Received: 16 November 2021 | Accepted: 18 November 2021

DOI: 10.1111/mmi.14849

MICROREVIEW

WILEY

Targeted protein degradation at the host–pathogen interface

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Funding information
National Health and Medical Research Council, Grant/Award Number: APP1177431

Abstract

Infectious diseases remain a major burden to global health. Despite the implementation of successful vaccination campaigns and efficient drugs, the increasing emergence of pathogenic vaccine or treatment resistance demands novel therapeutic strategies. The development of traditional therapies using small-molecule drugs is based on modulating protein function and activity through the occupation of active sites such as enzyme inhibition or ligand–receptor binding. These prerequisites result in the majority of host and pathogenic disease-relevant, nonenzymatic and structural proteins being labeled "undruggable." Targeted protein degradation (TPD) emerged as a powerful strategy to eliminate proteins of interest including those of the undruggable variety. Proteolysis-targeting chimeras (PROTACs) are rationally designed heterobifunctional small molecules that exploit the cellular ubiquitin-proteasome system to specifically mediate the highly selective and effective degradation of target proteins. PROTACs have shown remarkable results in the degradation of various cancer-associated proteins, and several candidates are already in clinical development. Significantly, PROTAC-mediated TPD holds great potential for targeting and modulating pathogenic proteins, especially in the face of increasing drug resistance to the best-in-class treatments. In this review, we discuss advances in the development of TPD in the context of targeting the host–pathogen interface and speculate on their potential use to combat viral, bacterial, and parasitic infection.

KEYWORDS
bacteria, drug, intracellular infection, parasites, pathogenic proteins, Proteolysis-targeting chimeras, resistance, viruses

1 | INTRODUCTION

Over the past two decades, there has been a paradigm shift in drug discovery toward an emerging field that holds great therapeutic promises: targeted protein degradation (TPD) using multi-specific drugs (Deshaies, 2020). These compounds function by impacting protein homeostasis by harnessing the intrinsic cellular mechanisms for protein regulation and degradation (Chen et al., 2011) to break down disease-causing or disease-related proteins (Alabi & Crews, 2021). Various concepts of using engineered multi-specific small molecules have emerged to hijack cellular pathways for TPD including autophagy (Li et al., 2019; Takahashi et al., 2019), the lysosomal pathways (Banik et al., 2020), and ubiquitin-dependent proteasomal degradation (Sakamoto et al., 2001). Of high significance are current TPD developments exploiting the cellular ubiquitin-proteasome system (UPS; Hershko & Ciechanover, 1998;

Komander & Rape, 2012) through a chemical approach wherein a bifunctional small molecule is designed to induce a favorable interaction between a target protein of interest (POI) and a component of the UPS machinery, typically an E3 ligase, triggering degradation of the target protein. Complementary in its effect to nucleic acid-based molecules that exploit RNA interference (RNAi) for modulation of intracellular protein concentration, TPD allows for selective chemical control ("knockdown") of any POI in the host cell and thus results directly in alteration of protein-related disease, while allowing for small-molecule in vivo pharmacology (Lai & Crews, 2017).

A plethora of small-molecule bifunctional protein degraders has evolved following the early reports of successful in vitro targeted protein degradation with proteolysis targeting chimeras (PROTACs). Using the chimeric peptide PROTAC-1 to recruit MetAP-2 to the Skp1-Cullin–F box complex, degradation of MetAP-2 was achieved in vitro (Sakamoto et al., 2001). Initial studies demonstrated the in vivo feasibility of de novo protein degradation with PROTAC technology (Schneekloth et al., 2008) and its potential therapeutic benefit (Sakamoto et al., 2003). But it was not until 2015 that increased interest in TPD emerged due to the success of more "drug-like" small-molecule PROTAC systems recruiting either the E3 ligases cereblon (CRBN; Winter et al., 2015) and Von Hippel Lindau (VHL; Bondeson et al., 2015), demonstrating the versatility of this technology, followed by studies that allowed for a better understanding of the molecular mechanism driving efficient TPD (Lai et al., 2016; Lu et al., 2015). The recent development and application of TPD using PROTACs and other related small-molecule degraders for various disease indications have been extensively reviewed previously (Burslem & Crews, 2020; Konstantinidou et al., 2019; Lai & Crews, 2017; Schapira et al., 2019; Sun et al., 2019).

The first PROTAC was administered to a human in 2019 when Arvinas Therapeutics advanced this paradigm-shifting technology into the clinic with two PROTAC candidates. ARV-110, targeting the androgen receptor localized at the nuclear membrane, was the first candidate to enter clinical trials, for treatment of metastatic, castration-resistant prostate cancer (NCT04072952), followed by ARV-471, targeting the nuclear-localized estrogen receptor in patients with metastatic (ER+/HER2−) breast cancer (NCT03888612; Mullard, 2019).

By the end of 2021, there are expected to be more than a dozen drugs falling into this class to enter the clinic (Mullard, 2021). Most of the more advanced molecules suitable for the clinical setting are in the oncology space for the treatment of blood cancer and solid tumors, on validated targets with proven therapeutic utility and known risks for safety and efficacy.

