, PROTACs induce the formation of a ternary complex that enables selective ubiquitination and proteasomal degradation of the target protein. Because PROTACs function catalytically and are regenerated after each degradation event, a single molecule can eliminate multiple copies of the target protein, thereby enhancing therapeutic efficiency. Despite these advantages, PROTACs face several limitations. Their relatively large molecular size and structural complexity often result in poor membrane permeability, reduced metabolic stability, and suboptimal pharmacokinetic properties. Furthermore, exposure to high PROTAC concentrations may produce the "hook effect," in which excessive binary complex formation interferes with productive ternary complex assembly, ultimately reducing degradation efficiency. To address these challenges, current research is focused on developing smaller, more stable PROTACs with improved bioavailability and drug-like characteristics.

In contrast, molecular glues are low-molecular-weight compounds that promote or stabilize protein–protein interactions between an E3 ligase and a target protein without requiring a bifunctional architecture. Their compact size generally provides superior cellular permeability and increases the potential for oral administration compared with PROTACs. Molecular glues also avoid the hook effect, making their degradation activity less susceptible to concentration-dependent loss of efficacy. However, the rational design of molecular glues remains difficult because their activity depends on inducing highly specific and often unpredictable protein–protein interactions. Consequently, many molecular glues have been identified through phenotypic screening or serendipitous discovery rather than structure-based design. Advances in high-throughput screening and computational approaches are expected to facilitate the identification of new molecular glue candidates capable of selectively modulating protein–protein interactions.

Although PROTACs and molecular glues employ distinct mechanisms to induce targeted protein degradation, they are complementary therapeutic strategies. Continued improvements in molecular design, screening technologies, and mechanistic understanding are expected to overcome their individual limitations and expand the clinical applications of targeted protein degradation for the treatment of a wide range of diseases.

PROTACs:

CRBN: CRBN, another critical substrate receptor of the CRL4 E3 ligase complex, is targeted by thalidomide and its analogs, also known as immunomodulatory drugs (IMiDs) like pomalidomide and lenalidomide. These drugs bind to CRBN, leading to the CRBN-dependent degradation of substrates such as IKAROS family zinc finger proteins 1 and 3 (IKZF1/3), pivotal in disease-related protein degradation.[63,64,65,66] Thalidomide gained infamy due to its teratogenic effects but has been repurposed in PROTAC technology for targeted protein degradation. In 2015, the first CRBN-recruiting PROTAC, dBET1, was generated,[67] following the report of co-crystal structure of DDB1–CRBN–thalidomide complexes.[68,69] dBET1 exhibited pronounced depletion ability of POIs at 100 nM in acute myeloid leukemia (AML) cell lines. Given the satisfactory clinical effectiveness and low molecular weight of IMiDs, many labs dedicated to explore novel CRBN modulators. Of note, TD-106, a pomalidomide derivative, was used to synthesize potent bromodomain-containing protein 4 (BRD4) and AR PROTACs.[70,71] While IMiDs were prone to hydrolysis,[67,72] this problem was effectively addressed by the development of phenyl-glutarimide analogs-based PROTACs, which maintained targeting

specificity without degrading IKZF1/3.[73] Another novel CRBN ligand, phenyl dihydrouracil, enhanced binding affinity, leading to highly potent degradation with low cytotoxicity.[74] Recently, achiral phenyl dihydrouracil (also called PDHU) derivatives were designed as CRBN ligands and corresponding PROTACs exhibited robust degradation at 569+picomolar concentrations.

Due to their stable metabolism, potent degradation capabilities, broad distribution, and relatively low molecular size, CRBN ligands are increasingly favored in the development of orally bioavailable PROTACs currently advancing in clinical trials.

Others: Researchers have uncovered that expression levels and types of E3 ligases vary across tissues, which affect the degradation activity of PROTACs.[75,76,77,78]

The concept of proteolysis-targeting chimeras (PROTACs) was first demonstrated by Sakamoto and colleagues using peptide-based molecules. Although these early PROTACs successfully induced targeted protein degradation, their relatively large molecular size and peptide backbone resulted in poor membrane permeability, limiting their therapeutic applicability. The introduction of fully small-molecule PROTACs in 2008 represented a major breakthrough by improving cellular uptake, pharmacokinetic properties, and overall drug-like behavior. Since then, the field has expanded rapidly, with numerous PROTACs being developed against diverse disease-associated proteins, several of which have progressed into clinical evaluation.

