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Review Article

PROTACs: Mechanism and Bioavailability enhancement strategies by nanotechnology, RNA viral infections (vaccine strategy) and Prodrug development

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Abstract

Proteolysis Targeting Chimera (PROTACs) are a brand-new concept of therapeutics that use the ubiquitin-proteasome system for selective degradation of disease-related proteins. Like other therapeutics, PROTACs function by recruiting a protein target and a ubiquitin ligase that leads to the degradation of the target protein; however, unlike most other drugs, PROTACs do not simply disable the target's function through steric hindrance. As a result of this present review, the application of PROTACs will be discussed in oncology, leukemia, and neurodegenerative diseases. However, there are considerable difficulties regarding the bioavailability of PROTACs; one of which is selecting the appropriate degron. This review describes the issues of bioavailability that are related with PROTACs, such as solubility and stability issues, and proposed tactical decisions, which could be used under those conditions. The newest approaches in nanotechnology-aided drug delivery and the use of nanocarriers to address the limitations in PROTACs and their pharmacokinetic and pharmacodynamic properties are discussed. They improve the solubility and stability of PROTACs, allow for targeted delivery to the tumour site and minimize toxicity to healthy cells. Moreover, arguably the most interesting virtue of PROTACs is its applicability for RNA viral infections (new vaccine design) based on the degradation of viral proteins. An idea for the field of antiviral therapy, this invention defines a new horizon for the management of diseases that have not responded to traditional methods. In addition, the review focuses on the advanced techniques on the application of prodrug strategies in PROTACs. These inactive PROTACs are then converted to prodrugs to enhance their uptake into target tissues thereby increasing the concentrations at the target site and at the same time minimizing on the adverse effects that may be caused by higher concentrations in the entire body. Also, when PROTACs are incorporated into sophisticated drug delivery systems, their desirability and selectivity are further improved beyond existing issues related to small-molecule inhibitors. This review also includes new progress, clinical applicability, and development trend of PROTAC for the change of disease therapy and drug delivery.

Keywords: PROTAC; targeted protein degradation; RNA viral infections; prodrug; Oncology; Alzheimer's disease; Parkinson's disease; nanocarriers

Introduction

Small molecule inhibitors and monoclonal antibodies are the two most used molecularly targeted types of drugs in the clinical treatments in today's global market. These drugs operate mechanism wise by counter looking for the active site of target proteins and functioning as competitive antagonist ligands thus restraining the proteins from binding to their subsequent targets. However, the binding sites can be altered through mutations of genes or the change in the conformation of the target protein leading to drug resistance. Most of the current research in target identification is therefore inclined to more conventional targets such as kinases and G protein coupled receptors. But this is slowly shifting

with technology crossing over to hard to 'drug' targets hence the name 'undruggable'. Approximately 80% of all proteins are targets and most of them include no enzymatic action [1].

PROTACs are a new generation of heterobifunctional small molecules that work on the basis of the UPS system [2]. These molecules consist of two ligands: one is to recognize the protein of interest (POI) and the other targets an E3 ubiquitin ligase. As the result, PROTACs effectively encourage the ubiquitination and, consequently, proteasomal destruction of the target protein by comingling the target protein and E3 ligase. This distinct outline of action gives several merits over conventional small-molecule inhibitors that often act by occlusion of the target protein's active site [27]. Studies of PROTACs as a replacement of the UPS in 2001 opened the chemical biology approach to regulate protein function for drug discovery in the current era [4-11]. This concept of drug discovery involves miniature molecules and is the only approach that has the possibility to overcome the widely documented shortcomings of targeted drug therapy [12]. Whereas traditional small molecule inhibitors are prone to off-target effects and drug resistance since they only inhibit target protein activity, PROTACs overcome these problems by degrading target proteins fully. This mechanism increases the selectivity and potency of therapeutic intervention and opens the prospects for using drugs for targets that were previously considered untouchable [13,14]. Because PROTACs are so potent the field of Targeted Protein Degradation (TPD) expanded and went beyond the proteasome [15]. An example is the Lysosome Targeting Chimeras (LYTACs) which translocate the lysosomal degradation pathway to export proteins labeled with a specific organelle targeting signal. Another is the Macroautophagy Degradation Targeting Chimera (MADTAC) platforms the AUTACs and ATTECs that subvert the autophagy pathway. This could help to disrupt organelles and macromolecular complexes with a view of eliminating them [16,17].