However, PROTACs targeting infectious disease-related proteins are still a nascent field of research. In this review, we focus on recent reports and speculate on potential applications of PROTAC and TPD technology to target infectious diseases for drug discovery.

2 | PROTAC TECHNOLOGY

2.1 | PROTAC mechanism of action—Hijacking of UPS

PROTACs exhibit their function through hijacking the UPS—essential machinery of the eukaryotic cell critical for cellular homeostasis and responsible for keeping misfolded or damaged proteins in check via degradation (Hershko & Ciechanover, 1998). The UPS' central element, ubiquitin, is a highly conserved, 8.6 kDa protein present in all eukaryotes (Zuin et al., 2014). The UPS recognizes damaged or misfolded proteins and marks them for proteasomal degradation through the conjugation of ubiquitin in a cascade event of enzymatic reactions: (a) ATP-dependent transfer of ubiquitin to form a thioester bond with a ubiquitin-activating enzyme E1, (b) activated ubiquitin is next conjugated to an E2 enzyme through a trans-thioesterification, and (c) last, ubiquitin is conjugated to the substrate protein on a surface lysine residue via an adaptor E3 ligase (Alabi & Crews, 2021). This cascade is repeated to form a specific poly-ubiquitin chain on the target protein, "tagging" it for its cellular fate (Komander & Rape, 2012). PROTACs and related small molecules (including molecular glues [Mayor-Ruiz et al., 2020]) can adapt this process to recruit novel substrates to the ubiquitin machinery to achieve their proteasomal degradation. A bispecific PROTAC targets the POI with one arm and recruit the E3 ligase with the other, resulting in a ternary POI-PROTAC-E3 complex. The hijacked E3 ligase transfers ubiquitin onto the neo-substrate POI, leading to poly-ubiquitination and subsequent degradation of the POI by the proteasome, while the PROTAC is released (Lai & Crews, 2017) and able to bind the next available POI substrate, repeating this catalytic cycle (Figure 1).

2.2 | PROTAC degrader design

As mentioned, a PROTAC structurally comprises a POI-binding element and an E3-recruiting arm, connected through a linker element. A number of ligands for a small subset of the overall estimated ~600 mammalian E3 ligases, notably VHL (Schneekloth et al., 2004), CRBN (Lopez-Girona et al., 2012), MDM2 (Schneekloth et al., 2008), and IAPs (Itoh et al., 2010) have been developed and incorporated into PROTACs and related small-molecule degraders (Ding et al., 2020). However, PROTAC design is not trivial. Each element is of critical importance in the PROTAC development and while ligands for the POI- and E3 ligase are often optimized independently, the linker length and composition can considerably influence the pharmacokinetic parameters of the overall small molecule, as well as the molecule's ability to induce ternary complex formation, required for efficient substrate ubiquitination and degradation (Alabi & Crews, 2021). A productive ternary complex is characterized by thermodynamically favorable protein–protein interactions between the E3 and the neo-substrate protein. This allows structural complementarity across the

