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Minireview

New therapies on the horizon: Targeted protein degradation in neuroscience

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This minireview explores the burgeoning field of targeted protein degradation (TPD) and its promising applications in neuroscience and clinical development. TPD offers innovative strategies for modulating protein levels, presenting a paradigm shift in small-molecule drug discovery and therapeutic interventions. Importantly, small-molecule protein degraders specifically target and remove pathogenic proteins from central nervous system cells without the drug delivery challenges of genomic and antibody-based modalities. Here, we review recent advancements in TPD technologies, highlight proteolysis targeting chimera (PROTAC) protein degrader molecules with proximity-induced degradation event-driven and iterative pharmacology, provide applications in neuroscience research, and discuss the high potential for translation of TPD into clinical settings.

INTRODUCTION

Targeted protein degradation (TPD) broadly refers to administering a chemical entity to cells or an organism to reduce the concentration of a specific protein or proteins through natural cellular protein degradation systems.[1,2] Because of the potential of TPD to decrease the levels of specific disease-associated proteins, it is a revolutionary small-molecule-based therapeutic strategy being applied in research and development programs by many biopharmaceutical companies.[1–3] This approach was first demonstrated with proteolysis targeting chimera (PROTAC) protein degrader molecules, which simultaneously bind to a target protein and an E3 ubiquitin ligase, bringing them in close proximity, leading to target protein ubiquitination and subsequent degradation by the proteasome.[4] PROTAC protein degrader molecules offer unique advantages over small molecule inhibitors, and this is exemplified by "event-driven" pharmacology as opposed to "occupancy-driven" inhibition, enabling the ability to act iteratively leading to substoichiometric potencies.[3] For example, kinase inhibitors prevent ATP binding by occupying the active site or an allosteric site that prevents or disrupts ATP binding. Thus, a one-to-one stoichiometry cannot be exceeded. In contrast, a PROTAC protein degrader can catalyze ubiquitination of multiple target proteins and improve stoichiometry. PROTAC protein degraders can also disrupt scaffolding function, degrade proteins that lack catalytic binding sites, and have many of the advantages of genomic modalities without the biodistribution challenges, including the potential of crossing the blood-brain barrier (BBB) with oral delivery.[2] Several PROTAC protein degraders and similar small molecule heterobifunctional compounds are in clinical development.[1,2] The most advanced compound, vepdegestrant, a PROTAC estrogen-receptor degrader currently in pivotal clinical trials for patients with ER+/HER2-breast cancer, has provided clinical proof of mechanism for the PROTAC approach (NCT05654623).[3,5]

Certain established drugs, such as the immunomodulatory imide compounds (IMiDs), also operate via TPD.[1] Initial examples of these molecules were not designed to be degraders but were retrospectively discovered to induce protein degradation via the ubiquitin-proteasome system (UPS). We will discuss how such compounds, classified as molecular glue degraders (MGDs) are distinguished from heterobifunctional degraders. We will also discuss non-clinical research examples of other MGDs and how MGDs could be applied to neuroscience.

TPD is not limited to the UPS and is part of a broader induced proximity (IP) concept.[6] Several modalities in which IP results in lysosomal-mediated degradation of the target protein have been reported in nonclinical research settings.[6–8] In general, these modalities are much less advanced than PROTAC technology. The most advanced is a molecular degrader of extracellular proteins (MoDE)[8]—BHV-1300, a heterobifunctional compound leading to plasma IgG protein degradation in liver lysosomes—which is currently in phase 1 clinical trials for rheumatoid arthritis.[92]

To date, most drugs utilizing TPD that are approved or in clinical development are for patients with cancer.[1,3] In February 2024, the first PROTAC protein degrader for neurological indications ARV-102—targeting the kinase LRRK2—entered phase I clinical trials (Figure 1). ARV-102 has the potential to treat Parkinson's disease (PD) and progressive supranuclear palsy (PSP), in which LRRK2 is hyperactive, induces scaffolding functions linked to neuronal cell death, and is upregulated in neuroimmune microglia.[9–14] Nonclinical research proof-of-concept studies have applied small molecule TPD to other neurological disorders, including cellular and rodent models of Alzheimer's disease (AD) and Huntington's disease (HD).[15–18] Macromolecule-based biodegraders, such as those based on peptides or antibodies, have also been reported for proteins linked to neurodegenerative disorders.[19–22] These and other TPD developments make this an exciting era for neuroscience therapeutics, which will be the focus of the current minireview.