In the subsequent decade there were major developments in PROTAC technology based on progress in the chemistry of linkers and in the characterisation of stronger E3 ligase ligands. The field was revolutionized with the advent of small-molecule PROTACs which are more drug-like and have preferable pharmacokinetic characteristics. These progress has boosted the general suitability of PROTACs to numerous target proteins that were once deemed as 'undruggable' [3,4]. Currently, several PROTAC drugs are under clinical trial examination. One of them is ARV-110 [18], that acts on the androgen receptor in the prostate cancer. Another is ARV-471 [19], a selective estrogen receptor antagonist indicated in treating breast carcinoma. Last is FHD-609 in which BRD9 is being targeted in synovial sarcoma [20].

Several PROTACs have thus been designed to target a variety of POIs involved in the development and progression of hematologic malignancies. Some of them are anaplastic lymphoma kinase (ALK) [21], Bcl-xL [22], breakpoint cluster region Abby like (BCR-ABL) [23], brutons tyrosine kinase (BTK) [24], bromodomain containing 4 (BRD4) [25], cyclin dependent kinase-6 (CDK-6) [26], FMS-like tyrosine kinase-3 (FLT Some agents are highly effective agents in eradicating leukemia and cancer cells in test tubes and can be capable of producing the complete remission and excision of tumors in animals [29]. Since their inception, PROTACs have shown the ability to degrade several and diverse target proteins associated with numerous disorders such as cancer, immunological diseases, neurological disorders, cardiovascular diseases and viral infections [30-32]. The degradation of target proteins by PROTACs has been proven to be efficient in sixty cases and two of them are in trial to treat prostate and breast cancer [33,34].

Recent advancements in PROTAC technology have focused on optimizing pharmacokinetics, bioavailability, and delivery mechanisms. The integration of PROTACs with advanced drug delivery platforms, such as nanoparticles (NPs) and lipid-based carriers, has significantly improved their stability and targeted delivery to disease sites has been a significant focus of recent research. These developments enhance the clinical potential of PROTACs and broaden their application across various medical conditions [35]. These efforts aim to improve the pharmacokinetics, bioavailability and targeted delivery of PROTACs. One notable advancement is the use of NPs as delivery vehicles. NPs can protect PROTAC molecules from degradation in the bloodstream, enhance their stability, and facilitate their

targeted delivery to diseased tissues. For instance, lipid-based NPs (LNPs) have been employed to encapsulate PROTACs, thereby improving their solubility and cellular uptake [36].

Furthermore, polymeric NPs have been fabricated so as to address the controlled release of PROTACs for constant therapeutic impact. These NPs can be designed to be sensitive to certain conditions in the tumor microenvironment including pH or enzymatic activity and thus, release the PROTACs in the right location [37]. To this effect, targeted delivery minimizes side effects that arise from the exposure of surrounding healthy cells and tissues as well as improves the effectiveness of the cure. Moreover, with Demdrimers, it has also been possible to fashion more elaborate delivery systems for PROTACs. Oleinik et al. described chemical knockdown method, which they called "ligation to scavenging", as an unconventional method for halting event-based proteolysis. This technique incorporates a ligation to a scavenging system of a tetrazine-functionalized BRD4 PROTAC 79 and PAMAM-G5-TCO to target the epigenetic regulation selectively. Being an efficient intracellular, nonspecific proteasome-targeted macro cationic dendrimer, PAMAM-G5-TCO quickly desorbs free PROTACs by the IEDDA approach. This in turn helps in preventing the degradation of the BRD4 protein and provides control of protein degradation termination. This is a novel chemical strategy that deserves about equal consideration as mechanical disruption methods and offers the possibility of modulating protein disintegration under command [38].