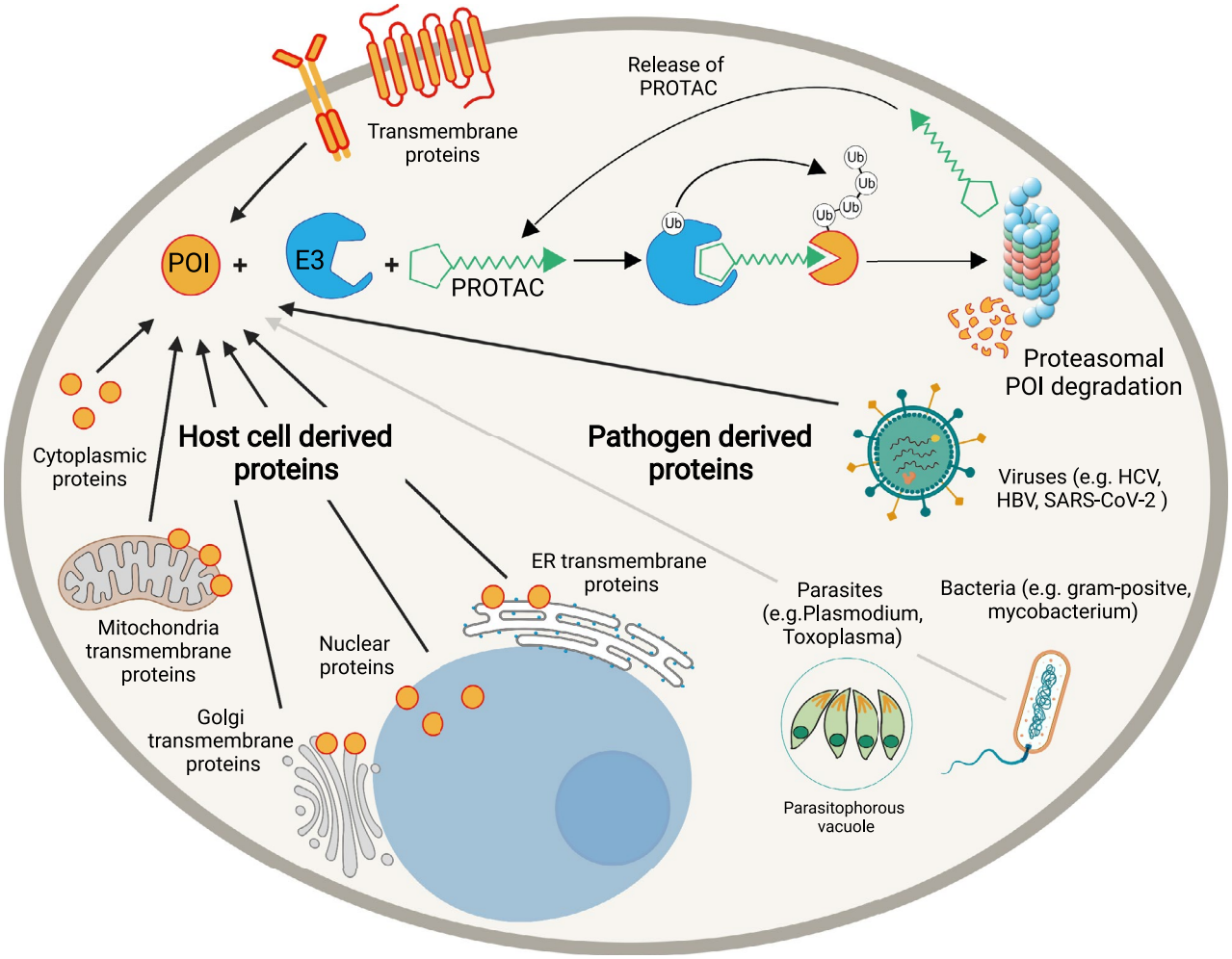

FIGURE 1 Schematic representation of the catalytic mode-of-action of PROTACs to induce proteasomal degradation of host and pathogen derived proteins in a mammalian cell. Current POIs amenable for TPD using PROTACs include host cell derived proteins and proteins derived from intracellular pathogens. With accessibility to the UPS being critical for TPD, cellular factors successfully used for TPD comprise cytoplasmic proteins, structural and scaffolding proteins, single or multi-pass transmembrane proteins including cell surface receptors, and cytosolic facing domains of mitochondria-, ER- and Golgi network derived proteins as well as nuclear proteins including transcription factors. Approaches that focus on the targeted degradation of intracellular pathogen derived proteins have been successfully demonstrated for various viruses. TPD approaches that affect bacterial pathogenicity factors are under development, but applicability has been already demonstrated in proof-of-principle studies using BACTACs (indicated by opaque arrow). Importantly, TPD has the potential to be extended to parasites by recruitment of host or parasite E3 ligases (opaque arrow), and other intracellular pathogens (Representation partly in style of review by (Burslem & Crews, 2020). [Corrections added on 26 December 2021, after first online publication: figure 1 and its citation has been added in this version.]

protein interface as well as the proper orientation of the E3 ligase to allow for ubiquitin transfer onto a suitable lysine residue on the POI. Another important aspect to consider is the induction of positive binding cooperativity (Roy et al., 2019). Cooperativity is defined as the measurable effect of protein association and affinity of the binary complex, PROTAC-POI (or PROTAC-E3), to the second protein, leading to a ternary complex; positive cooperativity results from stabilizing protein–protein interactions between E3 and POI substrate and negative cooperativity from destabilizing ones. Implementation of positive cooperativity is a desirable strategy as it often leads to a better degradation profile, although not guaranteeing to deliver the most efficient and potent PROTAC (Alabi & Crews, 2021). Ideal linker lengths vary for each POI and will need to be assessed individually when developing new PROTACs.

2.3 | Advantages of degraders over traditional inhibitors

When comparing PROTACs—and other protein degraders—to traditional small-molecule inhibitors, there are several potential benefits. First, the catalytic nature of the PROTAC mechanism is advantageous in a few regards: although small-molecule inhibitors require strong target occupancy of the POI-binding site for their

desired effect, PROTACs only require a transient binding event to form a productive ternary complex leading to POI degradation. Furthermore, the degraded protein needs to be resynthesized by the cell to regain function, therefore, its elimination from the system can effectively result in a more pronounced biologic phenotype than inhibition alone (Burslem et al., 2018). As it is recycled upon POI degradation, one PROTAC can degrade multiple POI molecules at much lower drug concentrations compared to traditional inhibitors which require a higher drug to POI stoichiometry for efficient inhibition of protein function (Lai & Crews, 2017).

The major advantage of PROTACs mechanism of action is the ability to degrade "undruggable" proteins. The current druggable proteome is limited by proteins with a defined active site or binding pocket—typically enzymes or receptors—that can be occupied by the drug to achieve the desired biologic effect. In contrast, PROTACs can use any available binding site on a protein as a handle to achieve efficient degradation of the POI in order to form a productive ternary complex. Consequently, PROTACs have been successfully used to target hitherto elusive drug targets such as tau (Kargbo, 2019) and KRAS (Zeng et al., 2020); (Bond et al., 2020).