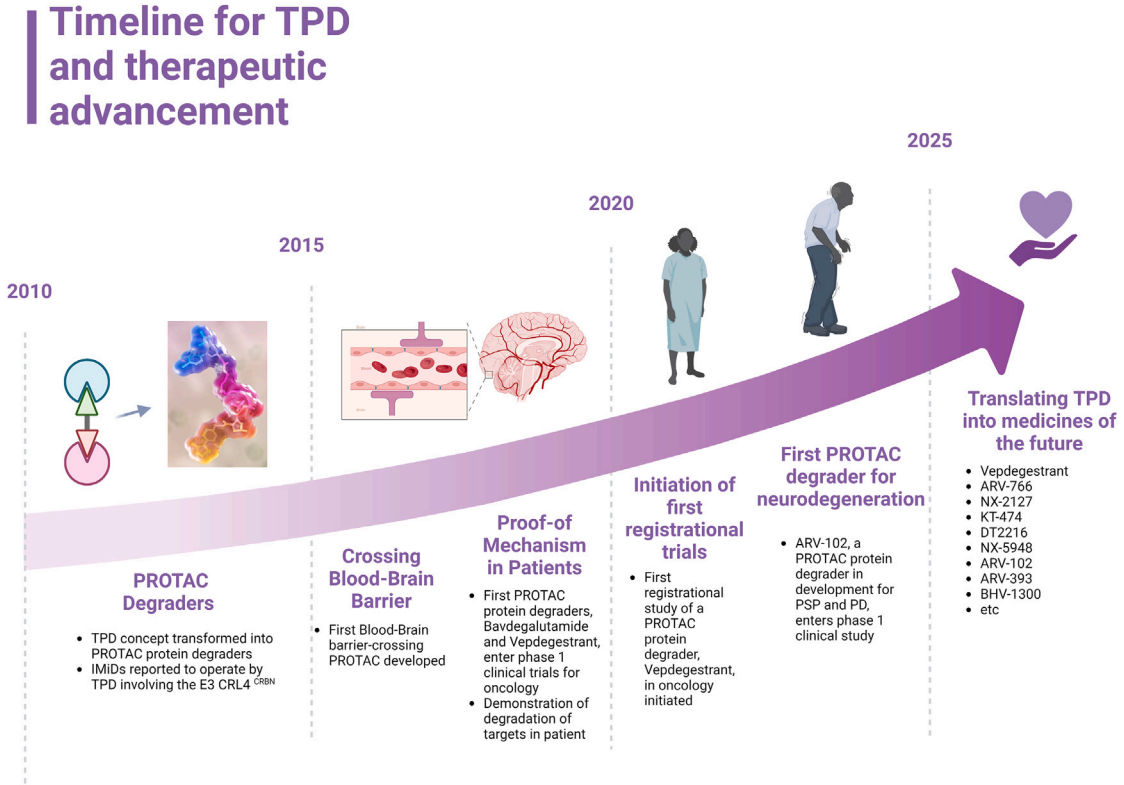

Figure 1. Timeline of key events leading to advancements in TPD for neurological disorders
Heterobifunctional degrader molecules in clinical development for a variety of diseases are included. Created with BioRender.com.

MECHANISMS OF TARGETED PROTEIN DEGRADATION

The UPS controls the majority of selective protein degradation in cells and can be divided into two parts: (1) the enzymatic machinery responsible for covalent modification of cellular proteins with the ubiquitin protein—often as a chain of multiple ubiquitins—and (2) the multi-protein proteasome that recognizes and degrades ubiquitinated proteins (Figure 2).[23,24] Ubiquitination requires the sequential activity of three enzymes: E1, E2, and E3. E1 activates the ubiquitin protein, and an E2 is an intermediate carrier of the activated ubiquitin. All eukaryotes express multiple E2s and many E3s, allowing ubiquitination of a diverse set of substrate proteins with spatial and temporal control. E3s typically interact directly with their substrates; in other cases, a chaperone protein binds both the E3 and substrate.[25] Humans encode approximately 600 E3s, only a fraction of which have been studied in detail.[24] E3s vary in architecture, but all have domains or subunits that allow the E3 to simultaneously interact with both an E2 and specific substrate proteins. The RING domain, with its main function to recruit an E2, is the most common domain found in E3s. A subset of RING domain-containing E3s are of the multi-subunit cullin RING ligase (CRL) class.[26] The human genome codes for over 200 CRLs with varying architectures and subunits. A CRL has a cullin protein as scaffold, with a RING domain protein interacting at one end of the cullin and a substrate recognition module on the other end. Substrate recognition modules can be a single protein or a combination of one or more adaptor protein(s) and a direct substrate recognition protein. Multi-subunit E3 ligases that are not CRLs also exist while other E3s are a single polypeptide.

At present, the most-used E3 for TPD, both for heterobifunctional degraders or IMiD-type MGDs, is $CRL4^{CRBN}$, a CRL with cereblon (CRBN) as the substrate recognition subunit, DDB1 as adaptor, RBX1 as the RING protein, and CUL4 as the scaffold subunit.[2] The other E3 currently used for clinical-stage heterobifunctional degraders is $CRL2^{VHL}$, in which the substrate recognition subunit is the Von Hippel-Lindau (VHL) protein and the scaffold subunit is CUL2. A few other CRLs, including those of the CRL4 class with substrate recognition subunits other than CRBN bound to DDB1, have been employed for TPD—as have some non-CRL E3s.[27,28] In addition, TPD has been demonstrated to work with compounds that co-opt E3 CRL complexes classically thought to be incomplete. For example, an MGD molecule that induces a protein-protein interaction (PPI) between DDB1 and the kinase CDK12 leads to degradation of the CDK12-interacting cyclin K protein.[29] This intriguing study—and others showing unexpected substrate recognition modes—highlight both the flexibility and unpredictable nature of TPD and the UPS.[1,27]

However, for many examples of TPD in the literature, the eventual utility of a strategy or employment of any new E3 as a therapeutic is unclear since exploration of chemical matter introduced is limited with respect to structure-activity delineation. Even the well-characterized PROTAC degraders based on CRBN and VHL recruitment may have a narrow property space for oral bioavailability.[30] Macromolecule-based biodegraders, whether