Similarly, the intracellular delivery of PROTACs has been advanced by conjugating them with cell-penetrating peptides (CPPs), thereby extending the capability of PROTACs to intercalate and degrade target proteins in cells [39]. Another interesting advancement is the application of exosomes to deliver PROTACs on target proteins. Exosomes are biomembrane-derived particles that are involved in the exchange of biomolecules across the cell membrane. By using loading PROTACs into exosomes, researchers make use of these vesicles to pierce barriers and deliver therapeutic agents right at the target cell. It may be worth emphasizing that this approach can enhance the therapeutic index of PROTACs, especially for diseases in which targeted delivery to specific cell types is difficult [40].

According to a recent bibliometric analysis retrieved from Pubmed, more than 2,251 papers on PROTAC were published from 1986 to 2024 [41]. Nevertheless, more candidates needed to enroll in clinical trials due to the small number of molecules that showed possible development. When rationally designing new candidates, a clearly defined experimental workflow using default protocols is essential [42]. There are at least 20 PROTACs in the clinical trials by the end of 2022 [43]. The review included clinical trials from 2022 to 2024, as outlined in Table 1.

Table 1. PROTACs in clinical trials.

Clinical trial no.	Sponser	Degrader	Target	Indications	phase	Study Start	Reference
NCT05501769	Arvinas	ARV-471 in combination with Everolimus	ER	Metastatic ER+, HER2- Breast Cancer	I	08.09.2022	[44]
NCT06206837	Pfizer	Vepdegestrant When Given With PF-07220060	ER+/HER2-	Metastatic Breast Cancer	II	19.02.2024	[45]
NCT06125522	Pfizer	Vepdegestrant When Given With Samuraciclib	ER+/HER2	Breast Cancer	II	10.01.2024	[46]
NCT05573555	Pfizer	Vepdegestrant When Given With Ribociclib	ER+/HER2	Breast Cancer	II	01.03.2023	[47]
NCT05548127	Pfizer	ARV-471 When Given With Abemaciclib	ER	Breast Cancer	II	23.02.2023	[48]
NCT05654623	Pfizer	ARV-471 When Given With Fulvestrant	ER+/HER2-	Advanced Breast Cancer	III	03.03.2023	[49]

Data source: https://clinicaltrials.gov [accessed: 06/06/2024]

PROTACs mechanism

The mechanism of action of PROTACs involves the formation of a ternary complex comprising the target protein, the PROTAC molecule, and the E3 ubiquitin ligase. The three steps (Figure 1) in this process are as follows: **Binding**: The targeting ligand of the PROTAC binds to the POI, while the E3 ligase ligand binds to the E3 ubiquitin ligase. **Ternary complex formation**: Upon simultaneous binding, a ternary complex is formed. The efficiency of this complex formation is critical for the subsequent steps. The structure and flexibility of the linker play a crucial role in facilitating this interaction. **Ubiquitination**: The formation of the ternary complex brings the POI in close proximity to the E3 ligase, allowing the transfer of ubiquitin molecules from the E2 ubiquitin-conjugating enzyme to the lysine residues on the POI. This process is mediated by the E3 ligase, which acts as a scaffold for ubiquitin transfer. **Proteasomal degradation**: The polyubiquitinated POI is recognized by the 26S proteasome, a large protease complex responsible for degrading ubiquitinated proteins. The POI is subsequently unfolded and degraded into small peptides, while the PROTAC molecule is released and can engage in additional rounds of degradation [18,50].

