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Review

Proteolysis Targeting Chimeras (PROTACs): A Perspective on Integral Membrane Protein Degradation

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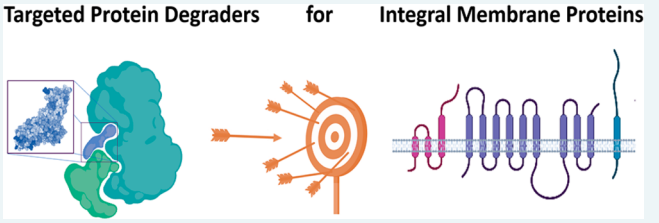
ABSTRACT: Targeted protein degradation (TPD) is a promising therapeutic modality to modulate protein levels and its application promises to reduce the "undruggable" proteome. Among TPD strategies, Proteolysis TArgeting Chimera (PROTAC) technology has shown a tremendous potential with attractive advantages when compared to the inhibition of the same target. While PROTAC technology has had a significant impact in scientific research, its application to degrade integral membrane proteins (IMPs) is still in its beginnings. Among the 15 compounds having entered clinical trials by the end of 2021, only two targets are membrane-associated proteins. In this review we are discussing the potential reasons which may underlie this, and we are presenting new tools that have been recently developed to solve these limitations and to empower the use of PROTACs to target IMPs.



KEYWORDS: *PROTAC, integral membrane proteins, targeted degradation, LYTAC, AbTAC, molecular glues*

Targeted protein degradation (TPD) is a promising therapeutic strategy to modulate abundance of disease-linked proteins. The attractive aspects of this strategy are the ability to address targets previously considered to be "undruggable" due to the lack of a therapeutically amenable active site, and the ability to overcome resistance to conventional therapy emerging from acquired mutations or compensatory feedback pathways.[1−3] Degradation of therapeutically interesting proteins allows instead a longer and more complete inactivation of the target(s), and by doing so it attenuates not only their enzymatic function but also any scaffolding roles they may have and the possibility of kinome rewiring.[4,5]

Among TPD strategies, proteolysis targeting chimera (PROTAC) technology has seen the most prominent development. Described already 20 years ago,[6] PROTACs are heterobifunctional molecules consisting of two ligands—also called warheads—jointed by a flexible chemical linker, thereby enabling them to simultaneously bind an E3 ubiquitin ligase and a protein of interest (POI). Target engagement of both warheads induces spatial proximity of the targets, leading to polyubiquitination of exposed lysines on the target protein by the E3 ubiquitin ligase complex, and the subsequent degradation of the POI by the ubiquitin−proteasome system (UPS) (Figure 1A).

A great advantage of using PROTACs is their inherent recycling: after dissociation from the target, the chimera can bind an additional free POI and proceed to stimulate its degradation in an iterative manner (Figure 2). This event-driven mechanism allows the degradation of the target in substoichiometric quantities, as long as target engagement is noncovalent.[7] Hence, PROTACs operate as catalytic molecules enabling the use of lower doses compared to the parent inhibitor (i.e., POI-specific warhead). This has the potential therefore to reduce undesired side effects and to allow a wider therapeutic window.[8]

Another important advance of PROTACs over conventional drugs is their ability to effectively degrade a target independently of an active site, as the mechanism of action relies on spatial proximity of the E3 and the POI.[9] This feature dramatically increases the druggable proteome. Furthermore, many studies highlighted how chimeras with low-affinity ligands to their cognate POI can still achieve potent degradation due to the positive cooperative interaction between the E3 and the target.[10,11] Thus, PROTACs lead to a stronger and longer lasting effect when compared to the

