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TOPIC SUMMARY

TARGETED PROTEIN DEGRADATION: NOVEL PHARMACOLOGICAL APPROACH FOR UNMET MEDICAL NEEDS

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Professor Alessio Ciulli, Director of the Centre for Targeted Protein Degradation at the University of Dundee, presented the conference "*Small-molecule Protein Degraders: Current State and Trends and Future Directions"* on 05 Dec 2024 at the School of Pharmacy of the University of Barcelona. Dr. Ciulli's conference was followed by the round table *"New Perspectives of Targeted Protein Degradation (TPD) in Therapeutic Approaches for Unmet Medical Needs"* that also included Dr. Mercè Pallàs (Director of the Catedra) and Dr. Carles Galdeano of the University of Barcelona, Dr. Jordi Bach of Almirall, and Dr. Carlos Plata who chaired the round table.

This is a brief summary of various general contemporary topics regarding TPD.

The human genome contains approximately 20,000 protein-coding genes. Because of mRNA processing, gene exons and alternative splicing (protein isoforms or variants), the number of different proteins in the human body is over 100,000 [15].

Proteins are necessary for many critical functions of the body including structure and regulation of the body systems. However, changes in protein conformation, excess of production and dysregulated proteins can result in pathologies due to abnormal protein function, aggregation and accumulation which disrupt cellular biological processes and interactions between cells, tissues, organs and systems. The current pharmacological armamentarium for the treatment of diseases includes a variety of protein inhibitors, such as the class of protein kinase inhibitors directed to different

kinases, which are extensively used in various types of cancer, inflammatory, dermatological and other diseases and disorders [13].

A new approach in pharmacology targeting proteins focuses on the down-regulation or elimination of a pathological protein, rather than its inhibition, by relying on the cell's intrinsic protein degradation and clearance mechanisms to dispose of the protein causing a disease. These small molecules (protein degraders) utilize the cellular ubiquitin-proteasome system to induce ubiquitination of a target protein leading to its subsequent degradation (Targeted Protein Degradation or TPD) [5, 12, 16]. That is, protein degraders take advantage of a critical cellular regulatory system involved in homeostasis and responsible for the regulated proteolysis that occurs to protect the cells from abnormal (unfolded or misfolded) proteins, which, if not dealt with, may result in e.g., impacts on cell function, protein aggregation and cell damage. In this intracellular process, the small protein ubiquitin acts as a molecular tag for protein degradation and the proteasome breaks down proteins that are tagged with ubiquitin by an E2 enzyme ligase (the E2 ligase interacts with a specific E3 partner and transfers the ubiquitin to the target protein).

Pathological proteins caused by mutations, changes in conformation, overexpression and aggregation can result in disease by causing gain of function or cellular damage due to biochemical dysregulation (e.g., due to aggregates) are currently being considered as preferable protein targets for TPD. Elimination of a pathological protein through TPD could offer advantages over the effects of a protein inhibitor, including to target undruggable proteins, increase specificity, and enhance tissue selectivity [12, 16, 19, 20]. Moreover, protein degraders, by inducing clearance of the abnormal pathogenic protein, terminate with all its activities, while a selective protein inhibitory mechanism is limited to acting at an active site of a protein. Hence protein degraders provide a holistic opportunity to impact the totality of a dysregulated protein causing disease since protein degraders will obliterate all protein activities (enzymatic and nonenzymatic; protein degraders can affect proteins that lack catalytic binding sites, compared to inhibitors that need to bind to a biologically functional active site). Furthermore, protein degraders are associated with a transient binding rather than competitive occupancy and are able to dissociate after promoting the polyubiquitination of an abnormal or disease-causing protein. Hence, overall, protein

inhibition and protein elimination as pharmacological approaches, could be positioned as complementary.

Two main types of current protein degraders are [5, 11, 12, 16, 18, 20]:

1) Small molecule “molecular glues” protein degraders that induce protein-protein interaction between a target protein and an E3 ligase, resulting in the ubiquitination and subsequent intracellular degradation of the protein causing a disease.

2) Proteolysis Targeted Chimeras (PROTACs) which are heterobifunctional degrader molecules that comprise a binder to a target protein and a binder to the E3 ligase that are joined by a linker. In general, the bifunctional PROTAC degraders known recruit Cullin RING E3 ubiquitin ligases (the von Hippel-Lindau and cereblon proteins), forming a ternary complex with the E3 ubiquitin ligase and a target protein, leading to polyubiquitination and degradation of the target protein by the proteasome within a cell. A single PROTAC molecule can operate in an iterative manner, enabling multiple ubiquitylation reactions (leading many pathogenic or disease-causing protein copies to destruction), consequently enhancing potency and efficiency while potentially reducing the amounts needed and doses required.

Although there are differences between small molecule “molecular glues” and PROTACs [16, 20], both result in a similar mechanism of action that involves the ternary complex degrader molecule-target protein-E3 ligase leading to target ubiquitination and protein degradation by the cellular proteasome. As more E3 ligases are being characterized, further opportunities may arise due to the fact that there are hundreds of individual human E3 ligases that may provide versatility to enhance selectivity [18, 20].

There are also other modalities of TPD with the overall similar objective of protein cellular degradation such as the endosome-lysosome and autophagy-lysosome systems [9, 12, 16], degron-driven pathways [4, 11], polymerization-induced degradation [19], as well as others, including biological proteolysis targeted chimeras [14] and the potential of ubiquitin-independent degradation [9].

