WHITE PAPER

A brand-new modality on the horizon: how targeted protein degradation can address the unmet need in drug discovery and development

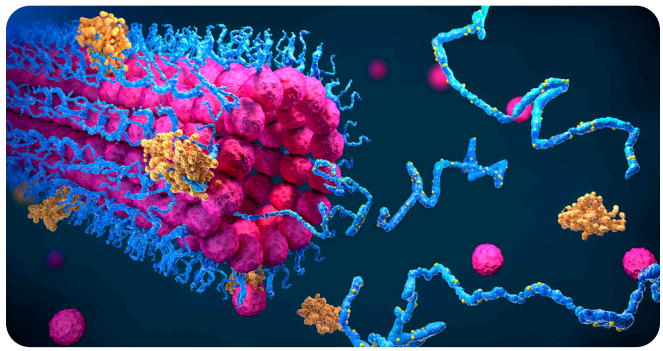
Addressing challenges to advance degraders into the clinic

Targeted protein degradation (TPD) is an emerging drug discovery modality that offers the potential to probe biological pathways and target proteins that have previously been considered "undruggable". Compared to traditional drug discovery approaches where small molecules bind and inhibit the function of a protein of interest (POI), TPD exploits the cell's own degradation machinery to selectively target a protein for degradation. This offers the potential for improved therapeutic efficacy and the possibility to discover and validate novel targets.

The field of TPD has grown rapidly since its initial development in the early 2000s, attracting interest from both industry and academia. Companies are dedicating significant resources to the development of TPD-based therapies and there are currently over 20 TPD degraders in clinical development.[1] In addition, there are numerous collaborations between industry and academia focused on the advancement of therapeutic degraders. In this review, we describe the principles and potential advantages of TPD, along with the challenges faced by the field to bring this modality into the clinic.

Targeting the undruggable proteome

Over the past century, traditional small-molecule drugs have dominated the therapeutic landscape due to their low molecular weight, oral bioavailability, and attractive



physicochemical properties. Just last year, nearly 60% of novel drugs approved by the FDA were small molecules,[2] demonstrating the significant value this drug class continues to bring to the field. Small molecule inhibitors typically exert their therapeutic effect by binding to an active or allosteric site on a target protein. Although this approach has proved incredibly successful, only 20% of proteins possess binding sites suitable for small-molecule interactions.[3] This leaves the majority of the proteome currently untapped.

Due to its unique mode of action, TPD offers opportunities to target many of these previously "undruggable" proteins. TPD involves the use of degraders which hijack the endogenous ubiquitin-proteosome pathway and selectively induce degradation of a target protein. Degrader molecules currently fall into two main classes: monovalent degradation-activating compounds (MonoDACs), also often referred to as molecular glues, or bifunctional degradation-activating compounds (BiDACs), also commonly referred to as PROTACs (PROteolysis TArgeting Chimeras). Both modalities form complexes with the target protein and an E3 ligase to initiate degradation. However, they are structurally very different.

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PROTACs are heterobifunctional molecules consisting of two ligands ("warheads") connected by a linker. One ligand binds a POI while the other recruits and binds an E3 ligase, resulting in the formation of a ternary complex. PROTAC binding induces ubiquitylation of the POI and subsequent degradation by the ubiquitin-proteasome system. By contrast, molecular glues induce or stabilize protein-protein interactions between the POI and an E3 ligase to form a ternary complex. This leads to ubiquitylation of the target and subsequent degradation. Molecular glues do not require a linker and are therefore much smaller in size then PROTAC degraders.

TPD degraders: Why the hype?