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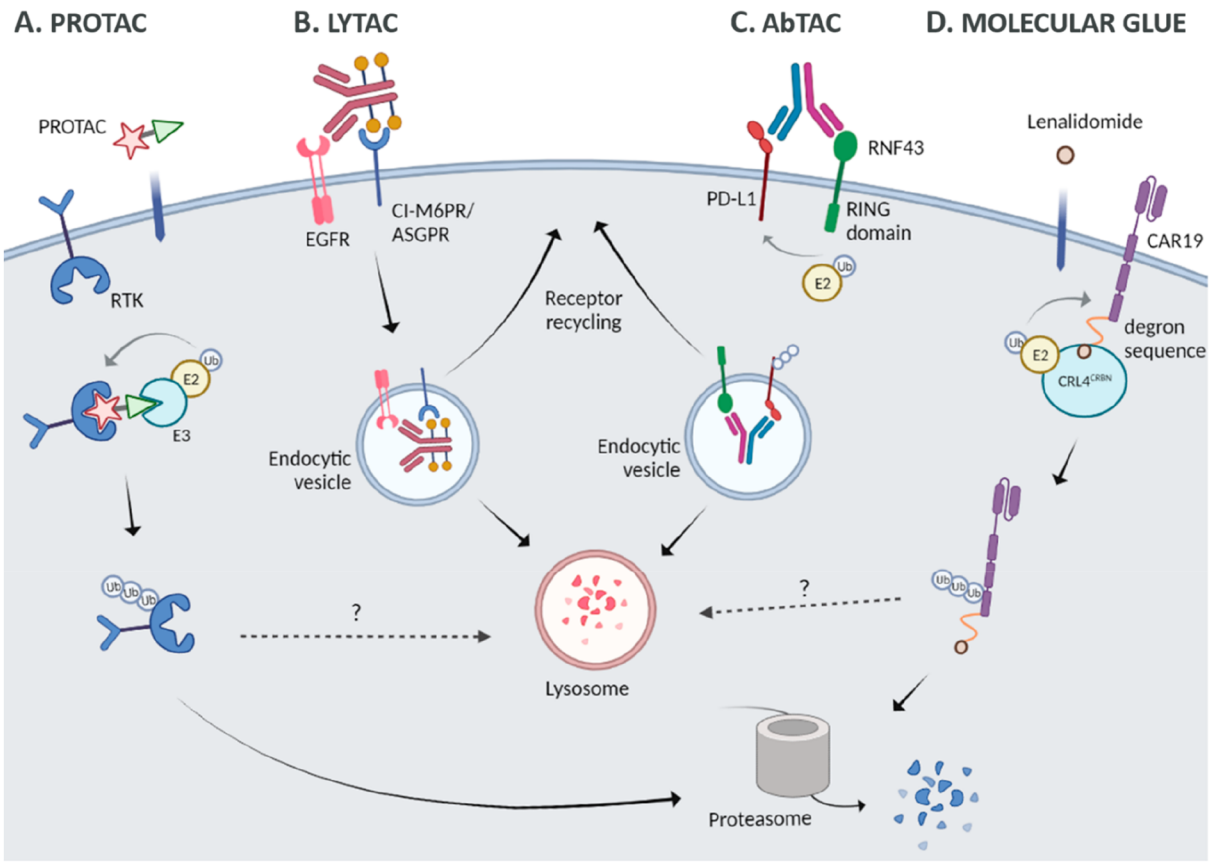

Figure 1. Integral membrane protein-targeted degradation technologies. (A) PROTAC technology brings polyubiquitination of the target and its proteasomal degradation. (B) CI-M6PR or ASGPR in LYTAC technology leads to the IMP degradation through the lysosomal system.[25,51] (C) Bispecific IgG of the AbTAC tool recruits RNF43 E3 ligase to polyubiquitinate PD-L1 and to induce its lysosomal degradation.[23] (D) Lenalidomide as a molecular glue binds the degron tag of CAR19 recalling the $CRL4^{CRBN}$ E3 ligase and inducing target polyubiquitination. Proteasomal degradation follows.[75]

parent molecule inhibition, with a major impact on the downstream signal(s) and the scaffolding function of the target.[5,8]

Another decisive point of strength that marks PROTACs is selectivity. Compared to the parent inhibitor, PROTACs often present a higher selectivity for their target, and this is achieved through linker optimization and ternary complex stabilization. Linker length and composition in fact impact on the binding, which is often not single but involves new positive cooperative protein−protein interactions between the E3 and the POI.[12] This is translated in the possibility of compensation for low binary affinity, allowing the formation of a stable ternary complex even in the presence of a low-affinity ligand for the POI.[13] In pharmacological optics, selectivity is a central characteristics for TPD compounds to avoid adverse side effects.

These features, together with a favorable absorption, distribution, metabolism, and excretion (ADME) profile,[8,14] have contributed to a profound interest in the therapeutic use of PROTACs for a broad array of diseases. This has already resulted in 15 PROTACs (Table 1) having entered clinical trials by the end of 2021.[15] It is noteworthy that the list is strongly biased toward kinases and nuclear factors, while only two integral membrane proteins (IMPs) are being pursued, despite the large availability of ligands[16] and the validated PROTAC development chemistry.[17] This clearly indicates that there is a challenge in targeting integral membrane proteins with PROTACs, largely inherent to their cellular localization,[18] and further research is required to overcome this. While publications showcasing the successful degradation of IMPs are limited, this Review will highlight those successful cases and discuss future perspectives.