PROTAC technology is also a promising way to overcome resistance to drugs that are evolving through either mutation of the drug target or changes in its abundance (Alabi & Crews, 2021). PROTAC-mediated TPD applications showed encouraging results in overcoming resistance in clinical trials for cancer therapy, where fast-occurring resistance is problematic (Hall et al., 2009). As only a transient interaction between the degrader, POI and E3 is required to form a productive ternary complex for degradation, PROTACs offer the potential to degrade mutant POI that is otherwise evading small-molecule inhibitor treatment. This was shown for the enzalutamide-PROTAC ARCC-4, which was effective in the degradation of androgen receptor (AR) in prostate cancer models presenting clinically relevant AR point mutations (Salami et al., 2018). Similarly, the estrogen receptor degrader ARV-471 is as mentioned before in advanced trials for therapy of heavily treated breast cancer patients (NCT03888612; Mullard, 2019). PROTACs and TPD are thus hoped to be effective in therapy for conditions where sustained small-molecule inhibition is ineffective or is overcome quickly by mutational resistance toward the target protein. Although resistance mechanisms to PROTACs have been observed in cancer cell lines, the underlying genetic alterations created mutations within the core E3 ligase complex components rather than the degraded target protein (Ottis et al., 2019; Zhang et al., 2019). This issue may be overcome by the recruitment of variant E3 ligases to achieve durable target degradation and compensate for the PROTAC resistance mechanism.

2.4 | Protein targeting in various cellular compartments and other TPD approaches

PROTACs have been demonstrated to act on proteins with a variety of solubility profiles and that are located within different cellular compartments (Figure 1) (Burslem & Crews, 2020). Most PROTAC-mediated degradation to date has been demonstrated within the cytoplasm and nucleus of mammalian cells. The targets of these PROTACS have included BET proteins (Lu et al., 2015; Raina et al., 2016; Zengerle et al., 2015), protein kinases (Burslem et al., 2018), and various molecules dysregulated in cancers (reviewed in (Li & Song, 2020)). The colocalization of the cytoplasmic proteasome, target POIs, and well-characterized ubiquitin E3 ligases such as cereblon and VHL could be reasons for the observed efficient degradation. In the absence of a known ligand for binding the target of interest, tag-based conditional protein knockdown and screening systems, including the cereblon-based dTAG and the VHL-based Halo-PROTAC techniques, have both shown remarkable efficiency in the degradation of proteins localized within multiple compartments, including the cellular cytoplasm, the nucleus, and transmembrane proteins that are accessible to the UPS, such as transmembrane proteins of the ER, Golgi, and cell surface (Bensimon et al., 2020; Buckley et al., 2015; Nabet et al., 2018, 2020; Ottis et al., 2017). For specific nuclear targeting, a variant of PROTACs known as TRAFTACs (TRAnscription Factor TArgeting Chimeras), that are guided via CRISPR/Cas9 to specific DNA elements, have been recently shown to induce the degradation of transcription factors (Samarasinghe et al., 2021). PROTACs have also shown efficacy in the degradation of single- or multi-pass transmembrane proteins including cell surface receptors. Early studies showed targeted degradation of receptor tyrosine kinases from the cell surface by PROTACs (Burslem et al., 2018).

Bensimon and colleagues were able to degrade multi-pass transmembrane proteins with 3–12 transmembrane domains either from the cell surface or from subcellular compartments, such as the ER and Golgi network, en route to its destination (Bensimon et al., 2020). Membrane proteins within the inner nuclear membrane can also be degraded in a ubiquitin and proteasome-dependent manner, demonstrating the role of E3 ligases in nuclear membrane protein homeostasis (Huang et al., 2020).

Approximately 40% of the proteome contains a secretion or transmembrane signal (Uhlen et al., 2015). These proteins include soluble cytokines, immune complexes, and extracellular aggregates, as well as membrane receptors and adhesion molecules. LYTACs (Lysosome-TArgeting Chimeras) that utilize either the Mannose-6-Phosphate or the asialoglycoprotein receptors have demonstrated the degradation of extracellular soluble proteins or membrane receptors via the lysosomal pathway (Ahn et al., 2021; Banik et al., 2020).

Other exciting TPD platforms and targeted clearance strategies are at various stages of the developmental pipeline. These approaches include AUTACs (AUtophagy TArgeting Chimeras), degraders that can induce TPD of disease-damaged organelles and dysfunctional fragmented mitochondria-associated proteins (Takahashi et al., 2019), as well as RNA-PROTACs, compounds that target defective RNA-binding proteins as the origin of various diseases (Ghidini et al., 2021). Akin to PROTACs and their ability to degrade target POIs, degraders targeting RNA via a cellular RNAse enzyme have also been reported (Costales et al., 2019). These degraders, termed RIBOTACs (RIBOnuclease TArgeting Chimera), comprise a heterobifunctional small molecule in which an RNA-binding moiety is linked to an

RNAse-binding one. Although their mechanism of action is similar to that of RNAi, RIBOTACs have superior pharmacodynamic properties, are accessible to a wider range of tissues in in vivo settings, and act catalytically similar to PROTACs (previewed in Dey & Jaffrey, 2019).