Both PROTACs and molecular glues offer numerous advantages over traditional small-molecule inhibition. For example, degraders do not need to bind within a biologically active site and do not require tight binding to exert their effects. These characteristics expand the druggable space beyond traditional small molecules. Indeed, more and more targets are proving to be degradable by PROTAC molecules, with recent figures showing that the number of PROTAC targets increased from 40 in 2019 to over 130 at the end of 2021.[4]

Besides their potential to address the undruggable, there are many reasons to attack clinically relevant targets by degraders. They can eradicate mutated targets such as resistance mutations and gene amplifications/protein overexpression, select isoforms specifically, target scaffolding functions, and remove protein aggregates.[5]

Another major benefit of PROTACS is their ability to improve selectivity compared to the small molecules used for their design.[6] Off-target degradation is less likely due to the intricate formation of a ternary complex containing the degrader, target protein, and E3 ligase. Extensive efforts have also been made to use rational discovery and design strategies to improve the properties of PROTAC degraders. Notably, PROTAC selectivity can be further improved through linker length optimization and stabilization of the ternary complex.[6] Such optimization not only provides a means to convert non-selective small-molecule inhibitors into more selective protein degraders but also affords the opportunity to target closely related members of protein families.

A unique property of degraders is that they can remove multiple copies of a target protein. Degraders are therefore expected to deliver longer-lasting effects at lower concentrations than small-molecule inhibitors—a property that is particularly attractive for drug development. This was demonstrated recently by You *et al.*, who developed a degrader for the popular cancer target AKT.[7] Not only did their degrader induce stronger anti-proliferative effects than direct AKT inhibition, but it also induced sustained AKT destabilization and inhibition of downstream signaling, even after compound washout.

Now that the field has a greater understanding of the mechanism of PROTAC degraders, and is realizing the technology's potential, collaborations have started to form between academia, biotech startups, and some of the world's leading pharmaceutical companies. In 2021, Novartis, Pfizer, and Bayer negotiated billion dollar deals with smaller biotechs including Dunad Therapeutics, Arvinas, and Vividion Therapeutics. And just last year Amphista Therapeutics caught the attention of Bristol Myers Squibb and Merck, the latter of which also struck a $554 million deal with Austrian biotech Proxygen. These are just a few examples, but it is no surprise that the technology has attracted the attention of industry giants. Notably, a surge in deals was observed following the entry of the first PROTACs into the clinic in 2019.[8] Both ARV-110 and ARV-471, developed by Arvinas, target well-established cancer targets and are now in Phase II trials for the treatment of prostate and breast cancer, respectively.

Although the majority of the 20 degraders currently in the clinic target solid or liquid tumors,[2] their application is beginning to extend beyond oncology indications. For instance, NX-5948 and KT-474 are currently in Phase I clinical trials for the treatment of immune inflammatory conditions targeting kinase BTK and IRAK4, respectively. Neurodegenerative proteinopathies such as Alzheimer's disease could also benefit from a TPD approach. Alzheimer's disease is characterized by the accumulation of misfolded and aggregated tau protein. However, despite being a well-defined target, successful tau-targeting therapies are yet to reach the clinic. In a recent study, Silva *et al.* converted a tau PET (positron emission tomography) probe into a heterobifunctional tau degrader.[9] The team successfully demonstrated that QC-01-175 preferentially degraded tau in patient-derived neuronal cell models with minimal effect on tau from neurons of healthy controls.

As TPD degraders continue to make progress through the clinic, they are also being used to validate therapeutic targets, such as synthetic lethal targets, and probe biological pathways. Degraders can be used to reversibly knock down a potential protein target, enabling exploration of the downstream phenotype. TPD can also be used as a complementary approach to genetic knockdown strategies such as CRISPR-Cas9 and RNAi.

Challenges in degrader development

Although TPD has shown great potential for addressing some of the inherent challenges of small-molecule drug discovery, workflows for degrader development need to extensively scrutinize every step of the degradation pathway. Penetration of the cell membrane can be particularly challenging due to the large molecular weight of PROTACs, which typically falls outside Lipinksi's "rule of five". Thus, poor oral absorption of PROTACs is a common and major problem. Molecular glues have lower molecular weight than PROTAC degraders making them more amenable to the "rule of five". In addition, several oral PROTAC degraders have been reported, a handful of which are currently in clinical development.[10]

Degrader development also demands comprehensive characterization of their mode of action in diverse cellular systems. Biological assays have been developed to interrogate PROTAC target engagement, ternary complex formation, target ubiquitination, and subsequent degradation and downstream effects. One way to study degradation is to follow the luminescent signal based on the formation of complementary partners (HiBiT system). This signal-off approach is impacted by compound toxicity and requires molecular engineering which can be challenging when using different cell types and primary cells. By contrast, no-wash immunoassays like Alpha and HTRF can be used to monitor the impact of degraders on a POI over time, in their cellular context, and without the need for molecular engineering. In addition to endogenous protein detection, these no-wash assays allow measurements of ternary complex formation in a biochemical setting.