Currently over 100 clinical trials (safety and tolerability, proof-of-concept and Phase 2 and 3) with a variety of protein degraders targeting different proteins have been reported [12, 16, 17, 19, 20, https://www.slideshare.net/slideshow/global-targeted-protein-degraders-2025-preview-copy-pdf/275451704]. The majority relate to cancer targets involving breast, prostate, lung, pancreatic, sarcoma, multiple myeloma, leukemia, lymphoma and other liquid and solid tumors, while there also ongoing trials for other therapeutic areas including autoimmune and neurological disorders [12, 16, 17, 19, 20].

Each therapeutic area offers opportunities for pharmacological interventions using TPD to address unmet medical needs. One example is neurodegenerative diseases, which have limited or no current treatments available that address the underlying pathophysiology of the disease, many of which have significant social implications due to the aging population. Key in these diseases is the presence of proteinopathies that by direct (aggregation, accumulation) or indirect (e.g., inflammation) toxic effects, contribute to neurodegeneration [7]. Alzheimer's and other types of dementias, fronto-temporal disorders, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, progressive muscular atrophy, Creutzfeldt-Jakob disease, and Picks disease, among others, are associated with a variety of potential protein targets depending of the disease (e.g., amyloid-beta, tau, alpha- and other synucleins, mutant Huntingtin, TAR DNA-binding protein 43, superoxide dismutase 1, prion protein, and others) [3, 7, 8]. Neurodegenerative diseases may also exhibit overlap of abnormal proteins; for example alpha-synuclein pathology (or alpha-synucleinopathy) is a feature that occurs in cases of Parkinson's disease, Lewy body dementia, and Alzheimer's disease [7].

Therefore, TPD in neurodegenerative diseases is an example of its potential applicability, as they are associated with proteinopathies resulting from an excess of a protein and/or proteins with abnormal conformations that lead to protein aggregates and accumulation of misfolded proteins that over time produce neuronal cell toxicity and a progressive loss of neurons in the affected brain regions [8]. The TPD pharmacology modality in neurodegenerative diseases would align with the therapeutic hypothesis that preventing, slowing, or removing aberrantly aggregated

proteins could impact (slow or halt) disease progression. Moreover, there could be several TPD approaches to explore, such as targeting different stages that involve a pathological form of a protein, from misfolded monomeric proteins to the aggregation phases to form dimers, oligomers, multimers, protofibrils and mature fibrils [3, 7].

Protein aggregation diseases also include peripheral amyloidogenic diseases such as hemodialysis-related disorders, type 2 diabetes, and other amyloidoses that result from changes in protein conformation (i.e., change from alpha-helix to beta-sheets forms is a characteristic of amyloid deposits) that lead to their aggregation and deposition [2].

Accordingly, TPD as a pharmacological modality offers multiple opportunities in drug discovery and clinical development to address unmet medical needs, particularly for challenging molecular targets for currently available therapeutic modalities (e.g., small molecules, peptides, antibodies, and nucleic acid-based therapies).

At the same time, there are challenges with TPD currently being investigated that include:

i) target engagement (e.g., the linker length, rigidity, and stereoselectivity have implications for affinity, and stability, and pharmacokinetics)[10, 12, 20];
ii) other ADME properties including bioavailability and metabolism of larger molecules (PROTACs) [12, 16, 20];
iii) defining specific ligand recognition motifs [11];
iv) assessment of the E3 ligase diversity [11];
v) activities and genetic differences that impact effectiveness of TPD as well as identification of novel effector sites for TPD that may have implications for addressing potential drug resistance [18];
vi) modeling and application of delivery technologies [20] to address challenges of cellular membrane permeability [10, 16], pathological tissue penetration such as tumors [17], and blood-brain barrier penetration [8]; and
vii) extending the spectrum of action of TPD to membrane-bound and extracellular proteins [16].

Progress has been made in the molecular mechanism and zone of ubiquitination associated with protein degraders [1], as well as in enhancing rational design approaches for protein degraders by studying the structural aspects of the ternary complex, including differences between *trans* and *cis* intermolecular interactions between the target protein and ligase [5]. In addition, advances in the development of new computational tools and the expansion of artificial intelligence are being applied to understand the structure−activity relationships of the ternary complex structure (protein-degrader-ligase) driven to intracellular degradation [10, 20].

Due to the mode of action of TPD, which can be iterative and prolonged [12], the pharmacokinetics-pharmacodynamics of TPD also require special attention since there are differences compared to small molecule protein inhibitors. This will aid in defining approaches and regimens to enhance target selectivity, maintain efficacy, minimize toxicity, and to prevent off-target effects and inhibition of efficacy caused by high concentrations of PROTACs, which can lead to the hook effect (i.e., a protein degrader could bind to each protein-binding partner individually leading to the formation of binary complexes that compete with the ternary complexes and negatively impact the degrader potency) [10, 20]. Additionally, the administration of a protein degrader molecule could elicit a prolonged pharmacodynamic response and potential drug-induced toxicity due to cumulative drug exposure [12], or due to the intrinsic mechanism of protein degraders resulting in complete reduction of a target protein (or of an unintended off-target protein) which may require an extended period of time for cellular recovery [12]. These challenges of the emerging TPD pharmacological modality are being extensively investigated.

Thus, continuous progress is expected in the drug discovery and basic and clinical pharmacology of the emerging TPD, particularly for proteins that cause diseases that have been challenging to target with well-established pharmacological approaches.

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