The efficiency of PROTAC-mediated degradation depends on multiple factors, including the binding affinity of the ligand for the protein of interest (POI), recruitment of the E3 ubiquitin ligase, linker length, flexibility, and chemical composition, as well as cellular factors such as E3 ligase expression and proteasome activity. Current research is therefore focused on optimizing these structural and biological parameters to improve degradation potency, selectivity, metabolic stability, and oral bioavailability.

E3 Ubiquitin ligases:

E3 ubiquitin ligases are indispensable components of the ubiquitin–proteasome system because they determine substrate specificity during ubiquitination by transferring ubiquitin from E2 conjugating enzymes to target proteins. Although the human genome encodes more than 600 E3 ligases, only a limited number have been successfully exploited in targeted protein degradation. Among these, mouse double minute 2 (MDM2), inhibitor of apoptosis proteins (IAPs), von Hippel–Lindau (VHL), and cereblon (CRBN) remain the most widely utilized. Expanding the repertoire of E3 ligases and developing high-affinity ligands for additional ligases are important strategies for broadening the applicability of PROTAC technology and improving therapeutic performance.

MDM2-Based PROTACs:

MDM2 is a well-characterized E3 ubiquitin ligase that negatively regulates the tumor suppressor protein p53 by promoting its ubiquitination and degradation. Consequently, inhibition of the MDM2–p53 interaction has long been pursued as a therapeutic strategy for cancers retaining wild-type p53. Although several small-molecule MDM2 inhibitors have entered clinical development, their therapeutic benefit has been limited by dose-related toxicity and the emergence of resistance, particularly in tumors carrying TP53 mutations.

The availability of potent MDM2 ligands, especially Nutlin-3, enabled the development of the first MDM2-recruiting small-molecule PROTACs. Early compounds demonstrated selective degradation of the androgen receptor, although their degradation efficiency was relatively modest. Subsequent optimization of ligand affinity and linker design substantially improved target degradation and produced pronounced cytotoxic effects in cancer cells while sparing normal tissues. An additional advantage of MDM2-based PROTACs is the stabilization of p53 following MDM2 recruitment, which enhances antiproliferative activity in several hematological malignancies.

A significant milestone in this field was the development of MDM2 homo-PROTACs, which recruit MDM2 to itself, triggering its self-ubiquitination and degradation. These studies provided proof-of-concept that E3 ligases can themselves serve as degradation targets. More recently, KT-253 has emerged as one of the most

potent MDM2-directed degraders, exhibiting markedly greater antitumor activity than conventional MDM2 inhibitors and producing sustained tumor regression in preclinical xenograft models. This promising candidate has subsequently advanced into Phase I clinical evaluation. Nevertheless, widespread application of MDM2-based PROTACs remains constrained by challenges including complex synthetic routes, high molecular weight, and excessive lipophilicity.

IAP-Based PROTACs (SNIPERs):

Members of the inhibitor of apoptosis protein (IAP) family possess dual biological functions, acting both as E3 ubiquitin ligases and as endogenous inhibitors of apoptosis through suppression of caspase activity. Among them, cIAP1, cIAP2, and XIAP are frequently overexpressed in malignant tissues, making them attractive targets for anticancer drug development.

Small-molecule IAP antagonists have been successfully adapted as E3 ligase-recruiting ligands in PROTACs, giving rise to a specialized class known as Specific and Non-genetic IAP-dependent Protein Erasers (SNIPERs). Unlike conventional PROTACs, SNIPERs often induce simultaneous degradation of both the protein of interest and cIAP1, thereby providing an additional mechanism for suppressing tumor cell survival.

Several IAP ligands have been incorporated into SNIPERs, including methyl bestatin, LCL-161, and MV1 derivatives. Bestatin primarily targets cIAP proteins, whereas LCL-161 and MV1 function as pan-IAP antagonists by interacting with both cIAPs and XIAP. The earliest SNIPERs, synthesized using methyl bestatin, demonstrated target protein degradation together with cIAP1 autoubiquitination. Subsequent structural modifications produced amide-based SNIPERs with improved selectivity toward target proteins. Later generations employing MV1 and LCL-161 achieved more efficient dual degradation and displayed superior antiproliferative activity compared with bestatin-derived compounds. Consequently, SNIPER technology has become a versatile platform for degrading a broad spectrum of disease-associated proteins while simultaneously eliminating cIAP1.