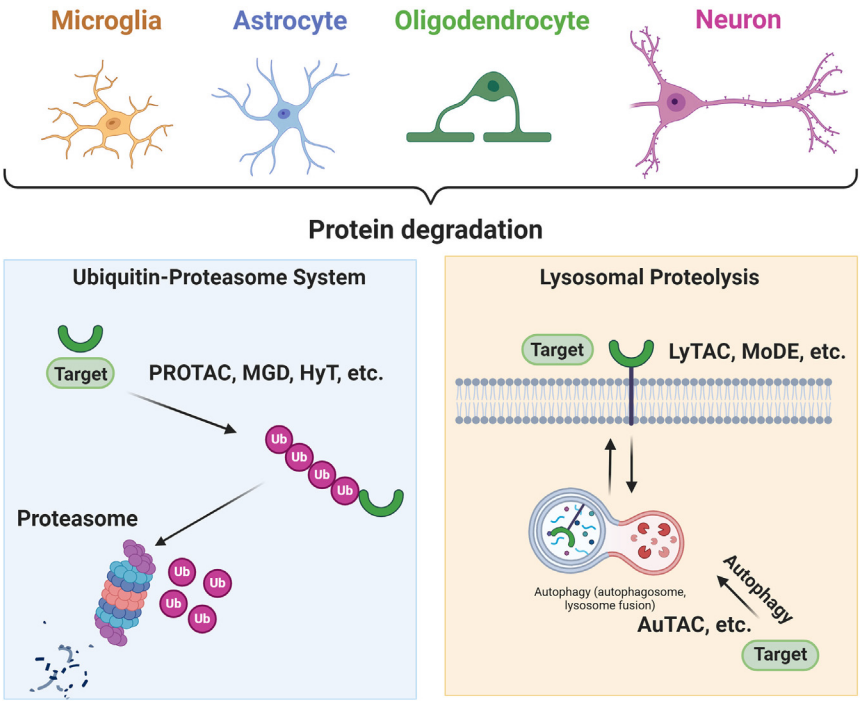

Figure 2. Illustration of potential pathways that could be leveraged for targeted protein degradation in central nervous system cell types

Like other cells, cells of the central nervous system (depicted at the top) have two main systems for protein degradation, the ubiquitin-proteasome system (UPS) and lysosomes. TPD modalities have been developed to co-opt each system, though most TPD currently uses the UPS. (Left) Induced by a PROTAC, MGD, hydrophobic tag (HyT), or other degrader, intracellular target proteins are modified by ubiquitin (Ub), which tags them for degradation by the proteasome. (Right, upper) Induced to bind recycling receptors (purple) by a LYTAC, MoDE, or similar degrader, extracellular or membrane target proteins are internalized and degraded in lysosomes. (Right, lower) Induced to bind autophagy machinery by an AUTAC or similar degrader, intracellular target proteins are degraded in lysosomes. See text for more details and relevant references. Created with BioRender.com.

genetically encoded or peptide/protein-based, have the most unclear therapeutic potential as they exhibit limitations in bioavailability and biodistribution. Despite these limitations, these proof-of-concept TPD research studies have shown that degradation of many different types of proteins can be induced—including proteins linked to neurological disorders.[19–21,31,32]

Small-molecule UPS-mediated degraders currently show the most promise for TPD therapeutics. IMiDs, such as thalidomide and lenalidomide, are FDA-approved MGDs that enhance PPIs between a set of target proteins and CRBN.[1] MGDs do not interact strongly with at least one of the proteins in the PPI they induce, rather they enhance the affinity of the two proteins for each other.[33] Conversely, direct PPIs are not necessarily needed for degradation via heterobifunctional degraders.[34] One potential advantage of MGDs is that proteins with relatively shallow pockets can still be targeted with TPD—whereas classic binder/inhibitor drugs require much higher-affinity interactions with the target. MGDs can also be of a lower molecular weight than heterobifunctional compounds, a potential advantage for drug administration and distribution.[30,35] On the other hand, heterobifunctional degraders offer superior rational design principles to MGDs.[1] Some examples of small molecule degrader compounds do not fit the classic MGD paradigm and were also discovered to be degraders retrospectively.[36,37] A recent study showed a compound, IBG1, operates as an intramolecular glue, binding simultaneously to the two different bromodomains of the BRD2 or BRD4 protein to induce a conformational change necessary for E3 interaction.[38] Similarly, certain binders of BCL6 induce its homodimerization, increasing recruitment of an E3.[39] Other small molecule degraders, such as those incorporating hydrophobic tagging technology, may lead to increased target engagement with cellular factors other than E3s.[40,41] Thus, many mechanisms are possible for small molecule degraders. Understanding the exact mechanism of each chemical entity requires detailed studies to deconvolve the cellular machinery employed to achieve the desired target degradation. Whether such mechanisms will allow rational design of potent degraders, as is possible for PROTACs, remains to be determined.

TPD has expanded beyond E3s and the UPS, as several TPD strategies that hijack the lysosomal degradation machinery have been reported (Figure 2).[6] These strategies may allow for degradation of proteins for which ubiquitination is not necessary or sufficient to induce degradation—as may be the case for certain proteins linked to neurological disorders.[23] One type of TPD modality that utilizes lysosomes, typified by MoDEs and lysosome-targeting chimeras (LYTACs), allows for degradation of extracellular proteins or proteins with domains on the surface of cells.[6–8] The other TPD arm with lysosomal proteases as the terminal degradation machinery induces proximity of a target to components of autophagy machinery. Such autophagy-targeting modalities provide another proximity mechanistic approach to induce degradation of intracellular proteins—and perhaps may show utility for proteins or protein complexes that are too large or aggregated for the UPS to handle.[23,25] However, compared to UPS-mediated degraders, these other TPD approaches are far less mature, and it remains unclear whether many of these alternative TPD strategies will result in the creation of drug-like molecules.[35] It is noteworthy that chemical matter reported to interact with LC3 (arylidene-indolinones),[42] and when incorporated into heterobifunctional molecules reported to target degradation through autophagy,[43] was recently reported to instead drive degradation via the E3 $CRL4^{DCAF11}$ in the context of heterobifunctional molecules.[44] Whether arylidene-indolinones, or similar relatively weak electrophilic ligands, have utility for mediating degradation through the UPS, autophagy, or both awaits further study.[45] Other approaches and chemical ligand tools reported to bind protein components of the autophagy/lysosomal system also await further study to assess their true utility. Results of several recent studies provide insights for TPD development