PROTACs AGAINST INTEGRAL MEMBRANE PROTEINS

PROTAC technology has gained rapid traction since its development, but its application to IMP-targeted degradation is still in its infancy. IMP overexpression or mutation is frequently linked to malignancies [e.g., epidermal growth factor receptor, EGFR[19−21]], providing the cells nutrients and all the needs for an uncontrolled growth.[14,22−29] The use of conventional IMP-targeting drugs to contrast this has proven beneficial in treating many diseases, yet often their long-term administration can lead to drug resistance. A prime example for this is the EGFR, which is implicated in the pathogenesis of nonsmall cell lung cancer (NSCLC).[19] Three generations of selective inhibitors have been approved by the FDA, each of which were developed to target specific receptor mutations attempting to overcome the resistance to the previous generation drug.[30]

PROTACs offer the opportunity to circumvent the complication of drug resistance as they promote degradation

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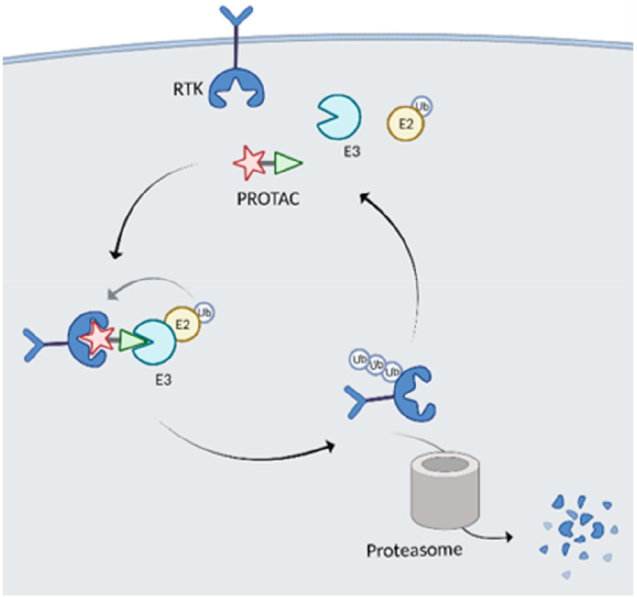

Figure 2. Inherent recycling of a PROTAC. Once a PROTAC molecule has engaged with both the target and the E3 ligase in a ternary complex, the target protein becomes ubiquitinated and degraded by the proteasome while the PROTAC molecule dissociates from the target and can be used for a new cycle of degradation.

of pathology-driving targets, instead of long-term use of conventional drugs. They are more effective and have a wider impact on the downstream signal(s);[5,8] therefore, a shorter application is needed without the risk of developing new mutations. Successful compounds have been reported (Table 2), and their observed effects on tumor growth and downstream signaling indicate a good prospect of membrane proteins being targetable with PROTACs. Multiple studies have used mass spectrometry assays as an efficient approach to estimate PROTAC selectivity, confirmed by a low number of off targets that are downregulated with lower fold change,[31−33] in some cases due to the E3.[34] These studies reporting IMP-targeting employ the E3 ligases cereblon (CRBN) or von Hippel−Lindau (VHL), which are well characterized and have a suitable druglike physicochemical profile.

Several of the reported PROTACs efficiently promote IMP degradation and consequently inhibit downstream signaling pathways. Zhang et al. reported a PROTAC that achieves more than 90% reduction in abundance of the ALK fusion protein, resulting in >90% reduction in ALK and STAT3 phosphorylation.[35] Another important receptor tyrosine kinase (RTK) that is being pursued using this methodology is the EGFR. In 2020 Zhao et al. synthesized PROTACs 3 and 4, which are capable of reducing the levels of the NSCLC oncogenic driver $EGFR^{L858R/T790M}$[36] in the H1975 cell line by 90.3% and 80.3%, respectively. Phosphorylations of EGFR itself and its downstream effector Akt were also dramatically reduced in the same cell line, in the absence of changes in Akt levels. The same compounds tested in A431, a cell line expressing high levels of wild-type EGFR, registered no alterations in the levels of the receptor.[37] Next to receptor tyrosine kinases such as ALK, G protein-coupled receptors (GPCR) are another large class of potential targets. Compound 9c is the first reported PROTAC directed against a GPCR, specifically against the α1 adrenergic receptor, and achieves a maximal degradation of 94% of the receptor.[27] Bensimon et al. also demonstrated the feasibility of targeted degradation via the proteasome of solute carriers (SLCs), multipass transmembrane proteins located in different compartments of the cell. This study showed successful degradation of SLCs located at the plasma membrane, lysosomes, Golgi, and endoplasmic reticulum. Furthermore, they showed that SLC amenability to degradation is not related to the topology.[38] This study used the dTAG system to tag the SLCs with an engineered variant of FKBP12 (the 12-kDa FK506-binding protein) that presents a cavity that can be selectively recognized by a synthetic $FKBP12^{F36V}$-directed ligand, AP1867.[39] Hence, this demonstrates that it is possible to synthesize a PROTAC that induces SLC degradation linking AP1867 to an E3 ligase warhead. These examples demonstrate that PROTACs may be able to target a wide variety of integral membrane proteins belonging to distinct families of receptors.