VHL-Based PROTACs:

Von Hippel–Lindau (VHL) is among the most extensively investigated E3 ligases used in targeted protein degradation. As the substrate recognition component of the Cullin-2 E3 ligase complex, VHL recognizes hydroxylated hypoxia-inducible factor-1α (HIF-1α) under normoxic conditions, directing it toward ubiquitination and proteasomal degradation. HIF-1α regulates numerous physiological processes, including cellular adaptation to hypoxia, erythropoiesis, angiogenesis, and tumor progression, making the VHL–HIF-1α interaction an attractive target for ligand development.

Structural characterization of the VHL–HIF-1α binding interface enabled the rational design of high-affinity VHL ligands. Initial ligands were generated by modifying peptide fragments derived from HIF-1α, but these compounds exhibited only moderate potency and poor membrane permeability. Continued medicinal chemistry optimization led to the discovery of potent small-molecule ligands such as VH032 and the second-generation analogue VH298, both of which bind VHL with nanomolar affinity.

These advances facilitated the development of the first VHL-recruiting PROTACs, which demonstrated highly efficient degradation of target proteins in cellular models. Since then, VHL has become one of the most widely employed E3 ligases in PROTAC design because of its well-characterized binding pocket, availability of potent ligands, and favorable degradation efficiency. An additional therapeutic advantage is the relatively low expression of VHL in platelets, which may reduce the risk of thrombocytopenia and other platelet-associated toxicities compared with degraders that recruit alternative E3 ligases.

Emerging E3 Ligases and Linker Optimization in PROTAC Design:

Although PROTAC technology has achieved considerable success, the emergence of acquired resistance remains a significant challenge. Recent studies have shown that resistance can arise from genomic alterations affecting components of the ubiquitin–proteasome system, particularly E3 ubiquitin ligases and their associated machinery. Such alterations impair the recruitment of E3 ligases, thereby reducing the efficiency of

target protein degradation. Consequently, expanding the repertoire of E3 ligases available for PROTAC development has become an important objective for overcoming resistance and broadening the range of proteins that can be therapeutically degraded.

To date, most clinically relevant PROTACs have relied on a small group of E3 ligases, including VHL, CRBN, MDM2, and IAPs. However, advances in chemical biology and ligand discovery have identified numerous additional E3 ligases that can be exploited for targeted protein degradation. More than a dozen alternative E3 ligases have now been incorporated into PROTAC design, providing new opportunities to improve tissue selectivity, circumvent resistance mechanisms, and target proteins that are not efficiently degraded using conventional ligases. A summary of these emerging E3 ligases is presented in **Table 2**.

Importance of Linker Design in PROTACs:

The linker is a critical structural element of PROTAC molecules because it connects the ligand for the protein of interest (POI) with the E3 ligase ligand, enabling productive formation of the POI–PROTAC–E3 ligase ternary complex. The physicochemical characteristics of the linker strongly influence degradation efficiency, selectivity, and pharmacological performance. Parameters such as linker length, flexibility, attachment sites, and overall chemical composition determine the spatial orientation of the two ligands and ultimately affect the stability and cooperativity of ternary complex formation.

Advances in structural biology, including co-crystal structures of ternary complexes, together with molecular modeling and computational simulations, have greatly improved the rational design of PROTAC linkers. These approaches facilitate identification of optimal attachment positions and appropriate linker lengths required to maximize target degradation while minimizing unfavorable molecular conformations.

Among the various linker chemistries explored, polyethylene glycol (PEG) and alkyl chains remain the most widely used because they provide synthetic versatility and permit fine-tuning of linker length and flexibility. PEG-based linkers generally enhance aqueous solubility, whereas alkyl chains contribute hydrophobic interactions that may improve membrane permeability. Owing to these favorable properties, both linker types are frequently incorporated into research-oriented PROTACs.

Despite their widespread use, flexible linkers may adopt multiple conformations that reduce the stability of the ternary complex. In contrast, rigid linkers restrict molecular flexibility and can stabilize the bioactive conformation of PROTACs, thereby improving degradation efficiency as well as pharmacokinetic properties such as solubility and bioavailability. A common strategy involves initially determining the optimal linker length using flexible PEG or alkyl chains, followed by replacement with structurally constrained linkers of comparable length to further enhance biological activity.