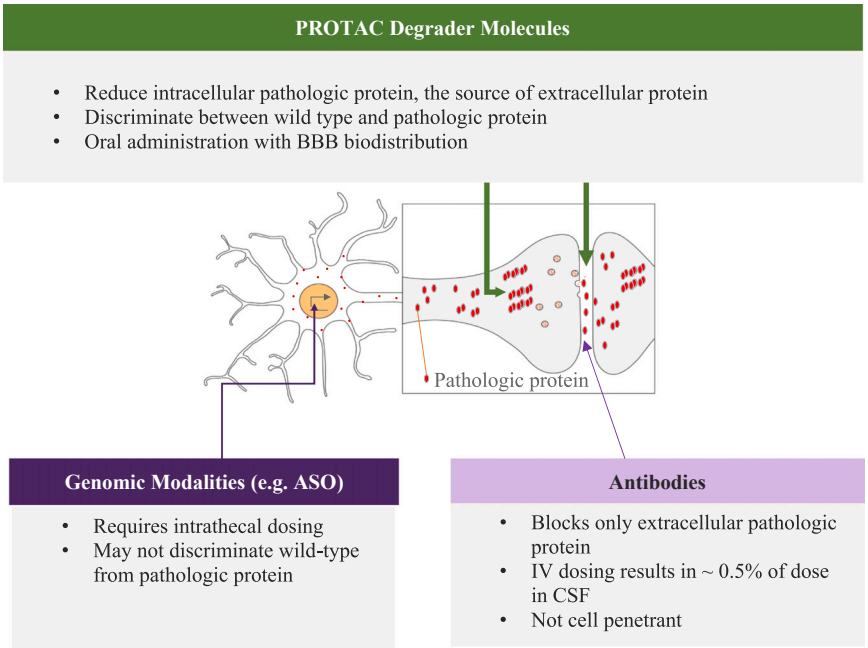

Figure 3. PROTAC protein degrader molecules may overcome the limitations of other therapeutic platforms for neurodegeneration
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going forward: (1) the mechanism of action cannot be assumed from heterobifunctional compound design, (2) more than one target protein should be assessed to validate a new modality or E3, and (3) selective ligands will likely garner the most attention for therapeutic potential.[38,44,46]

Drugs that operate via TPD offer several potential advantages over classical drugs.[2,6] Most drugs work by modulating the activity of a protein or proteins and require protein occupancy. With TPD, target protein concentrations decrease over time and therefore recovery of protein activities needed to drive disease depends on re-synthesis of the target. In addition, most degrader molecules can work iteratively, as they are released from the target protein molecule as it unfolds during the overall degradation process. These properties of degraders can impart superior potency. Also, protein degraders can be made from target ligands that do not affect target function. This dramatically expands what is considered "druggable" from a small-molecule perspective, including targets for multiple neurodegenerative diseases. Degraders can also confer superior specificity over inhibitors, as has been shown for several kinases.[47,48] Additional examples of superior specificity from degraders are likely given that degraders can theoretically use any target ligand, not only those for active sites. Agnostic of therapeutic area, ideal targets for PROTAC protein degraders fall into one or more of the "tenets of PROTACs."[2] In general, target proteins that contribute to disease through a toxic gain of function via overexpression, dominant driver mutations, scaffolding function, or aggregation or require isoform selectivity are good candidates for protein degraders.

APPLICATIONS OF TPD IN NEUROSCIENCE AND COMPARISONS TO OTHER MODALITIES

Innovative drug modalities developed to address the shortcomings of established small molecule inhibitors, recombinant proteins, and antibody-based therapeutics are bringing promising new potential treatment options to the clinic.[49] These new modalities, including small molecules with novel mechanisms of action (e.g., PROTAC protein degraders, molecular glues, etc.), genetic medicines (antisense oligonucleotides [ASOs], small interfering RNA [siRNA], clustered regularly interspaced short palindromic repeats [CRISPR], etc.), and cell replacement-based therapeutics (stem cell therapies, CAR-T, etc.) have the potential to target previously undruggable proteins, modify gene expression and/or splicing, or even replenish cells that are selectively vulnerable in specific diseases or have disease modifying function. Choosing the most appropriate modality to address a specific disease is frequently not straightforward. Instead, it is a balancing act to pair the disease biology and proposed target with the drug modality that achieves the best therapeutic window (Figure 3). While anti-amyloid antibodies have been approved by the FDA to slow AD progression, the clinical benefit has been debated, particularly in light of safety concerns. Moreover, antibody-based therapeutics cannot target intracellular proteins. Thus, there is a clear unmet need for improved therapeutics for AD and other neurodegenerative diseases.

AD, PSP, PD, and amyotrophic lateral sclerosis (ALS) are chronic neurodegenerative diseases and examples of proteinopathies.[50] As part of disease pathogenesis, Tau (AD, PSP), Alpha-synuclein (PD), and TDP-43 (ALS) undergo an irreversible conformational switch from their native intracellular PPIs and/or folded state into oligomeric and/or aggregated species. These misfolded proteins can then act as pathological seeds for further protein misfolding when transferred between cells (Figure 4).[50] The conformational plasticity of intrinsically disordered proteins (IDPs) such as Tau, Alpha-synuclein, and TDP-43 is driven by low-complexity domains (LCDs). LCDs mediate rapid and reversible condensation into phase-separated biomolecular condensates, a process referred to as liquid-liquid phase separation (LLPS).[51] LLPS is hypothesized to be important for spatiotemporal regulation of nucleoli, ribonucleic protein granules, microtubule dynamics,[52] and synaptic vesicle formation.[53] Highly penetrant point mutations (e.g. A53T Alpha-synuclein, P301L Tau, etc.) can impact key PPIs, disrupt the normally dynamic LLPS, and cause proteins to irreversibly aggregate. However, protein aggregation occurs in the absence of mutations in most cases and increases with age, which is the primary risk factor for neurodegenerative disease. Multiple mechanisms, including a breakdown of proteostasis with age, may contribute to protein misfolding in idiopathic neurodegenerative

