Targeted Protein Degradation:
New Promises for "Undruggable" Diseases

Commentary by Alessia Armezzani, PhD, & Amélie Rezza, PhD 16/02/2021

For the past 20 years, drug development has relied heavily on antibodies and enzymatic inhibitors, allowing some groundbreaking advances but also revealing limitations in these approaches.

Antibodies are highly selective, but because of their large molecular weight, they can only block extracellular domains of target proteins, which limits their therapeutic use for certain disorders. In addition, they require intravenous or subcutaneous administration, i.e., invasive delivery approaches that are not always well tolerated by patients and represent a drain on health care time and resources.[1,2]

Enzymatic inhibitors, on the other hand, have a low molecular weight (usually <600 Da, which makes them rightful members of the "small molecules" class), relatively simple chemical structures, and are cell permeable, allowing them to engage intracellular targets and, most importantly, cross the blood–brain barrier. They can be optimized for oral bioavailability and metabolic stability, thereby facilitating their administration and storage, as well as reducing costs for health care systems and patients.[3] However, these molecules possess important limitations. Owing to their occupancy-based model, they require high systemic drug levels for therapeutic efficacy, often leading to off-target and side effects. They typically disrupt the activity of a single domain, usually insufficient to inhibit all functions of the multidomain proteins. Most importantly, as inhibitors usually target active sites, they are unsuitable for targeting proteins lacking those (e.g., non-enzymatic proteins, or transcription factors), which represent 75% of the human proteome, and are therefore considered "undruggable."[4,5]

These limitations have prompted scientists to develop a new modality for potential therapy called "targeted protein degradation," whereby small molecules (referred to as "degraders") redirect newly translated proteins toward the proteasomal machinery. In other words, degraders selectively target proteins for removal, rather than inhibiting their activity.[3] As such, degraders offer a valid therapeutic alternative to inhibitors and antibodies, since they combine the drug-like properties of small molecules (e.g., solubility, permeability, metabolic stability) with the benefits of event-driven pharmacology, i.e., binding-induced elimination of the target protein. These specificities make them exceptional tools for the selective degradation of targets previously considered 'undruggable'.[6–9]

Several methods have been developed to artificially induce degradation of target proteins. Interestingly, they all aim to control E3 ubiquitin ligase activity. E3 ubiquitin ligases act sequentially,

together with E1 ubiquitin-activating enzymes and E2 ubiquitin-conjugating enzymes, to catalyze the covalent binding of multiple ubiquitin molecules to protein substrates targeted for degradation.[10–12] Ubiquitin ligase activity can be controlled through (i) small molecule degraders (i.e., bifunctional and molecular glue degraders), (ii) macromolecule conjugates, or (iii) genetically encoded degradation tags.[13]

Several small molecule degraders and macromolecule conjugates have been developed as therapeutic agents in the past few years. PROTACs (proteolysis-targeting chimeras or bifunctional degraders) were developed to simultaneously bind a protein of interest and a component of the ubiquitin E3 ligase complex (Figure 1).[14] Such molecules have the ability to degrade the entire target protein, its enzymatic and non-enzymatic domains, and can successfully dissociate from the target after promoting its polyubiquitination, suggesting a potential efficiency at very low doses. Additionally, because their inhibition process is event-driven, and not occupancy-driven (unlike enzymatic inhibitors), PROTACs are less susceptible to mutations and target protein's expression dysregulation, thus overcoming potential resistance observed for current therapeutic treatments.[15] At present, PROTACs have been successfully used against cancers, neurodegenerative disorders, viral infections, and immune diseases.[16]

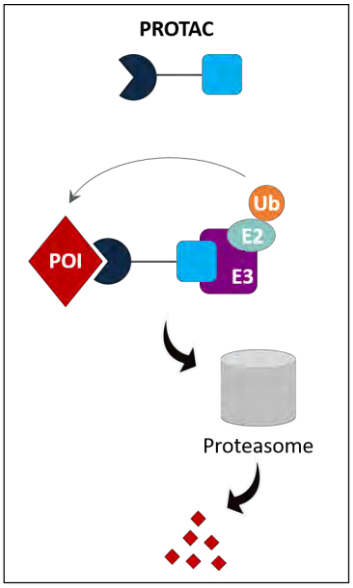

Figure 1 | Mechanism of action of PROTAC. POI, protein of interest; Ub, ubiquitin.