Due to their molecular weight, it is critical to ensure that the selected degrader can pass through the cell membrane to find its intended POI. CETSA® (Cellular Thermal Shift Assays) can provide the required information in a fast and easy way across different cell types without any modifications.

Experiments are also needed to distinguish whether an observed phenotype is due to protein degradation or inhibition. Investigations also need to explore any unwanted non-degradation-dependent effects caused by unspecific binding of the degrader to off-targets. The hook effect can be examined using technologies such as Alpha or HTRF in order to provide a concentration range in which the PROTAC can be used in cellular systems.

Another challenge in PROTAC development is the limited number of suitable E3 ubiquitin ligases available. Despite the human genome encoding over 600 E3 ligases, only a few corresponding E3 ligase ligands have been discovered. Notably, most PROTAC efforts have focused on ligands recruiting the E3 ligases von Hippel-Lindau protein (VHL) or cereblon (CRBN). One potential disadvantage of relying on a small pool of E3 ligases is the risk of resistance developing, particularly in the field of oncology. Efforts are currently underway to discover and optimize alternative E3 ligases to expand the scope of TPD and abrogate the risk of resistance.[11] There is also interest in the discovery of E3 ligases with unique expression profiles, which could enable tissue- and cell-specific target degradation and lead to the development of PROTACs for precision medicine.[12]

One of the major constraints of PROTAC-mediated TPD is that it is limited to proteins that can be bound by small organic molecules. Molecular glue degraders, on the other hand, do not require a binding pocket to exert their degradation effects, broadening the number of targets potentially amenable to degradation. But the discovery of molecular glue degraders has so far been serendipitous or relied on phenotypic screens. And despite efforts in the field, rational discovery and design strategies for molecular glues have proved difficult. To address this challenge, platforms are starting to emerge for the identification of novel molecular glue degraders. For example, Georg Winter and colleagues recently described a strategy for the discovery of molecular glues based on chemical profiling.[13] The team utilized quantitative proteomics and functional genomic strategies, drug-affinity chromatography, and cellular proximity assays to reveal a therapeutically unexplored mechanism for molecular glue-induced target degradation. Notably, the study successfully identified several compounds that induced ubiquitination and degradation of the transcriptional regulatory kinase cyclin K. Winter and colleagues predict that such an approach could help detect molecules with the potential to target unligandable, disease-relevant proteins.

Future outlook for TPD

The past few years have seen the field of TPD move from proof of concept to the development of degrader drugs which are now advancing in clinical trials. As our understanding of proximity-induced degradation grows and trial data are presented, it is predicted that investments will continue to sour, and breakthroughs will likely follow. In addition, concepts based on chimeric small molecules have started to emerge such as deubiquitinase-targeting chimeras (DUBTACs), PhIC—phosphorylation-inducing chimeras (PhICs), and phosphatase-recruiting chimeras (PhoRC).[14]

But while current degrader molecules offer numerous advantages over traditional small-molecule inhibitors, including their mechanism of action, improved selectivity, and potentially more sustained efficacy, there are still many challenges to be solved. Questions need to be asked relating to which proteins are best targeted by degradation over inhibition, whether there are any risks associated with whole protein depletion, and which tools are most suitable to evaluate the physicochemical properties of degraders. Future efforts need to focus on expanding the pool of E3 ligases, optimizing PROTAC linkers and ligands, and improving rational discovery and design strategies for molecular glues. By addressing these hurdles and through continued collaboration, TPD technologies will be able to realize their full potential and move closer toward safe and effective treatments for patients.

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