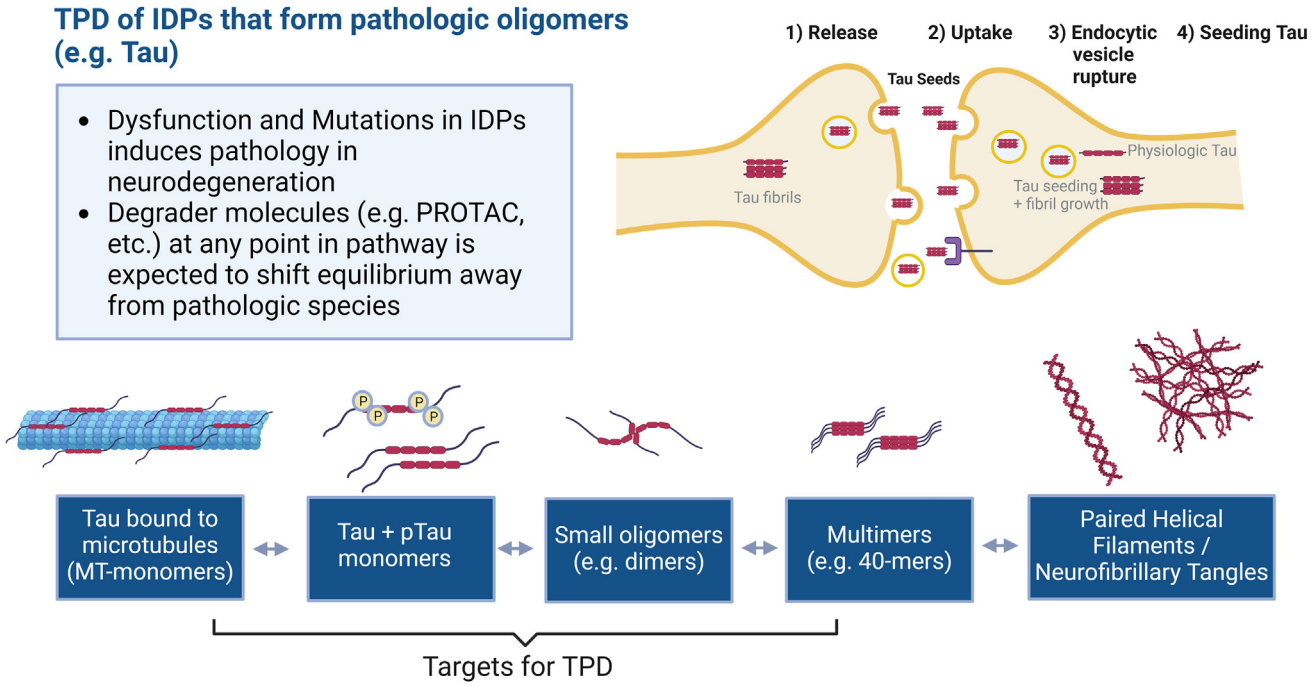

Figure 4. TPD strategy to degrade early pathologic species of templated IDPs and shift away from accumulating toxic oligomers to prevent trans-synaptic spread of pathologic protein (e.g. tau)
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disease.[54,55] TPD modalities may be able to leverage the UPS to restore cell health by removing toxic proteins that ultimately lead to neuronal loss. This approach contrasts with strategies in oncology that directly or indirectly aim to prevent cell proliferation by manipulating druggable proteins in pathways that drive uncontrolled cancer cell growth.

IDPs and the aggregated proteins derived from them are considered undruggable because they lack specific catalytic sites or defined enzymatic activity that can be inhibited, but it is generally agreed that they are toxic to cells when misfolded.[56,57] Thus, the prevailing therapeutic hypothesis is that preventing, slowing, or removing aberrantly aggregated proteins could halt or slow disease progression. Multiple recent discovery-stage biotechnology companies have focused on LLPS with the aim of rescuing the normal dynamic nature of biomolecular condensates. The major challenge will be to demonstrate that a small molecule can manipulate a condensate in a manner that would impact disease. Much further along in development, aggregation inhibitors for Tau and Alpha-synuclein are being tested in the clinic after showing promise in preclinical models.[58,59] Methylene blue derivative, HMTM, is reported to inhibit Tau aggregation.[60] Unfortunately, phase 3 clinical trials testing these methylene blue derivatives failed to demonstrate clinical benefit, although post-hoc subgroup analysis suggests neurofilament light chain (NfL), a neuronal damage biomarker, may accumulate more slowly in some patients. In development for PD is Emrusolmin (formerly Anle138b), an Alpha-synuclein aggregation inhibitor, that was well tolerated in patients with PD (NCT04685265) and healthy volunteers (NCT04208152) in a phase 1 study.[61] The Targeting Oligomer Pathology of Alpha-Synuclein in Multiple System Atrophy (TOPAS-MSA) study is currently enrolling patients with MSA, a rapidly progressive and debilitating synucleinopathy, in a phase 2 study. Small-molecule aggregation inhibitors continue to be of interest, but whether they will demonstrate clinical benefit remains an open question.