The primary readout with any PROTAC is a reduction in the cellular levels of its target. Next to this, many different cellular assays are used to test the effect of PROTACs on cellular physiology and health. Proliferation and cytotoxicity

Table 1. PROTACs in Clinical Trials by the End of 2021

Company	PROTAC	Target	Indication	E3 ligase	Target family	Target localisation
Arvinas	ARV-110	**AR**	Prostate cancer	CRBN	Nuclear receptor	Cytosol, nucleus
Arvinas/Pfizer	ARV-471	**ER**	Breast cancer	CRBN	Nuclear receptor	Cytosol, nucleus
Accutar Biotech	AC682	**ER**	Breast cancer	CRBN	Nuclear receptor	Cytosol, nucleus
Arvinas	ARV-766	**AR**	Prostate cancer	-	Nuclear receptor	Cytosol, nucleus
Bristol Myers Squibb	CC-94676	**AR**	Prostate cancer	CRBN	Nuclear receptor	Cytosol, nucleus
Dialectic Therapeutics	DT2216	**BCL-X_L**	Liquid and solid tumours	VHL	Antiapoptotic protein	Cytosol, MOM
Foghorn Therapeutics	FHD-609	**BRD9**	Synovial sarcoma	-	Nuclear factor	Nucleus
Kymera/Sanofi	KT-474	**IRAK4**	Autoimmune diseases	-	Kinase	Cytosol
Kymera	KT-413	**IRAK4**	Diffuse large B cell lymphoma	CRBN	Kinase	Cytosol
Kymera	KT-333	**STAT3**	Liquid and solid tumours	-	Nuclear factor	Cytosol, nucleus
Nurix Therapeutics	NX-2127	**BTK**	B cell malignancies	CRBN	Tyrosine kinase	Cytosol
Nurix Therapeutics	NX-5948	**BTK**	B cell malignancies and autoimmune diseases	CRBN	Tyrosine kinase	Cytosol
C4 Therapeutics	CFT8634	**BRD9**	Synovial sarcoma	CRBN	Nuclear factor	Nucleus
C4 Therapeutics	CFT8919	**$EGFR^{L858R}$**	NSLC	CRBN	Cell surface receptor	Cell membrane
Cullgen	CG001419	**TRK**	Cancer and other indications	CRBN	Tyrosine kinase	Cell membrane

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Table 2. Published Integral Membrane Proteins (IMPs) Targeted for Degradation through Different Technologies

Technology	Target	E3 ligase	Reference
PROTAC	$EGFR^{L858R}$, $EGFR^{del19}$	VHL	[5]
PROTAC	$EGFR^{L858R/T790M}$	VHL	[5]
PROTAC	EGFR, HER2	VHL	[5]
PROTAC	cMET, $cMET^{del14}$	VHL	[5]
PROTAC	MELK	CRBN	[33]
PROTAC	ALK	CRBN	[35]
PROTAC	FLT-3, FLT-3 ITD	VHL	[29]
PROTAC	SLCs	CRBN	[38]
PROTAC	SLC9A1	CRBN	[38]
PROTAC	$EGFR^{del19}$	VHL	[44]
PROTAC	ALK	CRBN	[14]
PROTAC	α_{1A}-adrenergic receptor	CRBN	[27]
PROTAC	$EGFR^{L858R}$, $EGFR^{del19}$	VHL, CRBN	[31]
PROTAC	$EGFR^{L858R/T790M}$, $EGFR^{del19}$	VHL	[37]
PROTAC	$EGFR^{L858R/T790M}$, $EGFR^{L858R/T790M/C797S}$, $EGFR^{L858R/T790M/L718Q}$	CRBN	[45]
PROTAC	$EGFR^{L858R/T790M}$	VHL	[28]
PROTAC	TRKA	CRBN	[24, 32]
1st generation LYTAC	EGFR	-	[51]
1st generation LYTAC	CD71	-	[51]
1st generation LYTAC	PD-L1	-	[51]
Dual PROTAC	IGF-1R and Src	CRBN	[22]
PROTAC	FGFR1, FGFR2	VHL	[34]
PROTAC	$EGFR^{L858R/T790M}$, $EGFR^{del19}$	CRBN	[42]
PROTAC	$EGFR^{L858R/T790M}$, $EGFR^{del19}$	VHL	[46]
PROTAC	PD-L1	CRBN	[41]
AbTAC	PD-L1	RNF43	[23]
Molecular Glue	CAR19	CRBN	[72]
2nd generation LYTAC	EGFR	-	[25]
2nd generation LYTAC	HER2	-	[25]
2nd generation LYTAC	$\alpha_v\beta_3$ integrin, $\alpha_v\beta_5$ integrin	-	[25]
PROTAC	FLT3-ITD, $FLT3\text{-}ITD^{D835V}$, $FLT3\text{-}ITD^{F691L}$	CRBN	[40]
PROTAC	$EGFR^{del19/T790M/C797S}$	VHL	[77]
PROTAC	CCR9	VHL	[43]