Similarly, molecular glue degraders can force an interaction between the protein of interest and a component of an E3 ubiquitin ligase complex. Thalidomide and indisulam, both examples of molecular glues, induce the degradation of their substrates by redirecting them to this very complex. Withdrawn from the market due to its teratogenic effects, thalidomide has now been approved by the FDA for the treatment of leprosy complications and multiple myeloma, while indisulam is currently being tested for the treatment of solid tumors.[6,13]

An example of macromolecular conjugates is TRIM21. This ubiquitin ligase displays a broad antibody isotype specificity, and the ability to ubiquitinate itself. These properties have been exploited by scientists to redirect target proteins-antibody complexes to the ubiquitin-proteasome system, thus degrading endogenous proteins for which specific antibodies are available (Figure 2).[17] However, it has been shown that using TRIM21 can induce target protein's binding partners degradation, as well as a rapid recovery of the main target.

As previously mentioned, ubiquitin ligase activity can also be controlled through genetically encoded degradation tags. Although not fitted for therapeutic use, these approaches provide optimal preclinical models when developing treatments through targeted-protein degradation. Different methods have emerged in the past 10 years, including the small molecule–assisted shutoff system, or SMASh.

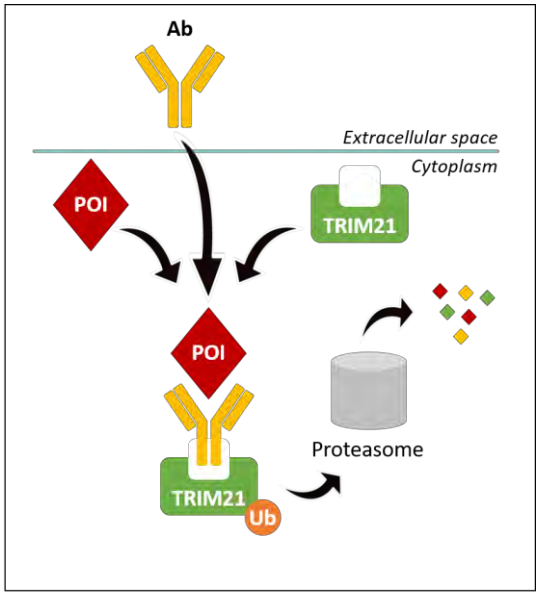

Figure 2 | Mechanism of action of TRIM21. Ab, antibody; POI, protein of interest; Ub, ubiquitin.

This system, a novel tag comprising the hepatitis C virus (HCV) NS3 protease, was developed in 2015 by Michael Z. Lin and his team.[18] In the absence of the protease inhibitor, the SMASh tag self-cleaves, producing an intact protein, whereas in its presence, all newly synthetized tagged proteins are rapidly degraded (Figure 3).[18] The SMASh system possesses many advantages over other targeted protein degradation methods: it is inducible, reversible, and requires only limited engineering.

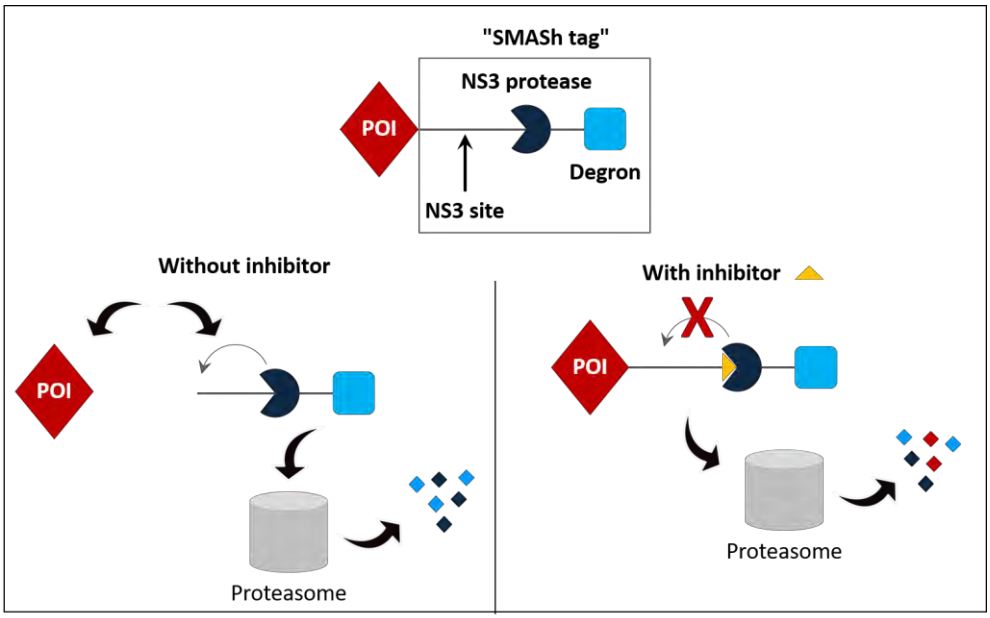

Figure 3 | Mechanism of action of SMASh. POI, protein of interest; Ub, ubiquitin.