Other modified TPD concepts exploiting various degradation strategies with exciting application spectrum include the already well-defined SNIPERs (Specific and Nongenetic Inhibitor of Apoptosis Protein (IAP)-dependent Protein ERasers) that engage an IAP (inhibitor of apoptosis protein) as E3 ubiquitin ligase and can induce simultaneous degradation of IAPs themselves along with other target proteins (Ishikawa et al., 2020; Itoh et al., 2010; Naito et al., 2019; Ohoka et al., 2016). Another approach to induce a suicide-type TPD-mediated knockdown is to induce dimerization of E3 ligases by using homo-PROTACs, an approach that has been initially demonstrated by using active compounds that dimerize and degrade VHL (Maniaci et al., 2017) and CRBN E3 ligases (Steinebach et al., 2018).

3 | TARGETED PROTEIN DEGRADATION AT THE HOST–PATHOGEN INTERFACE

3.1 | PROTAC applications for targeted therapy of infectious diseases

In pathogenic human infections, the interplay between pathogenic biomolecules, the host's immune system, and therapeutic treatment regulate the natural course of the disease. However, this balance may be tipped in the pathogen's favor by a combination of factors including immune evasion strategies, immunologically silent life cycle stages, and key virulence factors that are undruggable by existing inhibitors. In addition, the rapid emergence of drug resistance to the current best-in-class compounds is of extreme concern in the cases of pathogenic viruses, bacteria, and parasites (Heymann, 2006).

Compared with traditional small-molecule treatments, PROTACs have several advantages (Lai & Crews, 2017), which are also beneficial for the application against infectious pathogens. As outlined above, ligands binding the protein of interest (POI) do not necessarily require strong binding kinetics or achieve sustained target occupancy, thereby even weak target-binding ligands and early drug discovery hits represent valuable starting points for successful PROTAC design. Depending on the capability of the host cell and the precise nature of the host–pathogen interface, PROTACs can be rationally synthesized to recruit either host- or pathogen-derived E3 ligases to degrade pathogenic effector molecules. In either case, selection of the E3 ligase binder requires an understanding of the E3 ligases expressed both by the host cell and the infectious pathogen and the precise molecular events leading to disease pathogenesis. When considering pathogen encoded E3 ligases, however, our knowledge of the respective protein degradation systems in pathogens is hitherto very limited due to their evolutionary divergences, in the case of parasites and bacteria, or virtually nonexistent, as is the case for viruses.

PROTACs can also expand the targetable landscape of pathogenic proteins beyond those of traditional inhibitor drugs. Most clinically relevant inhibitors of bacteria, parasites, and viruses target either enzyme, ion/solute channels, and membrane receptors, however, these targets only represent a minor fraction of the total pathogenic proteome. Virulence proteins also include host cell invasion and adhesion ligands, translocons, transcription factors, and toxins which function through protein–protein interactions rather than enzymatic activity. As TPD mediated by PROTACs only requires a transient protein-binding event, rather than inhibition of a specific active site, they are amenable to target a much larger pathogenic proteome repertoire than that available to traditional inhibitors.

A critical advantage of PROTAC technology is its ability to overcome drug resistance. Drug resistance in various infectious pathogens has been shown to be due to (a) mutation of the target protein such that the inhibitor no longer binds with the required kinetics, (b) the amplification of the target gene, thereby allowing "quenching" of the inhibitor by excess protein targets, and (c) drug efflux pumps that actively shuttle away from the inhibitors from the site of action and limit exposure to the target (Reygaert, 2018). PROTACs derived from compounds against which pathogens display drug resistance due to mutation or overexpression may offer a novel route of re-targeting virulence proteins. As an example, Ibrutinib-based PROTACs can bind and degrade mutant Bruton's Tyrosine Kinase while Ibrutinib alone remains ineffective in functional inhibition of the target mutant protein (Buhimschi et al., 2018). This phenomenon offers a great opportunity and second chance for antipathogenic compounds that failed in previous trials due to the emergence of drug resistance to reenter the drug development pipeline as PROTAC-based derivatives. It should be noted that to date, PROTACs have been unable to overcome resistance due to the activity of drug efflux pumps unless the efflux mechanism is in itself a direct PROTAC target (Reygaert, 2018). Finally, PROTACs can also be used to identify the unknown binding targets of known antipathogenic drugs. The design of a PROTAC molecule linking an E3 ligase to the drug of interest can create a degrader capable of binding and depleting the protein(s) targeted by the compound (Huang et al., 2018; Jiang et al., 2021). An added advantage of PROTAC design, as evidenced by the incorporation of the nonselective kinase inhibitor Foretinib into a selective PROTAC, is the creation of highly selective degraders from promiscuous parent warheads through harnessing positive cooperativity of protein–protein interactions (Bondeson et al., 2018). However, as the E3 ligase engaged by a particular PROTAC affects the efficiency of degradation, a trial and error method is often necessary when determining the optimum E3 ligase–small-molecule PROTAC pairing (Kannt & Dikic, 2021).