assays are particularly used with PROTACs developed for treating malignancies as they allow the study of the consequences of compounds on cell replication and growth.[27,40−43] Some compounds have been demonstrated to induce apoptosis,[29,37,42,44] whereas others stop the cell cycle in the G_0/G_1 phase.[37,42,44] In the case of cancer-associated targets, evaluating the effect of the PROTAC on cell invasion and migration capacity is another important measure.[22,42] Of note, it is not rare for PROTACs to be less potent against their cognate IMP as compared to the parent inhibitor in biochemical assays. However, this usually reverses in cellular assays, where the PROTACs exhibit a much stronger effect, thereby demonstrating that degradation of the target has a stronger and wider impact on the cell than its inhibition. Clearly, PROTACs not only inhibit the enzymatic activity due to decreased abundance of the integral membrane protein but also attenuate any scaffolding role the POI may have had and prevent development of compensatory feedback pathways.[29,35,38,45,46]

A major gap exists between the development of PROTACs and their testing in cell-based models and eventual evaluation *in vivo* in animal models, which may be arduous due to possible interspecies alterations in target sequence and structure. Nevertheless, several pharmacokinetic studies reported high plasma concentrations of the chimeras after intraperitoneal administration with a half-life of up to 3−4 h in some cases.[14,29,34] In every reported case IMP PROTACs were well tolerated by mice and did not result in weight loss or any adverse clinical complications.[14,27,29,31,32,35,41] Where evaluated, these PROTACs show an important inhibitory effect on tumor growth,[14,27,40,41] and immunohistochemical assays confirm a remarkable degradation of the IMP of interest.[27,29,34,41] These limited studies indicate that PROTACs appear efficient and nontoxic and are thus a promising venue for novel treatments in humans.

Despite the reported successes listed in this Review (Table 2), the number of publications reporting PROTACs targeting IMPs remains limited, and most of these studies do not report on the *in vivo* data of the investigated PROTAC. As such, it seems that targeting IMPs using PROTACs remains a challenging task. Several potential reasons may underlie this. The localization of the IMP within the membrane and the presence of hydrophobic transmembrane domain(s) and microenvironment may represent an obstacle for PROTAC-induced target internalization, with a relevant impact on target orientation and presentation. Furthermore, current reported PROTACs recruit either VHL or CRBN. Both E3 ligases are cytosolic, and the whole UPS machinery recruitment to the cell membrane might be challenging. Hence, a large potential lies in the large untapped pool of human E3 ligases, among which

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those physiologically addressing integral membrane proteins are potentially hijackable for IMP-targeted degradation.[47−50] Finally, the IMP degradation mechanism is not fully clear. It might require directing the target toward lysosomal degradation pathways, which represents a more substantial challenge than directing proteins toward proteasomal degradation. To solve these limitations and to empower the use of PROTACs to target IMPs, new tools have been recently developed, which we summarize in this Review (Table 2).