Today, targeted-protein degradation has become a promising field in drug development. Combining optimized preclinical models using genetically encoded degradation tags such as SMASh

with small molecule degraders or macromolecule conjugates as therapeutics promises to expand the list of druggable proteins. This should provide new opportunities for novel treatments for diseases previously considered to be “undruggable.”

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References:

1. Lu R-M, Hwang Y-C, Liu I-J, et al. Development of therapeutic antibodies for the treatment of diseases. *J Biomed Sci*. 2020;27(1):1. doi:10.1186/s12929-019-0592-z

2. Holdgate GA, Meek TD, Grimley RL. Mechanistic enzymology in drug discovery: a fresh perspective. *Nat Rev Drug Discov*. 2018;17(2):115-132. doi:10.1038/nrd.2017.219

3. Kostic M, Jones LH. Critical Assessment of Targeted Protein Degradation as a Research Tool and Pharmacological Modality. *Trends Pharmacol Sci*. 2020;41(5):305-317. doi:10.1016/j.tips.2020.02.006

4. Ngo HX, Garneau-Tsodikova S. What are the drugs of the future? *Medchemcomm*. 2018;9(5):757-758. doi:10.1039/c8md90019a

5. An S, Fu L. Small-molecule PROTACs: An emerging and promising approach for the development of targeted therapy drugs. *EBioMedicine*. 2018;36:553-562. doi:10.1016/j.ebiom.2018.09.005

6. Chamberlain PP, Hamann LG. Development of targeted protein degradation therapeutics. *Nat Chem Biol*. 2019;15(10):937-944. doi:10.1038/s41589-019-0362-y

7. Cromm PM, Crews CM. Targeted Protein Degradation: from Chemical Biology to Drug Discovery. *Cell Chemical Biology*. 2017;24(9):1181-1190. doi:10.1016/j.chembiol.2017.05.024

8. Di L, Kerns EH, Carter GT. Drug-like property concepts in pharmaceutical design. *Curr Pharm Des*. 2009;15(19):2184-2194. doi:10.2174/138161209788682479

9. Lai AC, Crews CM. Induced protein degradation: an emerging drug discovery paradigm. *Nat Rev Drug Discov*. 2017;16(2):101-114. doi:10.1038/nrd.2016.211

10. Chondrogianni N, Gonos ES. Structure and function of the ubiquitin-proteasome system: modulation of components. *Prog Mol Biol Transl Sci*. 2012;109:41-74. doi:10.1016/B978-0-12-397863-9.00002-X

11. Gong B, Radulovic M, Figueiredo-Pereira ME, Cardozo C. The Ubiquitin-Proteasome System: Potential Therapeutic Targets for Alzheimer’s Disease and Spinal Cord Injury. *Front Mol Neurosci*. 2016;9. doi:10.3389/fnmol.2016.00004

12. Zheng N, Shabek N. Ubiquitin Ligases: Structure, Function, and Regulation. *Annu Rev Biochem*. 2017;86(1):129-157. doi:10.1146/annurev-biochem-060815-014922

13. Wu T, Yoon H, Xiong Y, Dixon-Clarke SE, Nowak RP, Fischer ES. Targeted protein degradation as a powerful research tool in basic biology and drug target discovery. *Nat Struct Mol Biol*. 2020;27(7):605-614. doi:10.1038/s41594-020-0438-0

14. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc Natl Acad Sci U S A*. 2001;98(15):8554-8559. doi:10.1073/pnas.141230798

15. Sun X, Gao H, Yang Y, et al. PROTACs: great opportunities for academia and industry. *Signal Transduct Target Ther*. 2019;4:64. doi:10.1038/s41392-019-0101-6

16. Gao H, Sun X, Rao Y. PROTAC Technology: Opportunities and Challenges. *ACS Med Chem Lett*. 2020;11(3):237-240. doi:10.1021/acsmedchemlett.9b00597

17. Clift D, McEwan WA, Labzin LI, et al. A Method for the Acute and Rapid Degradation of Endogenous Proteins. *Cell*. 2017;171(7):1692-1706.e18. doi:10.1016/j.cell.2017.10.033

18. Chung HK, Jacobs CL, Huo Y, et al. Tunable and reversible drug control of protein production via a self-excising degron. *Nat Chem Biol*. 2015;11(9):713-720. doi:10.1038/nchembio.1869