An innovative example of linker engineering was reported for the BET degrader MZ1, in which a macrocyclic architecture was introduced by connecting the VHL ligand to the original PEG linker through an additional covalent bridge. This conformational constraint stabilized the active ternary complex while maintaining cellular degradation activity. Although the resulting macroPROTAC-1 exhibited somewhat lower binding affinity than its parent compound MZ1, it retained comparable target degradation potency in cellular assays, highlighting the potential of macrocyclization as an effective strategy for next-generation PROTAC optimization.

Molecular Glues (MGs)

Molecular glues (MGs) represent a distinct class of targeted protein degradation (TPD) agents that function by promoting or stabilizing protein–protein interactions (PPIs). Unlike bifunctional PROTACs, MGs are single, low-molecular-weight compounds that induce the association of two proteins that would otherwise interact weakly or not at all. Their ability to remodel protein interfaces has attracted considerable interest as an alternative strategy for selective protein degradation.

Subsequent studies revealed that many molecular glues exert their biological activity by facilitating interactions between E3 ubiquitin ligases and specific target proteins. This induced proximity promotes

ubiquitination of the target protein, followed by its degradation through the ubiquitin–proteasome system. Owing to this mechanism, molecular glues have emerged as an important complement to PROTAC-based approaches for targeted protein degradation.

A major advantage of molecular glues lies in their favorable physicochemical properties. Because they are small, monovalent molecules, most MGs conform to Lipinski's Rule of Five, resulting in improved membrane permeability, enhanced oral bioavailability, and more desirable pharmacokinetic characteristics compared with the larger and more structurally complex PROTACs.

Despite these benefits, the rational discovery of molecular glues remains considerably more difficult than that of PROTACs. Their activity depends on identifying suitable protein surfaces capable of forming productive induced interactions, many of which are cryptic or transient. Consequently, numerous molecular glues have historically been discovered through phenotypic screening or serendipitous observations rather than through conventional structure-based drug design.

To date, only three amide-containing molecular glues have received regulatory approval for the treatment of multiple myeloma (MM) and myelodysplastic syndromes (MDS). Nevertheless, rapid advances in screening technologies, structural biology, and medicinal chemistry have accelerated the discovery of new molecular glue candidates. As a result, increasing numbers of these compounds are progressing through preclinical development and clinical trials, underscoring their growing potential as a therapeutic modality for targeted protein degradation.

Non-degradative MGs:

Early Development of Molecular Glues

The concept of molecular glues (MGs) originated from studies of naturally occurring microbial products that were initially developed as immunosuppressive agents. Among the earliest and most influential examples are cyclosporin A, FK506 (tacrolimus), and rapamycin (sirolimus), all of which have played a pivotal role in understanding induced protein–protein interactions.

Cyclosporin A was first identified in 1971 by researchers at Sandoz during the search for biologically active microbial metabolites. Its potent immunosuppressive activity soon established it as a promising therapeutic agent. Subsequently, FK506 and rapamycin were discovered and found to exhibit similar immunomodulatory properties. Structurally, FK506 and rapamycin are characterized as large macrocyclic polyketides and share several mechanistic features with cyclosporin A.

Intensive investigations throughout the 1980s and early 1990s clarified the molecular basis of their biological activities. Cyclosporin A was shown to bind the intracellular receptor cyclophilin, whereas FK506 interacts with FK506-binding protein 12 (FKBP12). Importantly, neither cyclophilin nor FKBP12 alone is capable of binding calcineurin. Instead, the drug–protein complexes create a new interaction interface that enables high-affinity binding to the phosphatase calcineurin, thereby inhibiting its activity and suppressing T-cell activation. These findings provided one of the earliest demonstrations that a small molecule can induce productive interactions between proteins that do not naturally associate.

This unique mechanism inspired the term "molecular glue," which was introduced in 1992 to describe compounds that stabilize or promote protein–protein interactions by acting as molecular adhesives rather than conventional inhibitors. Additional evidence supporting this concept emerged in 1994, when rapamycin was shown to form a ternary complex with FKBP12 and the mammalian target of rapamycin (mTOR). The observation that mutations in either FKBP12 or mTOR confer resistance to rapamycin further confirmed that its biological activity depends on induced protein complex formation, establishing rapamycin as a prototypical molecular glue.