LYTAC

In 2020 Banik et al. developed a new strategy to specifically target IMPs and more generally extracellular proteins for degradation. This new method makes use of lysosome-targeting chimeras (LYTACs), which are small molecule−antibody conjugates that simultaneously bind a cell-surface lysosome-shuttling receptor and the extracellular domain of a target protein. The former is the cation-independent mannose-6-phosphate receptor (CI-M6PR) which is engaged through an agonist, whereas a small molecule or an antibody is applied as the binding moiety for the POI.[51] This strategy relies on the ability of the CI-M6PR to bind proteins bearing N-glycans capped with mannose-6-phosphate residues and to subsequently promote their endocytosis and lysosomal degradation, while the receptor is recycled to the membrane (Figure 1B).[52] Like PROTACs, LYTACs represent an event-driven system that allows catalytic degradation of the POI through reiterative CI-M6PR cycles (Banik et al. 2020). As a proof-of-concept, Banik et al. tested three compounds directed against EGFR, transferrin receptor-1 (CD71), and PD-L1, using specific antibodies for each target as the POI-specific ligand. Degradation of the POI was observed with all three compounds, validating the LYTAC technology. Furthermore, mass spectrometry approaches assessed the stability of CI-M6PR levels in EGFR-LYTAC treated cells, confirming the catalytic nature of the strategy.[51]

This same group developed a second generation of LYTACs using the liver-specific asialoglycoprotein receptor (ASGPR) as a lysosome-trafficking receptor. As ASGPR is exclusively expressed in the liver, this targeting strategy allows liver-specific IMP degradation.[25] Accordingly, linking an ASGPR agonist to specific antibodies, LYTACs that successfully degrade EGFR, the human epidermal growth factor receptor 2 (HER2), or the integrins $\alpha v\beta 1$, $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 6$, and $\alpha 5\beta 1$ were reported. Assays conducted with CI-M6PR- and ASGPR-mediated LYTACs directed against EGFR reveal a comparable performance of the compounds in terms of target degradation and effects on downstream signaling.[25] On the other hand, the kinetics of HER2 internalization induced by first and second generation LYTACs were different, highlighting that two IMPs will not necessarily behave the same way.[25]

Taken together these observations suggest that the success of any developed LYTAC results from a combination of factors, including the endogenous kinetics of protein trafficking and turnover, the amount of the recycling receptors on the surface, their susceptibility to induced endocytosis, and their distinct sorting.[25,51] Despite their promise, LYTACs have several notable disadvantages. They have a higher molecular weight compared to PROTACs, which may hamper delivery. Moreover, using an antibody as the POI-specific ligand may require humanization of the antibody and development of antibodies that can break self-tolerance. On the other hand, these molecules bind extracellular domains of receptors, overcoming any cellular permeability problems. The possibility of antibodies targeting neo-antigens represents a potential venue for application as this may allow reduction of off-target effects in healthy cells. LYTACs appear therefore to be a promising new strategy for IMP-targeted degradation, and the liver-specific second generation has opened the possibility of synthesizing cell-type-specific LYTACs, that target organ-specific IMPs.[26]

AbTAC

Recently, an alternative strategy has been developed by Cotton et al. to target membrane-associated proteins. They used a bispecific immunoglobulin G (IgG) to generate an antibody-based version of PROTAC called AbTAC.[23] Bispecific IgGs are recombinant antibodies which are able to selectively recognize two different antigens and colocalize them. The advantage of these molecules lies in their long serum half-lives and their rapid generation through phage display. Their mechanism of action relies on the ability of the AbTAC to simultaneously recognize the target protein that is to be degraded and the membrane-resident E3 ligase that drives target polyubiquitylation (Figure 1C).

In their work Cotton et al. used the single-pass transmembrane E3 ligase, RNF43, to provide the ubiquitylation activity. Their hypothesis that RNF43's structured ectodomain could facilitate phage display antibody generation drove this decision. Furthermore, this E3 is widely expressed in the human body, allowing general use of this approach.[53] The AbTAC generated consisted of a recombinant antibody to recruit RNF43 and the FDA-approved drug atezolizumab for PD-L1 binding. PD-L1 degradation was assessed in different cell lines, and no other cellular perturbations were detected using global proteomics, confirming that the developed AbTAC was highly selective.[23] Inhibition assays confirmed the RNF43 dependence for PD-L1 degradation and further suggested that the lysosomal degradation pathway is involved. However, the mechanism of action of this AbTAC remains largely unknown, as it remains unclear how RNF43 interacts and acts on the target, and how the trafficking of the target toward the lysosomes proceeds. Further elucidation of the process is needed to completely assess the potential advantages of AbTAC in TPD.