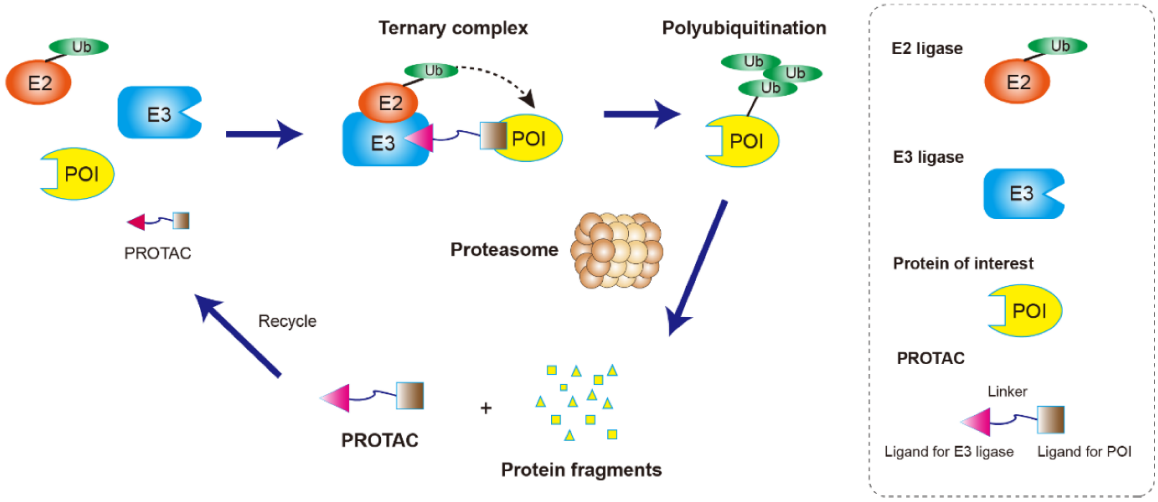

Figure 1. PROTAC mechanism. A PROTAC consists of a ligand that attaches to a protein of interest (POI) and another that connects to the ubiquitin ligase enzyme via a small chemical linker. By forming a POI-PROTAC-E3 ligase ternary complex, the target protein is tagged with ubiquitin groups. The proteasome recognises the polyubiquitination signal and facilitates the protein's breakdown. To facilitate further breakdown cycles, the PROTAC molecule can be recycled. Reprinted under the terms of the Creative Commons Attribution 4.0 International (CC BY) license [1].

The original proposal for PROTACs was made in 2001 by Sakamoto et al. [5]. They developed and synthesized the first bifunctional PROTAC molecule to degrade methionine aminopeptidase 2 (MetAP-2). In its initial form, the PROTAC molecule consisted primarily of peptides. A portion of the PROTAC-1 molecule that directed its attention to MetAP-2 bound to ovalicin, an inhibitor of angiogenesis, and another portion bound to the E3 ubiquitin ligase complex SCF (Skp1-Cullin-Fbox), an IPP derived from the IKB protein that β-TRCP uniquely identifies. Next, it was shown that PROTAC-1 could recruit MetAP-2 and SCFβ-TRCP. Different PROTACs have been created for numerous proteins since the preliminary result, including the BET protein family [5], EGFR [25], and aperiodic cyclin-dependent kinases (CDKs) [51]. The PROTAC approach has also been improved. Many of these proteins are currently being investigated as possible therapeutic targets or linked to cancerous tumors' progression and metastasis. In contrast to other approaches that have been developed to control tumor-related protein expression or function, such as CRISPR, siRNA, small molecule inhibitors, monoclonal antibodies, etc., PROTAC offers numerous benefits, such as a wide range of targets, rapid action, and high specificity in degrading pathogenic variant proteins while leaving normal proteins alone [52,53].