3.2 | TPD-based antiviral strategies

In traditional antiviral strategies, inhibition of viral replication is achieved by the development of high-affinity small molecules and drugs that inhibit the enzymatic function of viral polymerases and proteases. The re-occurring emergence of drug resistance to this class of compounds, during monotherapy using viral enzyme inhibitors, as well as the need for the development of drugs that tackle

non-enzymatic pathogenic proteins, led to the idea of developing alternative antiviral therapies with increased barriers to resistance and alternative mode of action. Chronic hepatitis B virus (HBV) infection is the leading cause of cirrhosis and liver cancer (Ganem & Prince, 2004) and remains a global health threat (WHO, 2017). The HBV-encoded X-protein, essential to facilitate HBV replication (Lucifora et al., 2011), is also involved in the development of virus-induced liver disease through regulation of cancer-associated gene expression, cell survival, cell death, and metabolism (Kew, 2011) and has become an attractive target for novel therapies to treat chronic HBV infection, including PROTAC-mediated degradation and regulation. Montrose and Krissansen reported a peptide-based PROTAC-like molecule that can induce the degradation of the X-protein in the liver cancer cell line HepG2, however, the PROTAC's impact on HBV replication or liver disease development has not yet been shown (Montrose & Krissansen, 2014). Hepatitis C virus is yet another pathogen that causes chronic hepatitis, liver cirrhosis, and liver cancer. In more recent work, de Wispelaere and colleagues used Telaprevir, a first-generation peptidomimetic protease inhibitor approved for the treatment of HCV, for the development of a PROTAC targeting the HCV NS3/4A protease. Interestingly, the developed degrader retained antiviral activity and reduced Telaprevir-resistant mutant virus production. The study provided proof of concept that small-molecule degraders can overcome viral drug resistance emerging under classic antiviral therapy targeting enzymatic activity (de Wispelaere et al., 2019). The global spread of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the resulting Covid-19 pandemic resulted in the very fast development and approval of a few highly effective vaccines, however effective therapeutic approaches for the treatment of severe COVID-19 are still urgently needed. Worldwide efforts include the development of small-molecule inhibitors and the repurposing of existing compounds that target various steps of the viral replication cycle. However, Martinez-Ortiz and Zhou proposed the development of an antiviral PROTAC specific for the SARS-CoV-2 structural envelope protein E, which targeted degradation would result in the inhibition of the viral life cycle, including viral entry, replication, and assembly (Martinez-Ortiz & Zhou, 2020). Chatterjee et al. engineered ACE-2-derived peptides bringing the SARS-CoV-2 spike protein receptor-binding domain (RBD) and an E3 ubiquitin ligase in close proximity to promote the proteasomal degradation of SARS-CoV-2 RBD and inhibition of infection (Chatterjee et al., 2020). Haniff and colleagues applied the aforementioned RIBOTAC strategy to impact viral replication by targeting the SARS-CoV-2 RNA genome (Haniff et al., 2020). Notably, other SARS-CoV-2 structural and nonstructural proteins display valid targets for TPD using PROTACs to affect viral replication.

3.3 | TPD-based antibacterial strategies

Bacterial degrader signals target bacterial polypeptides toward specific endogenous proteases for proteolysis to regulate intracellular protein levels. The bacterial degron ssrA tag is widely used in the fusion protein tagging systems for the modification of protein stability in bacteria (reviewed in Fritze et al., 2020). Even though the ubiquitin system is restricted to eukaryotes and prokaryotes lack E1/E2 enzymes, bacteria do possess the capacity to express ubiquitin effector molecules in the form of E3 ligases and deubiquitinating enzymes (DUBs) to hijack the hosts' ubiquitination system and promote infection and pathogenicity (reviewed in Maculins et al., 2016). Induced targeted bacterial protein degradation remains a challenging endeavor, but the first proof of principle studies has already demonstrated its applicability and value. Long et al. applied ubiquitin-independent hydrophobic tagging (reviewed in Izert et al., 2021) and linked trimethoprim, a widely used antibiotic to treat a variety of infections caused by gram-positive bacteria, which inhibits the bacterial dihydrofolate reductase (DHFR), to a hydrophobic Boc3-Arg (tert-butyl carbamate protected arginine) moiety, recognized by the proteasome as a degradation signal. The result was a "degrader" (Trimethoprim-Boc_3-Arg/TMP-Boc_3-Arg) that induced proteasomal degradation of *E. coli*-derived DHFR (Long et al., 2012). However, bacterial DHFR degradation was shown in a heterologous system following overexpression of the bacterial target protein and treatment with TMP-Boc_3-Arg in fibroblasts and remains to be demonstrated in bacterial cells.

Pyrazinoic acid is the bioactive form of the prodrug pyrazinamide, an anti-tuberculosis drug that interrupts the biosynthesis of coenzyme A, essential in *Mycobacterium tuberculosis* (Mtb), the causative agent of TB (Lee et al., 2013). Pyrazinoic acid itself targets the aspartate decarboxylase PanD, essential for coenzyme A biosynthesis (Shi et al., 2014). Gopal et al. (2020) recently characterized that although the binding of pyrazinoic acid to Pan D is weak, it is able to induce the degradation of PanD via activation of the protease ClpC1-ClpP.