In their initial report, a maximal PD-L1 degradation of 63% was achieved with the RNF43 AbTAC.[23] A similar result was observed in a murine colon adenocarcinoma cell line after 48 h of incubation with a PD-L1-directed PROTAC,[41] while the CI-M6PR-mediated LYTAC reduces PD-L1 levels to close to 45%.[51] For these three different approaches, the maximal degradation achieved represents the steady state between synthesis and degradation rates. Multiple factors influence this steady state, and the target turnover and its endogenous trafficking kinetics are shared among these TPD strategies. Furthermore, the three PD-L1 TPD approaches differ in the E3 that is recruited, its turnover and its levels, in the E3:PD-L1 stoichiometry, and in the binding properties of the various ligands. Direct comparison is further complicated by the fact that the three strategies employ different modes of PD-L1 recruitment as well as degradation pathways and thus different trafficking kinetics. The combination of these factors may explain why the reported PROTAC, LYTAC, and AbTAC strategies directed against the same target reach different maximal degradation. It remains to be seen whether these

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differences also hold in the *in vivo* setting, where delivery and targeting represent an additional layer of variability.

MOLECULAR GLUES

Molecular glues are monomeric small molecules that either induce or reinforce interactions between two proteins that typically do not interact directly or strongly. These "glues" were discovered by Schreiber's group in 1991,[54] but deeper knowledge has been achieved only in the past decade.[55,56] Generally, molecular glues target an E3 ligase, altering its surface to recruit in a ternary complex a neosubstrate which is then polyubiquitinated and degraded through the proteasome (Figure 1D). Similar to PROTACs, molecular glues allow the targeting of proteins considered not-druggable and also work in a catalytic and substoichiometric manner.[57] Compared to PROTACs, which are bivalent degraders, molecular glues are single low-molecular-weight molecules with enhanced pharmacokinetics and pharmacodynamic properties.[58] Their development is also different. PROTACs require a rational design and precise modeling approaches and in fact can use molecular glues as warheads.[59] On the other hand, molecular glue discovery is mainly serendipitous or a consequence of unbiased screening approaches. Thalidomide,[60] indisulam,[61] auxin,[62] and jasmonate[63] are all examples of molecules for which the capability of inducing interactions between the E3 and its target has been discovered "accidentally" as secondary properties during their characterization.

Despite their efficacy in degrading specific proteins, e.g., Ikaros and Aiolos degradation mediated by thalidomide and its derivatives, molecular glues provide a binding platform on E3 ligases for a wider variety of neosubstrates[64−67] whose mechanism of action is often not clear, making them less controllable than PROTACs. Equally, this increases the difficulty in designing molecular glues using conventional medicinal chemistry approaches, as neosubstrates can rarely be identified *a priori*. Thalidomide analogues, for instance, has successfully been applied in clinics as antitumor agents for years before their molecular mechanism was elucidated.[67−70] For molecular glues to achieve widespread adaptation, the serendipitous mode of compound discovery requires a shift toward a more rational design approach. Accordingly, major improvements have recently been achieved in the discovery of molecular glues through phenotypic screens, facilitating their development toward therapeutic strategies.[56,71]

Among the available degraders, immunomodulatory drug (IMiD)-derivatives of thalidomide are known to act on CRBN as molecular glues.[72] These compounds "reshape" the E3 and induce the recruitment and subsequent polyubiquitination of neosubstrates, including the two zinc finger transcription factors IKZF1 and IKZF3.[73] IKFZ3 is recognized by CRBN through a specific sequence in its N-terminus,[74] which has been used for the development of an IMiD-induced system that leads to the degradation of chimeric antigen receptor (CAR) proteins. This was made possible by appending the 6-kDa IKZF3-based degron to the C-terminus of the targets through a linker that guaranteed the flexibility and accessibility needed for the molecular glue mechanism.[75]

Building on this development, Hild's group proved that an IKZF3-based degron successfully allows for the control of CAR levels, leading to the reversible degradation of type I transmembrane proteins with different characteristics including size, length of cytosolic tail, and number of cytosolic lysine residues. Using this approach, promising results were achieved in controlling CAR expression and activity dosing IMiDs *in vivo*, with a consistent antitumor response preserved also after discontinuation of drug administration.[75] Although this system relies on an FDA-approved IMiD drug, and translation into the clinic should be more straightforward, this strategy requires a heterologous degron to be present on the targeted protein which will limit usage and bring an extra layer of complexity to TPD.

CONCLUSIONS

Selective degradation of disease-causing proteins brings undoubtable advantages compared to their inhibition and promises to minimize the undruggable genome. A longer lasting target inactivation is reached through target elimination, with a more significant impact on its enzymatic function and any scaffolding roles.[29,35,38,45,46] This approach may also circumvent drug-induced target mutation and acquired drug resistance following long-term drug use,[1−3] which mandates a constant development of new molecules to target emerging target variants.