Application of PROTAC

Oncology

As we know, cancer is one of the dangerous diseases which can threat human life. A cancer patient is not restricted to surgical intervention, radiation, or chemotherapy as the only ways to cure. Another essential component of cancer treatment is a targeted treatment and immunotherapy. Such 'un-druggable' sites like KRAS and TP53 for instance, are still unlabeled by effective targeted drugs [54]. The main advantage of PROTAC technology is the shift of focus from "no drug" to "drug. " Affinity to the target protein is critical for conventional targeted drugs. Due to its capability to selectively and weakly interfere with target proteins, the PROTAC degradation agent can address many of the "undruggable" proteomes today, that is, around 80%. Literally it proves to be a lifesaver when conventional targeted therapy is not possible for a particular patient [55]. There are many oncogenic proteins, for example, kinase, transcription factor, and epigenetic enzymes, which play a significant role in carcinogenesis. Several PROs have been demonstrated to be targeted by PROTACs, which destabilise these proteins, hence suppressing cancer cell proliferation and survival. For instance, ARV-110 and ARV- 471 is two PROTACs discovered by Arvinas targeting androgen receptor and estrogen receptor respectively. These receptors are very essential in cases of prostate and breast cancer. These and other PROTACs have been proven to selectively degrade their targets and suppress tumor cells within vitro clinical trials, thus, the innovation has potential to be a new form of targeted cancer therapeutics [56,57].

Leukemia

Several PROTACs have demonstrated high efficacy in leukemic cells by providing a new approach for the degradation of proteins that are associated with the formation of the disease. Leukemia, the cancer of the white blood cells, is generally associated with the mutations that affect intrinsic factors such as the genetic code and protein functions, which cannot be effectively treated with conventional approaches. The problem of synthesising and purifying such proteins can be a concern and therefore PROTAC technology offers a solution that reacts with the cell's own machinery of protein degradation [58].

PROTACs targeting BCR-ABL in chronic myeloid leukemia

Chronic Myeloid Leukemia (CML) is associated with increased activity of BCR-ABL fusion protein that is a consequence of the translocation t(9;22). Conventional therapies for CML, especially TKIs including imatinib, have shown remarkable success for the initial years but increasing resistance resulting from BCR-ABL gene mutations poses a problem. PROTACs targeting BCR-ABL represent a more favorable strategy. For instance, it has been shown that the approach using both BCR-ABL1 kinase inhibition and protein degradation is currently a promising approach in overcoming BCR-ABL1-mediated drug resistance. Targeting BCR-ABL1 for degradation in CML through small molecules is better than targeting it and offers information about CML stem cells [59].

PROTACs in acute myeloid leukemia

Acute Myeloid Leukemia (AML) is another form of leukemia where PROTACs have shown promise. AML is characterized by the rapid proliferation of immature myeloid cells, often driven by mutations in various signaling proteins and transcription factors. Targeting these proteins with PROTACs can induce their degradation and inhibit leukemic cell growth. One study described an oral activity BRD9 PROTAC C6 by recruiting the highly efficient E3 ligase. C6 exhibited superior oral activity, with a Cmax value of 3436.95 ng/mL. These findings demonstrated that C6, as a novel BRD9 PROTAC with remarkable pharmacodynamic and pharmacokinetic properties, had the potential to be developed as a promising therapeutic agent for AML treatment [60]. An internal tandem duplication (ITD) in the juxta-membrane domain is a common mutation in FLT-3, a therapeutic target in acute myeloid leukaemia (AML) [61,62]. One study found that a chemical that triggers the degradation of the FLT-3 ITD mutant at low nanomolar doses was produced by converting the FLT-3 inhibitor quizartinib into a PROTAC. Compared to the warhead alone, the PROTAC can limit cell growth with greater potency and fewer off-target kinases inhibited. The increased level of apoptosis induction suggests that the FLT-3 ITD protein

has nonkinase roles. This explains why the PROTAC's kinase inhibitory action is slightly reduced while the antiproliferative activity remains unchanged. The PROTAC can also induce the degradation of FLT-3 ITD in living organisms. Based on these findings, FLT-3 ITD degradation could be a promising therapeutic target [27,63].