By the end of the twentieth century, cyclosporin A, FK506, and rapamycin had all received approval from the U.S. Food and Drug Administration (FDA) for clinical use, primarily to prevent organ transplant rejection.

Beyond their therapeutic importance, these landmark discoveries laid the conceptual foundation for the modern development of molecular glues as targeted protein degradation agents**.**

Degradative Molecular Glues:

Degradative molecular glues (MGs) represent an important class of small molecules that induce selective protein degradation by promoting interactions between target proteins and E3 ubiquitin ligases. Unlike PROTACs, which rely on bifunctional molecules, degradative MGs remodel protein interfaces through a single small molecule, thereby triggering ubiquitination and subsequent proteasomal degradation. The success of this strategy has established molecular glues as a promising therapeutic platform for treating cancer and other diseases.

Among the best-characterized degradative molecular glues are the immunomodulatory drugs (IMiDs), including thalidomide, lenalidomide, and pomalidomide. Thalidomide was originally introduced as a sedative but was withdrawn after its association with severe congenital malformations. Interest in the compound was later revived following the discovery of its efficacy in treating erythema nodosum leprosum, which stimulated the development of safer and more potent analogues. Lenalidomide and pomalidomide subsequently demonstrated enhanced anti-inflammatory and anti-angiogenic activities and became important therapeutic agents for several hematological malignancies, including multiple myeloma (MM), myelodysplastic syndromes (MDS), chronic lymphocytic leukemia (CLL), and certain B-cell lymphomas.

A major breakthrough occurred in 2010 with the identification of cereblon (CRBN) as the primary molecular target of thalidomide and its analogues. This discovery revealed that IMiDs function as molecular glues by altering the substrate specificity of the CRBN E3 ubiquitin ligase complex. Upon binding CRBN, these compounds promote the recruitment and ubiquitination of neosubstrates such as the lymphoid transcription factors IKZF1 and IKZF3, as well as casein kinase 1α (CK1α) in del(5q) myelodysplastic syndrome. Understanding this mechanism transformed the field of targeted protein degradation and stimulated extensive efforts to identify additional molecular glues with similar modes of action.

The repertoire of degradative molecular glues was further expanded through the discovery of arylsulfonamide compounds, including indisulam and E7820. These molecules recruit the RNA-binding protein RBM39 to the CUL4–DCAF15 E3 ubiquitin ligase complex, resulting in selective ubiquitination and degradation of RBM39. Although indisulam demonstrated proof of concept for this degradation mechanism, its clinical efficacy has remained modest, and arylsulfonamide-based molecular glues have not yet received regulatory approval.

Another important advance was the identification of BI-3802, initially developed as an inhibitor of the transcriptional repressor BCL6. Subsequent mechanistic studies demonstrated that BI-3802 functions as a molecular glue by inducing BCL6 oligomerization, thereby promoting its recognition by the E3 ubiquitin ligase SIAH1. This interaction triggers ubiquitination and proteasomal degradation of BCL6, illustrating that molecular glues can exploit diverse mechanisms beyond simply redirecting substrate recognition by E3 ligases.

Recent research has increasingly shifted from the serendipitous discovery of molecular glues toward systematic screening strategies. A notable example is the large-scale chemical screening performed by Słabicki and co-workers, who evaluated thousands of small molecules across hundreds of cancer cell lines to identify previously unrecognized degradative mechanisms. This effort led to the identification of CR8, originally developed as a cyclin-dependent kinase (CDK) inhibitor. CR8 was subsequently shown to function as a molecular glue by promoting assembly of a complex containing CDK12–cyclin K and DDB1, an adaptor of the CUL4 E3 ligase complex. Formation of this induced complex results in selective ubiquitination and degradation of cyclin K, producing potent antitumor activity.

Natural products have also emerged as valuable sources of novel molecular glues. Advances in chemoproteomics have facilitated the identification of structurally diverse compounds capable of inducing previously unrecognized protein–protein interactions. For example, the polyketide natural products asukamycin and manumycin A promote interactions involving the E3 ligase UBR7, leading to activation of

the tumor suppressor TP53 through a molecular glue-like mechanism. These findings demonstrate that natural products represent an important reservoir for discovering new degradative molecular glues with unique biological activities.