In gram-positive bacteria and mycobacteria, phosphorylated arginine residues (pArg) serve as a degradation signal that is recognized by the bacterial ClpCP protease, a proteasome-like complex required for bacterial protein quality control, stress regulation, and pathogenicity. Very recently, Morreale and colleagues proposed in a yet to be peer-reviewed study the development of BacPROTACs, chimeric bifunctional small-molecule degraders that bind to the substrate receptor of the ClpCP protease and thus priming those neo-substrates or POIs for degradation, demonstrating the potential of inducible and selective degradation of target proteins in bacteria in a proof of principle study. Linking the BET bromodomain inhibitor JQ1 to the mycobacterial antibiotic sCym-1 recruiting the ClpC1P1P2 complex to give BacPROTAC-3, they are able to show selective degradation of $BRDT_{BD1}$ proteins expressed in *Mycobacterium smegmatis*. Compared with their eukaryotic PROTAC analogs, rather than relying on the UPS machinery to induce lysine chain ubiquitination for degradation, BacPROTACs function in a more straightforward manner by directly trafficking their substrates to the proteasomal ClCP complex. This study is of exceptional significance, being the first example of TPD in a prokaryotic pathogen by reprogramming the bacterial proteasome. Further developed, this strategy could be used to eliminate certain

virulence proteins and lead to the development of novel antibiotics (Morreale et al., 2021).

3.4 | The potential application of TPD as an antiparasitic strategy

Human parasites are responsible for considerable morbidity and mortality, especially in vulnerable populations of the developing world (Khalil et al., 2018; Mitra & Mawson, 2017; Torgerson et al., 2015; WHO, 2020). Malaria parasites of the genus *Plasmodium* resulted in 229 million cases and 409,000 deaths in 2019 (WHO, 2020). The ubiquitous *Toxoplasma gondii* parasite, the causative agent of Toxoplasmosis, affects approximately a third of the world's human population (Torgerson & Mastroiacovo, 2013) and can be severe and often fatal in immunocompromised patients. The related apicomplexan parasites of *Cryptosporidium* spp. spread the gastrointestinal disease Cryptosporidiosis. In developing countries, as many as 45% of children under the age of 2 contract cryptosporidiosis (Valentiner-Branth et al., 2003), a disease that can cause life-threatening complications and increases the risk of digestive cancers, childhood malnutrition, and impaired fitness (Benamrouz et al., 2014; Checkley et al., 1997, 1998; Molbak et al., 1997). Although steady progress has been made in averting many more cases and deaths by these and other clinically relevant parasites, the emergence of drug-resistant parasite strains forewarns a need for better control of parasite spread and disease symptoms. To this purpose, novel drug therapies that can subvert resistance mechanisms must be explored to achieve eradication and elimination goals. PROTAC-mediated degradation of parasite virulence proteins could become a valuable component by which to achieve these goals.

On the warhead side of antiparasitic PROTAC design, the scope of POI ligand binders can include many, if not all, of the clinically relevant inhibitors. These include drug inhibitors at various levels in development and, given the catalytic nature of PROTACs and their ability to overcome resistance mechanisms, may include those molecules that were initially deselected due to the emergence of drug-resistant parasite strains even while exhibiting desirable pharmacodynamic properties.

At the opposite end of the antiparasitic PROTAC, suitable ligands could recruit either host or parasite-derived E3 ligases to degrade the POI. As *Toxoplasma gondii* and *Cryptosporidium parvum* parasites export a subset of virulence factors into the host cell upon infection (Bougdour et al., 2013, 2014; Dumaine et al., 2021), these proteins could be targeted for degradation by PROTAC ligands that bind well-characterized human E3 ligases such as CRBN and VHL. All apicomplexan parasites reside within a membrane-bound vacuole within the host, thereby effectively shielding most virulence proteins from host cell detection. This shielding effect is more pronounced in the case of *Plasmodium* spp., where at the symptomatic stage of infection, the parasites infect a terminally differentiated erythrocyte lacking an efficient ubiquitin-proteasome pathway (UPP). Parasite-specific E3 ligases may provide access to this larger proteome for TPD, however, this approach requires a more comprehensive understanding of the UPP in parasites.

The presence of a parasite-encoded UPP including a narrow repertoire of E1, E2, and E3, ubiquitin ligases have been bioinformatically identified and functionally characterized, although to a limited extent (Ponts et al., 2008, 2011; Silmon de Monerri et al., 2015). RING, Ring-between-RING, HECT, and U-box E3 ligases can be bioinformatically identified for all three of the Apicomplexan species discussed here (Ponts et al., 2008). Mutagenesis and gene knockout studies have identified essential roles for the UPP in both *Plasmodium* and *Toxoplasma* species (Sidik et al., 2016; M. Zhang et al., 2018). Treatment of parasites with E1 and proteasomal inhibitors has also identified key checkpoints that require a functional UPP pathway (Green et al., 2020; Kreidenweiss et al., 2008). Most excitingly, TPD using the TIR1/auxin-induced degradation (AID) system has been successfully adapted for use in both *Toxoplasma* and *Plasmodium* spp. (Brown et al., 2017; Kreidenweiss et al., 2013; Liu et al., 2021; Philip & Waters, 2015).