Neurodegenerative diseases

There are currently no effective therapies or drugs for several refractory diseases, including neurodegenerative ones such as Alzheimer's, Parkinson's, progressive supranuclear palsy, and frontotemporal dementia. Conventional medications have failed miserably at targeting a large number of neurodegenerative disease-causing proteins. None of the compounds that reduced the rate of amyloid-b (Ab) accumulation have been authorized for usage in clinical trials, and numerous therapeutic trials for Alzheimer's disease have been unsuccessful. Novel developments in PROTACs provide an alternative perspective on this problem [64,65]. So far, PROTACs have effectively targeted a wide variety of proteins associated with neurodegenerative diseases, such as a-Synuclein [66], mHTT [67], GSK-3 [68], LRRK2 [69], Tau [70], TRKA [64], and TRKC [71].

PROTACs in Alzheimer's disease

Alzheimer's disease is a type of dementia disorder in which people experience progressive cognitive decline due to deleterious deposition of the amyloid beta (Aβ) and neurofibrillary tangles (tau protein) in the brain. PROTACs have been revealed as an effective therapeutic strategy for selectively and ubiquitinate these pathological proteins [72]. Targeting Tau and Amyloid Precursor Protein (APP) are considered to be of paramount importance in the development of AD. Tau protein leads to the formation of neurofibrillary tangles in AD which is toxic to the neurons. Antibodies against IST tau would decrease ist levels and prevent tau aggregation and formation of neurofibrillary tangles, which presumably should attenuate disease progression. In one study, Authors used PROTAC to selectively degrade tau in neuronal cells with reduced tau aggregation and toxicity. In this case, the selective degradation of tau has the potential to alleviate tau-mediated neurodegeneration [73]. Specific for the elimination of intracellular tau proteins, Chu et al. synthesized a small peptide, called TH006, which is a PROTAC that enhanced poly-ubiquitination of its target. TH006 also had effects on tau decrease in primary neuron cells and a mouse model of AD, and the inhibition of cytotoxicity induced by Aβ12 [74]. Wang et al.'s similar work contributes to this conclusion. Another compound was synthesized and it was confirmed to enhance the tau degradation via UPS and lessen the cognitive impairments in Alzheimer-like models [70], all of which indicating that protein degradation is advantageous in order to combat aggregation problems [75].

PROTACs in Parkinson's disease

Parkinson's disease (PD) is an ongoing neurodegenerative and movement ailment that has been established by the degeneration of the dopaminergic neurons that are found in the substantia nigra coupled with the α-synuclein proteins. In PD, PROTACs are employed to degrade α-synuclein directly, bringing down the toxic levels of the protein [76-79]. One of the protein aggregates evident in PD is alpha synuclein which is known to be damaging and fatal to neurons. Recently, Sun et al. established a PROTAC for degradation of α-synuclein in dopaminergic neurons and alleviation of their neurotoxicity. This work also showed that via the generation of PROTACs, α-synuclein aggregation as well as ist-associated neurotoxicity can potentially be managed [31]. LRRK2 gene polymorphism is perhaps one of the most popular and best-studied genetic factors contributing to PD. Here, we shown that LRRK2 can be targeted for degradation using a well-studied technique called proteolysis-targeting chimeras (PROTACs) which provides a new therapeutic strategy. These mutations are identified mostly in LRRK2 gene but most especially the G2019S mutation which increases the kinase activity of the protein and is toxic to neurons hence causing Parkinson's disease. Small molecule chimeric inhibitors of LRRK2 have been explored and while they have their limitations such as partial inhibition and off target toxicity. PROTACs on the other hand, which use the cell's ubiquitin–proteasome system to target their protein and degrade it selectively, provides an answer to these limitations. In the recent past, much

research effort has been directed towards designing and fine tuning PROTACs for the selective degradation of LRRK2. Liu et al. described the research done in identifying LRRK2 proteolysis targeting chimeras (PROTACs) to devise degrader XL01126. Altogether, these experiments demonstrate that XL01126 can be considered as an appropriate degrader probe for dissecting the non-catalytical and scaffold functions of LRRK2 in vitro and in vivo [80].