Protein lowering strategies using antisense modalities (e.g. ASOs, siRNA, etc.) have been successful in AD, PD, and ALS preclinical models and more recently in the clinic for ALS, thus providing a proof of concept for the protein-lowering paradigm of disease modification. ASOs are synthetic single- or double-stranded oligodeoxynucleotides that selectively bind a target mRNA via complementary base-pairing and inhibit translation, induce splice switching, or modify message stability.[62] The mode of binding enables precision-medicine approaches based on the sequence of the target gene, including disease-associated alleles or specific haplotypes. In 2023, the FDA approved Tofersen, an mRNA-lowering ASO, for the treatment of ALS patients with mutations in superoxide dismutase 1 (SOD1). Multiple unique point mutations in SOD1 impart a propensity for aggregation and a toxic gain-of-function resulting in a genetically defined subtype of ALS. Tofersen mediates the degradation of SOD1 transcript and resulted in cerebrospinal fluid (CSF) SOD1 reductions of 30%–40% after 28 weeks of treatment in a randomized double-blind placebo-controlled phase 3 trial (VALOR study) in patients with confirmed SOD1 mutations.[63,64] Although Tofersen did not improve clinical endpoints, it reduced concentrations neurofilament light chains in plasma over 28 weeks. The approval of Tofersen was supported by 12-month integrated results from the phase III VALOR clinical trial and the open-label extension (OLE) comparing the efficacy of Tofersen early initiation with delayed initiation. A second phase 3 trial called ATLAS is enrolling presymptomatic SOD1 ALS patients and

aims to assess whether Tofersen can delay the onset of symptoms (NCT04856982) with expected results anticipated in 2027.

Biogen is developing BIIB080, a Tau ASO licensed from Ionis for the treatment of AD. Unlike Tau antibodies that target extracellular Tau tangles that have thus far failed to demonstrate cognitive or functional improvement,[65] Tau ASOs target intracellular Tau. Dose-dependent reductions of total Tau, pTau, and tau positron emission tomography (PET) imaging were observed after a 13-week treatment period of BIIB080 (NCT03186989).[66] These results are particularly promising because tau PET imaging correlates with cognitive function in AD.[67] A major challenge for ASOs is bioavailability within target tissues, particularly for the brain. Tofersen and BIIB080, for example, are delivered intrathecally to bypass the BBB, but even then, they are not uniformly distributed in the central nervous system (CNS).[68,69]

BBB permeability, or a strategy to circumvent the BBB, is essential for targeting proteins in CNS cells. An intact BBB prevents passive diffusion of most small molecules and nearly all proteins and macromolecular complexes into the brain.[70] Although strategies to overcome BBB impermeability for oligonucleotide therapeutics, antibodies (including antibody-based TPD modalities), and recombinant proteins are being investigated, bioavailability in the brain after systemic dosing currently hinders therapeutic use unless the BBB is significantly compromised. A promising approach leverages transcytosis via the transferrin receptor. JR-141, a recombinant iduraonate-2-sulfatase fused to an antibody against the human transferrin receptor delivered via infusion, was approved by the FDA in 2023 for the treatment of Hunter syndrome.[71]

Physiochemical properties can be used to guide the design of BBB penetrant small molecules.[30] Despite falling outside of the Lipinski rule of five with respect to molecular weight, PROTAC protein degraders can cross the BBB and induce degradation of a target protein.[14] A number of small-molecule intrinsic features, including lipophilicity, molecular weight, number of H-bond donors, polar surface area, and solubility, can impact both oral bioavailability and may impact BBB permeability.[30] The combination of these small-molecule features and transporter mechanisms related to compound efflux, including P-glycoprotein and other ATP-binding cassette (ABC) transporters, and compound influx such as glucose and amino acid transporters have been reported to impact small molecule BBB permeability.[70] Large datasets examining BBB permeability of small molecules have been used to build tools that aim to predict BBB permeability.[72] Similar tools will undoubtedly aid PROTAC protein degrader design as more structures become available. Combined with oral bioavailability, a CNS penetrant PROTAC targeting disease-associated proteins is highly desirable for treating slow-progressing neurodegenerative diseases that could require patients to remain on treatment for decades.

PROTAC protein degraders that target Tau, Alpha-synuclein, mutant Huntingtin (mHTT), and other aggregation-prone proteins associated with neurodegeneration have been reported in the literature and are being pursued by biotechnology companies.[73] There are at least two possible approaches: (1) target the monomeric form of the protein prior to misfolding or (2) target pathogenic conformers (Figure 4). From a structural standpoint, targeting monomeric protein is more straightforward. A PROTAC targeting monomeric Tau reduced phospho-Tau species and moderately improved cognitive function in the hTau mouse model.[16] However, reducing the monomeric protein could be problematic if doing so hampers normal cellular function. Phase 2 studies of BIIB080 indicate this may not be an issue for Tau.[66] In contrast, development of Tominersen, an ASO targeting both the wild type and mutant HTT mRNA, was stopped after an analysis found no clinical benefit and poor safety signals, including cognitive decline and more rapid accumulation of neurofilament light chain in a Phase 3 study.[74] One interpretation is that the loss of the wild-type allele is detrimental to normal cellular function, although off-target aptameric or inflammatory effects were not ruled out. Specifically targeting the pathogenic protein in these scenarios would be a preferred strategy.