Despite the rapid progress in this field, the discovery of degradative molecular glues remains considerably more challenging than that of PROTACs. Many currently known molecular glues were identified retrospectively after unexpected biological activities were observed rather than through rational design. Consequently, only a limited number of degradative molecular glues have successfully advanced into clinical use. Continued integration of structural biology, chemoproteomics, artificial intelligence, and high-throughput screening is expected to accelerate the rational identification of new molecular glues, expand the diversity of exploitable E3 ligases and target proteins, and ultimately broaden the therapeutic applications of targeted protein degradation.

Lysosome-Based Protein Degradation:

Although the ubiquitin–proteasome system (UPS) serves as the principal pathway for targeted protein degradation, lysosome-mediated degradation has emerged as an important complementary strategy that expands the scope of degradable substrates. Unlike the UPS, which primarily eliminates short-lived intracellular proteins, the lysosomal system is capable of degrading a much broader range of cellular components.

Lysosomes are membrane-bound organelles containing hydrolytic enzymes that degrade proteins and other macromolecules delivered through endocytosis, autophagy, and related trafficking pathways. In addition to long-lived and aggregated proteins, lysosomes participate in the turnover of nucleic acids, lipids, damaged or dysfunctional organelles, and even intracellular pathogens. Owing to their broad substrate specificity and high degradative capacity, lysosome-based approaches have become an attractive addition to targeted protein degradation technologies, particularly for eliminating proteins and biomolecular complexes that are not readily accessible to the ubiquitin–proteasome system.

Mechanism of Protein Degradation Through Lysosomes:

Lysosomes are specialized membrane-bound organelles that function as major degradative compartments within cells. Their ability to break down diverse biological materials is attributed to the presence of more than 60 hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. These enzymes belong to the class of acid hydrolases and exhibit optimal catalytic activity within the highly acidic lysosomal lumen, which maintains a pH of approximately 4.5.

The acidic environment required for lysosomal enzyme activity is established and preserved by vacuolar H^+-ATPases (V-ATPases) located in the lysosomal membrane. By continuously transporting protons into the lysosomal lumen, V-ATPases create the optimal conditions necessary for efficient degradation of various cellular substrates.

Compared with the ubiquitin–proteasome system, lysosomes possess a broader degradation capacity, allowing the removal of both intracellular and extracellular materials. Extracellular components are delivered to lysosomes primarily through endocytic pathways, whereas damaged organelles, protein aggregates, and other intracellular materials are transported through autophagic processes.

Taking advantage of these natural clearance pathways, lysosome-based targeted degradation technologies have been developed by harnessing mechanisms such as autophagy and endocytosis. These approaches provide an expanded platform for eliminating a wider range of biological targets, including substrates that may be unsuitable for conventional proteasome-mediated degradation.

Autophagy:

The autophagy–lysosomal pathway is an evolutionarily conserved intracellular degradation system that maintains cellular homeostasis by removing unnecessary or damaged components. Beyond its role in protein

turnover, autophagy regulates diverse biological processes, including cellular differentiation, stress adaptation, immune defense, development, and tissue remodeling. Through this pathway, cytoplasmic materials are selectively or non-selectively delivered to lysosomes, where they undergo degradation by hydrolytic enzymes. Due to its ability to regulate protein and organelle quality control, manipulation of the autophagy–lysosome system has attracted considerable attention as a therapeutic strategy for diseases such as cancer and neurodegenerative disorders.

Autophagy can be broadly categorized into three major pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Among these mechanisms, current lysosome-based targeted degradation approaches primarily exploit macroautophagy and CMA because of their potential for selective intracellular substrate removal.

Novel lysosomal targeting degradation technologies:

In recent years, the emergence of TPD strategies via the lysosomal pathway has been witnessed, including AUTAC, LYTAC, ATTEC, CMA-based degraders. These developments are driven by extensive research into the endosome-lysosome and autophagosome-lysosome pathways. Unlike proteasome-based TPD, which targets specific intracellular proteins, lysosome-based TPD can eliminate protein aggregates, damaged organelles, membranes, and extracellular proteins.

Autophagy-targeting chimeras (AUTACs):

Autophagy-tethering compounds (AUTACs) are a class of lysosome-based targeted degradation molecules designed to eliminate intracellular proteins and damaged organelles, including fragmented mitochondria, through selective autophagy pathways. Unlike proteasome-dependent degradation systems, AUTACs utilize the cell's endogenous autophagic machinery to direct specific substrates toward lysosomal clearance.