Although this preliminary evidence points to a functional UPS system in parasites (Baptista et al., 2019; Paul et al., 2020; Sidik et al., 2016; Zhang et al., 2018), detailed multi-omics-based functional characterization of parasite E3 ligases and the subsequent identification of specific E3 ligase-binding small molecules remain the priority for the synthesis of antiparasitic PROTACs.

4 | CONCLUSION

PROTAC-induced TPD has emerged over the last years as a powerful screening and therapeutic strategy for drug development. However, despite several advantages such as enhanced potency, specificity, and efficacy offered by PROTACs, and other degrader concepts over traditional inhibitors, TPD can potentially cause toxicity issues in the clinic (Raina et al., 2016). To overcome this limitation, a number of laboratories designed trifunctional PROTACs that additionally contain a photoswitch that allows light-activated spatiotemporal fine-tuning of PROTAC activation and were labeled PhotoPROTACs (Pfaff et al., 2019; Xue et al., 2019), Azo-PROTACs (azobenzene-proteolysis-targeting chimeras; Jin et al., 2020), PHOTACs (Reynders et al., 2020; Wu & Manna, 2020), or opto-PROTAC (Liu et al., 2020). Furthermore, the large number of yet to be characterized E3 ligases will potentially allow to enhance specificity by designing PROTACs to recruit E3 ligases in a tissue and cell-type manner. PROTACs and other TPD degraders can deplete proteins within multiple cellular compartments as well as extracellular proteins. Pilot studies targeting membrane proteins within mitochondria, ER, and Golgi hint at the potential expansion of the targetable degradome of the eukaryotic cell, with the important caveat of UPS accessibility to the target of interest (Alabi & Crews, 2021; Bensimon et al., 2020). As our knowledge of ubiquitin E3 ligases and targeted protein degradation expands, so will the expected PROTAC repertoire capable of targeting multiple compartments. Although TPD for targeting the

host–pathogen interface is still in its infancy compared with oncology applications, there are inherent aspects of this technology that makes it highly promising for future developments.

Apart from the advantages that PROTAC technology offers compared with traditional small-molecule inhibitor drug discovery, including the great benefits of overcoming drug resistance, there is a potential immunogenic aspect to capitalize on when combatting viral pathogens with PROTACs. Cellular immunity provides a defense mechanism against pathogens through cytotoxic T cells via recognition of foreign peptides presented by major histocompatibility complex (MHC) proteins on antigen-presenting cells (Yewdell et al., 2003). These peptides are presented on cell surfaces via MHC as a result of proteasomal degradation. Given the correlation between ubiquitination of viral proteins and MHC-mediated peptide presentation, PROTACs may offer a novel therapeutic avenue to specifically prime the immune system against pathogens via targeted protein degradation (Hahn et al., 2011). PROTAC-mediated degradation of pathogenic proteins and their subsequent presentation on the cell surface via MHC proteins could stimulate a potent immune response leading to specifically enhanced T-cell response against infected cells during overwhelming infections, cell death, and immune-mediated elimination of the pathogen. The demonstrated capability of PROTACs to induce MHC presentation of POI-derived peptides (Jensen et al., 2018) and the proposed dual action of PROTACs to inhibit viral proliferation in combination with the promotion of immune-mediated viral clearance, support the utility of antipathogenic PROTACs to overcome intracellular infections and related disease.

Although resistance to classical inhibitors is typically caused by mutations in the target protein, the mechanisms of PROTAC resistance in pathogens will be of keen interest as it may arise through alternate means such as differential expression or mutation of the PROTAC-recruited degradative E3 complex. The potential to use a variety of different E3 ligases targeting the same POI could be crucial to overcome this potential issue.

Next-generation antibiotics and antiparasitic molecules can be generated through the novel mode of action provided by TPD in pathogens through hijacking the pathogens' own proteasomal machinery, as highlighted by BacPROTACs (Morreale et al., 2021). Therefore, a comprehensive understanding of pathogen biology in concert with discovery chemistry will be required to develop suitable small-molecule inhibitors or interactors amenable to TPD.

Collectively, all these approaches will support our endeavors to develop small-molecule compounds that promote TPD of pathogenic proteins or infectious disease-related host factors with enhanced selectivity, tissue specificity, and reduced off-target toxicity. These novel TPD-based approaches will provide us with the opportunity to develop a repertoire of screening and therapeutic strategies capable of identifying and targeting pathogenic proteins that have been previously elusive to drug discovery or coined "undruggable." It will ultimately support our common aims of eliminating infectious diseases that remain a global health threat and reducing the associated human morbidity and mortality.

ACKNOWLEDGMENTS

D.M. is supported by an Emerging Leadership Fellowship by the National Health and Medical Research Council Australia (APP1177431). The figure in this manuscript was created with BioRender.com. We thank Dr Rebecca Feltham and Professors Guillaume Lessene, Alan Cowman, David Komander and Marc Pellegrini for their past and ongoing support. We thank Associate Professor Chris Tonkin for his critical reading of the manuscript. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no data were analysed in this study.

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How to cite this article: Grohmann, C., Marapana, D.S. & Ebert, G. (2021) Targeted protein degradation at the host–pathogen interface. *Molecular Microbiology*, 00, 1–12. https://doi.org/10.1111/mmi.14849