A major challenge for PROTAC discovery is understanding the most pathologically relevant conformers (Figure 4) and developing methods to produce and screen against them *in vitro* in a higher-throughput manner. PROTAC protein degraders targeting pathogenic Tau using the F-T807 PET ligand have been shown to be active against P301L and A152T Tau in neuronal cell models.[15] Cryoelectron microscopy (cryo-EM) structures of Tau paired helical filaments (PHFs), the large aggregates found in postmortem AD brain and other tauopathies, could inform further warhead optimization. Attempts to determine the binding mode of T807 to the core of Tau fibrils have been inconclusive,[75] but it's possible that T807 binds Tau in a sub-stoichiometric fashion, additional amino acids outside of the core are required, or other proteins that bind Tau fibrils are required. Smaller prefibrillar Tau oligomers are also an attractive pathological target for a PROTAC protein degrader. Preclinical studies suggest these may be PHF precursors and the most toxic of the Tau conformers (Figure 4).[76] Importantly, Tau oligomers can be purified, propagated *in vitro*, and tracked using antibodies to various phospho-species of Tau.[77]

Missense mutations and multiplications of SCNA, the gene that encodes Alpha-synuclein, are linked to autosomal dominant forms of PD. Alpha-synuclein is a small intrinsically disordered protein that moves between cytoplasmic, and membrane associated conformers.[78] Multiple cryoEM structures of Alpha-synuclein aggregates, the predominant component of Lewy bodies, have been reported.[79,80] Similar to Tau, the most toxic conformer may be the lower ordered oligomers, which may precede or be released from Alpha-synuclein fibrils.[81,82] These oligomers exert their toxicity at least in part by disrupting biological membranes.[83] Small molecule inhibitors of Alpha-synuclein aggregation have been reported to bind monomeric[84] and fibrillar forms of Alpha-synuclein,[85] which may provide a starting point for TPD development.

The differentiated mechanism of action and pharmacology of PROTAC protein degraders may outperform conventional inhibitors that target proteins in CNS cell types. Occupancy-driven pharmacology often requires high levels of drug to maintain inhibition. Combined with typically lower drug concentrations in the brain compared to the periphery due to the BBB, inhibitors may not achieve satisfactory safety margins or sufficient inhibition for clinical benefit. For example, nilotinib development in PD was halted after clinical trials failed to show clinical benefit at any dose.[86] However, CSF levels of nilotinib were dose independent, and the ratio of unbound CSF to plasma nilotinib of 0.5%–1% may be insufficient.[87] K0706, a c-Abl inhibitor with improved

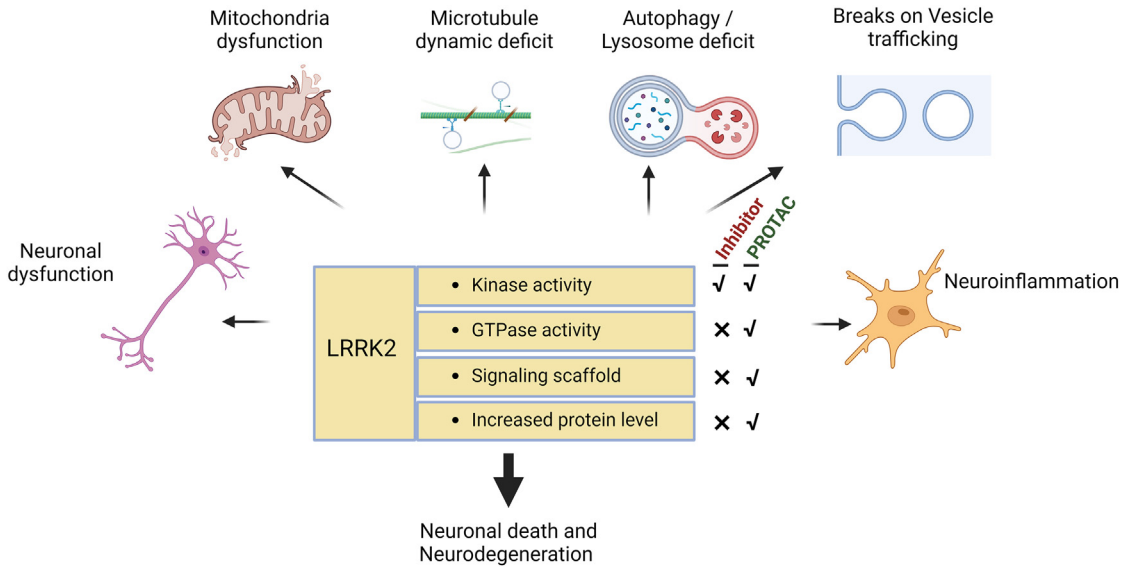

Figure 5. Roles of LRRK2 in familial and idiopathic Parkinson's disease
Kinase inhibitors only impact LRRK2 activity. PROTAC-targeted protein degraders will impact all LRRK2 activities that contribute to neuronal death and neurodegeneration. Created with Biorender.com.

BBB permeability,[88] is expected to complete phase 2 trials in 2024. The catalytic mechanism of a PROTAC protein degrader could, in theory, induce sustained suppression of c-Abl activity. This could be particularly true for long-lived proteins that have a slow resynthesis rate. For example, degradation of RIPK2 results in an extended pharmacodynamic response compared to inhibitors.[89]

LRRK2 is an attractive therapeutic target in PD and PSP.[90] Rare coding mutations in LRRK2 account for approximately 5% of familial PD cases and genome-wide association studies (GWASs) identified noncoding variants that upregulate LRRK2 in microglia increase PD disease risk.[9] Additionally, genetic variation at the LRRK2 locus suggests LRRK2 levels impact PSP disease progression.[10] LRRK2 is a 286 kDa multidomain protein with dual GTPase and kinase activity as well as domains associated with protein scaffolds.[12] LRRK2 has been linked to multiple cellular processes, including endolysosomal and mitochondrial function, interferon signaling, and neuronal morphology (Figure 5).[90] Pathogenic mutations clustered in the ROC-COR and kinase domains (e.g., G2019S) increase kinase activity resulting in a hyperactive protein, suggesting the kinase activity alone can contribute to disease. Whether the remaining domains also contribute to pathogenicity or to what extent they are needed for normal function is unclear. Large-scale human genomic analysis has shown that loss of function *LRRK2* variants that reduce LRRK2 protein levels are not associated with any specific phenotype or disease state.[91] Microvacuolation was observed in some peripheral cells in LRRK knockout animals but are otherwise viable. LRRK2 kinase inhibitors and ASOs that knockdown LRRK2 rescue Th+ neurons in rodent PD models.[11] Most recently, a potent, selective PROTAC degrader of LRRK2 employing VHL, XL01126, has been demonstrated to cross the BBB following IV administration.[15] Validation of LRRK2 as a disease-modifying target in the clinic is ongoing, with inhibitors and a PROTAC protein degrader in early clinical development. The rationale to focus on PSP and PD is based on human genetics, transcriptomics, and nonclinical research suggesting that LRRK2 is hyperactive in disease, induces scaffolding functions linked to neuronal cell death, is upregulated in microglia, and puts the breaks on the lysosomal protein clearance system.[9–14] ARV-102 LRRK2 targeting PROTAC protein degrader is the first oral brain penetrant PROTAC to enter clinical development for neurodegeneration.