The development of AUTAC technology was inspired by studies of innate autophagic responses against bacterial infection. Arimoto and colleagues identified the role of 8-nitroguanosine 3′,5′-cyclic monophosphate (8-nitro-cGMP) in promoting selective autophagy through induction of Lys63-linked polyubiquitination. This modification enables damaged or unwanted cellular components to be recognized by the autophagy receptor SQSTM1/p62, which subsequently interacts with LC3 on autophagosomal membranes, facilitating substrate sequestration and delivery to lysosomes.

Mechanistically, 8-nitro-cGMP modifies cysteine residues through S-guanylation, generating an endogenous degradation signal that marks proteins and mitochondria for autophagic elimination. Based on this principle, AUTAC molecules were developed by incorporating a guanine derivative-based degradation tag, a chemical linker, and a targeting ligand (warhead) that selectively recognizes a protein of interest (POI) or a specific organelle. Following target engagement, AUTACs promote K63-linked polyubiquitin chain formation, thereby triggering recognition by the autophagy machinery and subsequent lysosomal degradation.

In 2019, Arimoto's group reported the first generation of AUTAC molecules (AUTAC1–4), establishing proof-of-concept that chemically induced autophagic tagging could achieve targeted degradation. However, early AUTACs showed limitations, including activation of protein kinase G (PKG) due to the cGMP-derived tag and insufficient cellular permeability, which restricted their degradation efficiency.

To overcome these challenges, second-generation AUTACs were developed in 2023 through optimization of several structural components, including refinement of the guanine-based degradation tag, adjustment of linker length, and incorporation of an L-cysteine-derived connector. These modifications substantially enhanced intracellular activity and improved degradation performance, demonstrating the potential of AUTACs as a versatile platform for lysosome-mediated targeted degradation of proteins and organelles.

Lysosome targeting chimeras (LYTACs):

Lysosome-targeting chimeras (LYTACs) represent an emerging class of lysosome-dependent targeted protein degradation (TPD) technologies designed to eliminate extracellular proteins and cell-surface membrane

proteins that are generally inaccessible to conventional proteasome-based approaches. LYTACs exploit endogenous lysosomal trafficking pathways by recruiting lysosome-targeting receptors (LTRs), which naturally mediate the internalization and delivery of extracellular cargo to lysosomes for degradation.

The first LYTAC platform was introduced in 2020 by Bertozzi and colleagues, who developed bifunctional chimeric molecules capable of simultaneously binding an extracellular protein of interest (POI) and a cell-surface LTR. This dual-recognition strategy promotes the formation of a ternary complex consisting of the LTR, LYTAC molecule, and target protein. Following receptor-mediated endocytosis, the complex is transported through the endosomal pathway to lysosomes, where the POI is degraded by lysosomal hydrolases.

Structurally, LYTACs contain three essential components: a ligand that recognizes and binds the extracellular or membrane-associated target protein, an LTR-binding element responsible for recruiting the lysosomal trafficking machinery, and a linker that connects these two functional groups. Through the formation of the LTR–LYTAC–POI complex, target proteins are selectively directed toward lysosomal compartments and subsequently eliminated.

Subsequent studies expanded the LYTAC platform by developing GalNAc-conjugated LYTACs, which selectively engage asialoglycoprotein receptors expressed predominantly on hepatocytes. These liver-targeted degraders broadened the available lysosomal receptor repertoire and demonstrated the possibility of designing cell-type-specific LYTAC systems with improved tissue selectivity.

Further mechanistic investigations have provided important insights into the regulation of LYTAC activity. In 2023, Bertozzi and colleagues demonstrated that intracellular trafficking factors significantly influence LYTAC-mediated degradation. Activation of the retromer complex, which promotes recycling of LYTAC–cation-independent mannose-6-phosphate receptor (CI-M6PR) complexes back to the cell surface, can reduce degradation efficiency by limiting receptor availability. Additionally, neddylation of cullin 3 (CUL3) was identified as an important factor involved in efficient lysosomal delivery and may serve as a biomarker for evaluating LYTAC activity. Competitive occupation of CI-M6PR by mannose-6-phosphate (M6P) can also interfere with LYTAC function by blocking receptor engagement.

These mechanistic discoveries provide valuable guidance for improving LYTAC design and developing next-generation lysosome-targeting degraders with enhanced efficiency, specificity, and therapeutic potential.