Among TPD techniques, PROTACs have seen rapid development, with an established design strategy and a small-molecule nature that facilitates an appropriate ADME profile.[8,14] Their synthesis often involves an inhibitor as binding moiety to target the POI. The resulting chimera typically presents different properties from the parent molecule, with a higher selectivity for the target reached through the optimization of the linker and the positive cooperative interaction between the E3 and the POI.[12]

Despite recent advances in rational use of PROTACs, their great potential, and evident success in degrading kinases and transcription factors, this technology does not address membrane-associated proteins with the same efficiency. Notwithstanding, IMP dysregulation and mutation are established key factors that promote and drive many disease indications; thus, their targeted degradation still remains an auspicious objective. It is unclear if this lack of success is due to limited testing, or an inherent difficulty associated with TPD of membrane-associated proteins. IMPs establish strong interactions with the membrane, and the E3 ligases usually involved are cytosolic and physiologically target proteins belonging to different families. Investigating the human proteome for IMP-targeting E3s can be a successful outset to broaden TPD technologies.

The success of PROTACs has inspired the rise of the other approaches discussed in this Review, each with their (dis)advantages. Technologies that employ antibodies, like LYTAC and AbTAC, avoid any cellular permeability issues and have the potential to also target a specifically mutated POI (e.g., neo-POI). The possibility to recruit tissue-specific E3s for AbTAC and tissue-specific lysosome trafficking receptors for LYTAC represents another attractive, enhancing feature. Molecular glues are instead monomeric degraders, with a low molecular weight which benefits their ADME profile. The recent progress achieved in their rational design encourages the development of specific degraders with major therapeutic advantages.

Another contribution to improve IMP-targeted degradation could come from understanding the mechanisms that lead to the actual degradation of the POI in more detail. Many reports have demonstrated the involvement of the proteasome in the degradation of IMPs,[27,28,38,41,43,44] while others have shown that the endolysosomal system is required.[23,25,46] Others have

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instead suggested that TPD involves the cooperation of the autophagic-lysosomal system and the UPS together,[37,42] though this could be induced by the inhibition of the proteasome, which can switch from a proteasome-dependent pathway to a lysosomal-dependent protein degradation pathway.[76,77]

As targets, each membrane-associated protein presents unique properties, such as its endogenous turnover and trafficking kinetics, and its susceptibility to be internalized, which do not hinge on the technology applied. On the other hand, TPD strategies differ in the E3 employed, its endogenous levels, turnover, and stoichiometry with the target. This entails different ways of recruiting and sorting the POI, different trafficking kinetics, and different degradation pathways. All these variables combined together define the maximal target degradation achieved, which is the result of the steady state between synthesis and degradation rates.

For these reasons, further elucidations of the molecular mechanism induced by TPD tools could represent a first step toward the development of new successful strategies. Efficient and more precise technologies to target membrane-associated proteins for degradation keep standing as an important turning point for many diseases, and investigating the human proteome for new "hijackable" E3s can contribute to this cause, making a decisive impact in therapeutic intervention.

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Notes

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ABBREVIATIONS

α1-AR	α1 adrenergic receptor
ADME	absorption, distribution, metabolism, and excretion
ALK	anaplastic lymphoma kinase
ASGPR	asialoglycoprotein receptor
CAR	chimeric antigen receptor
CI-M6PR	cation-independent mannose-6-phosphate receptor
cMET	mesenchymal-epithelial transition factor
CRBN	cereblon
EGFR	epidermal growth factor receptor
FGFR1/2	fibroblast growth factor receptor 1/2
FKBP12	12-kDa FK506-binding protein
FLT3-ITD	FMS-like tyrosine kinase 3 protein with an internal tandem duplication
GPCR	G protein-coupled receptor
HER2	human epidermal growth factor receptor 2
IGF-1R	insulin-like growth factor 1 receptor
IgG	immunoglobulin G
IKZF1/3	IKAROS family zinc finger 1/3
IMiD	immunomodulatory drug
IMP	integral membrane protein
LYTAC	lysosome-targeting chimeras
NSCLC	nonsmall cell lung cancer
PD-L1	programmed cell death ligand 1
PEG	polyethylene glycol
POI	protein of interest
PROTAC	proteolysis targeting chimera
SLC	solute carrier
TPD	targeted protein degradation
UPS	ubiquitin-proteasome system
VHL	von Hippel−Lindau

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