CLINICAL DEVELOPMENT AND THERAPEUTIC POTENTIAL

Patients suffering from neurodegenerative disease associated with misfolded protein aggregates other than AD and SOD1-ALS have few or no options for effective disease modifying treatments. TPD is among the recent innovations in therapeutic modalities that could address the shortcomings of other approaches such as immunotherapy or antisense oligonucleotides, particularly when targeting intracellular proteinopathies. Similar shortcomings exist when it is important to differentiate mutant from wild-type proteins.

Small molecule TPD therapeutic strategies warrant traditional approaches for first-in-human (FIH) studies, including evaluation of single and multiple ascending doses for the purpose of assessing initial safety and tolerability and for initial characterization of pharmacokinetic/pharmacodynamic (PK/PD) relationship. The latter requires careful selection of target engagement and pharmacodynamic biomarkers to be measured systemically (i.e., blood/plasma) and in the cerebrospinal fluid. Depending on the target, the FIH studies could be conducted in either healthy volunteers or patients.

Many small molecule TPD strategies are amenable to formulation for oral dosing, an important consideration when developing potential disease-modifying therapies for neurodegenerative diseases that will likely require long-term or even life-long

dosing. Furthermore, oral dosing allows for better dose optimization, dose adjustments (e.g., titration), and treatment holidays, if necessary, given shorter half-life compared to other modalities such as ASOs. A potential drawback for an oral medicine targeting the CNS is the systemic exposure needed to achieve effective BBB penetration. Careful evaluation to rule out off-target effects and potential systemic toxicities will be critical for the success of TDP as a therapeutic modality targeting the CNS.

Other routes of drug administration, such as intravenous injection, may allow wider chemical properties for degraders—including biodegraders. An important consideration here, however, is that target proteins with fast re-synthesis rates (and concomitant short half-lives) may not be amenable to the intermittent dosing required for most infusion strategies. The relationship between disease-causing target protein levels and disease progression is another consideration. For neuronal health, intermittent clearance of specific pathologic protein species could be therapeutic.

A better understanding of the pathophysiology of neurodegenerative disease, particularly as it relates to LLPS and protein aggregation, will inform strategies for disease modification and hypotheses in support of specific modalities and promising targets for TPD. Incorporation of novel E3s and non-UPS degradation mechanisms may further expand the number of degradable targets for neurodegenerative diseases.

FUTURE DIRECTIONS, CHALLENGES, AND CONCLUSIONS

The potential of TPD, in particular PROTAC protein degrader molecules, in neuroscience is increasingly promising as recent clinical proof of mechanism has been demonstrated in cancer and immunology, and nonclinical research studies have successfully demonstrated the degradation of key neurodegenerative disease targets. However, significant challenges persist in discovering small-molecule ligands or MGDs that are suitable for degradation for IDPs that cause neurodegeneration in the CNS. The chemical characteristics of TPD molecules require a deep understanding of the intricate biochemical and mechanistic interactions that drive ternary complex cooperativity and ultimately intracellular degradation. The discovery of MGDs to new disease targets requires careful identification of effective degraders and the detailed understanding of the specific protein-protein interactions they facilitate. On the other hand, PROTAC protein degrader molecules offer the opportunity for rational design considerations that can be uniquely applied to these heterobifunctional molecules, and companies that have deep expertise are beginning to understand how to engineer these molecules to cross the BBB.

Advancements in co-opting CNS-enriched E3 ligases would be transformative for the application of both PROTACs and molecular glues in neurodegenerative diseases. Despite these challenges, the unique event-driven pharmacology offered by these degraders aligns well with the dynamic nature of CNS diseases, presenting an opportunity for groundbreaking therapeutic innovations in an area with substantial unmet medical needs.

The path to successful application of PROTAC technology in neurodegenerative diseases mirrors the well-documented innovation cycles seen in other areas of drug development. The remarkable success of TPD in oncology, with several candidates advancing to pivotal clinical trials, sets a precedent for what could come in neurodegeneration. Extending this technology to tackle proteins that form neurotoxic oligomers and aggregates that promote pathologic glial inflammatory processes, a hallmark of many neurodegenerative diseases, offers promising advantages over current antibody-based approaches. Nevertheless, the complexity of bringing CNS-targeted PROTACs to clinical fruition should not be underestimated.

Despite these hurdles, the growing research and collaborative efforts in this field are cause for optimism. The potential to develop effective, disease-modifying therapies for neurodegenerative diseases through targeted protein degradation could herald a new era in CNS therapeutics. With continued innovation and perseverance, the transformative power of these technologies is poised to unlock new treatment paradigms, ultimately meeting the profound need for effective CNS disease interventions.

DECLARATION OF INTERESTS

J.G., C.H., J.C., and A.C. are employees and shareholders of Arvinas. Arvinas funds research on and holds patents and trademarks for PROTAC protein degraders.

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