Conclusion and perspectives:

Identification of biological processes and their functional effectors is invaluable not only to understand how our bodies function at the molecular level but also for the development of new therapeutic modalities. Induced protein degradation by heterobifunctional PROTACs is designed to work by co-opting the natural cellular ubiquitination process. PROTACs are composed of two ligands that bind to the POI and an E3 ligase, tethered together. Subsequent recruitment of these two entities in proximity induces ubiquitination of the POI and directs it to the cellular proteolytic machinery. In this way, PROTAC technology has been widely applied against various disease-causing proteins with different biological roles and has demonstrated multiple advantages over conventional inhibitory strategies. Since PROTAC is an *event*-driven modality, when a noncovalent POI ligand is used in PROTAC design it can induce the degradation of the POI in a sub-stoichiometric manner. Thus, administration of relatively low doses might provide prolonged effects on the target protein compared with small molecule inhibition. Also, application of TPD in oncology might overcome resistance mechanisms, such as overexpression of oncogenic proteins, and potentially reduce adverse effects compared with conventional inhibitor drugs due to the reported higher specificity of PROTACs. Furthermore, recent advances in the TPD field indicate that exploitation of tissue-specific E3 ligases (e.g., KLHL41 in skeletal muscle, RNF112 in brain tissues, TRIM69 in testis, ASB9 in pancreas) or tumor-specific E3 ligases (e.g., TRIM50 and FBXO2) will significantly improve tumor-selective mode.

PROTACs, by definition, are capable of eradicating target proteins from the cellular environment. Unlike conventional inhibition, induced disposal of proteins not only interferes with catalytic activity of POI but also eliminates any other associated scaffolding roles upon which cancer cells rely. Since simultaneous ligand-dependent engagement of the PROTAC with the target protein and the E3 ligase is the first step involved in a successful PROTAC mechanism, targeting proteins that lack a well-defined catalytic pockets (such as TFs) has been challenging. Only a few TFs, principally nuclear receptors, have been targeted by PROTACs due to the limited availability and difficulty in developing small molecule ligands for TFs, leaving a large portion of TFs still remaining to be targeted by direct methods. Recently, we reported a generalizable strategy for targeted degradation of TFs: TRAFTACs—chimeric oligonucleotides composed of double-stranded DNA and crRNA—were designed by harnessing the DNA binding ability of TFs and a cellular E3 ligase machinery. Due to the flexibility of the proposed TRAFTAC strategy, TFs with known DNA binding sequences can be targeted for proteasomal degradation by incorporating those DNA sequences into TRAFTAC design. Thus, this method provides a general way to directly target previously undrugged TFs. Further, simple modification to this strategy has the potential to induce the selective degradation of only the promoter-bound pool of a TF to achieve specific downregulation of an individual gene (Figure 6B). This could be achieved by using a traditional gRNA that targets a specific locus neighboring the TF-binding promoter site of the targeted gene. Introduction of the dCas9-HaloTag7 fusion protein and a HaloPROTAC will recruit the E3 ligase complex to the vicinity of the promoter-bound TF to induce its ubiquitination and rapid degradation by the proteasome. Thus, direct targeting of TFs by TRAFTAC-like strategies may have profound effect toward cancer cells compared with the targeting of upstream signaling effector proteins or indirect strategies. Furthermore, future advancements to such direct technologies will ultimately provide more efficient and safe therapeutic avenues to explore different disease conditions where TF functions are dysregulated. Overall, 2 decades of TPD have shown us a remarkable progress toward precision targeting of disease-causing proteins for proteasomal degradation, and have proven that this technology has a great promise in the drug discovery world.

Significance:

Intracellular disease-causing proteins targeted by current treatment modalities are just a small population compared with the number of proteins that are yet to be drugged. A large portion of the proteome performs noncatalytic scaffolding roles in biological pathways that are crucial to the initiation and progression of certain diseases. Due the lack of well-defined active sites within them, such proteins long have been considered undruggable. However, recent advancements in the medicinal chemistry and chemical biology fields have contributed significantly to change the traditional view of targeting such proteins. Since 2012, more than a decade after the PROTAC technology was introduced, the TPD field has started to rapidly evolve to successfully target numerous disease-relevant proteins. The continued progress in the field has led to the development of direct and generalizable strategies to target hard-to-drug proteins such as TFs for proteasomal degradation. Thus, TPD holds a great promise to overcome obstacles that are inherently linked with troublesome proteins and for the future of medicines.

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