PROTACs bioavailability: Challenges and strategies

It is the molecular structure that distinguishes PROTACs from traditional chemo-drugs as far as their physicochemical properties are concerned. This is because, although PROTACs display a lot of promise in the treatment of many diseases, the bioavailability is a major barrier. The issue of providing sufficient bioavailability to drugs derived from these types of compounds is difficult because of their size, multifunctionality and other chemical properties [81-84].

Factors affecting PROTAC bioavailability

Molecular size and complexity

PROTACs are bispecific molecules consisting of two functional ligands linked by an intervening chain. This structure often leads to high molecular weights and molecular mass which in a way negatively affects their absorption and distribution. In general, PROTACs have MWs between 800 to 1,200 Da, which are larger than traditional small molecule drugs. This size can sometimes hinder their entrance into the cellular matrices via passive diffusion [85]. Moreover, several polar groups in PROTACs could enhance the hydrophilicity, which subsequently results in a decrease in membrane permeability [13].

Solubility

The appreciable solubility of PROTACs in biological fluids makes the absorption and bioavailability functions a key factor here. PROTACs are also insoluble in aqueous media and therefore poorly dissolve in gastrointestinal tract and can be absorbed [86-88]. Solubility of PROTACs may be further improved by complicated formulation strategies, that is utilizing solubilizers or employing proteolysis-targeting chimeras with the help of nanocarrier systems [89]. Most PROTACs face issues related to intracellular delivery and having a low bioavailability. Many approaches have been attempted to boost their performance by prognostically modifying the scaffold of PROTACs to remove their relatively poor properties mainly due to the chemical characteristics of PROTACs. Klein et al., further explaining the method of construction of the PROTACs, believed that one way of lowering the amount of intramolecular hydrogen bond donors and improving the cell permeability of the PROTACs might be done by the use of ester groups to replace the amide groups in between the linkers and the warheads [90]. Another correlation that was discovered regards the increase of cell permeability and the decrease of molecular polarity where large linkers with lipophilic side chains or the substitution of more polar E3 ligase recruits might be beneficial [91,92]. However, it is important to remember that as TPSA is directly related to water solubility raised cell permeability by reduced molecular polarity can affect the water solubility of PROTACs and their pharmacokinetic response. This has called for more research in the development of theories in this area.

Stability

The stability of PROTACs present in any of the body fluids also determines the problem of bioavailability. As a result of this, PROTACs may be chemically unstable and could be degraded in the stomach before getting absorbed [15]. In the same regard, enzymatic Degradation is an issue because PROTACs are vulnerable to being broken down by proteolytic enzymes hence limiting the quantity of the active drug in the systemic circulation [93].

Strategies to improve PROTAC bioavailability

Nantechnology-based delivery systems

NPs represent a promising approach to improving the solubility and, therefore, the efficacy of PROTAC compounds. The encapsulation of PROTACs in NPs may enhance solubility, stability, and bioavailability besides shielding the complex from degradation and elimination within the cells. These nanoparticle based delivery systems are capable of releasing the PROTACs in a controlled and more informed manner to influence the positive change in the pharmacokinetic characteristics of the PROTACs. Some of the delivery strategies include antibodies, small-molecular targeting agents, organic NPs, liposomes, polymeric NPs, inorganic NPs, and lipid NPs of PROTACs, as illustrated in Figure 2 [84].

NPs and PROTAC molecules are at the crossroads between nanobiotechnology and targeted protein degradation for therapeutic uses. However, issues such as the poor solubility and cell permeability characterise traditional PROTACs as well as off target effects. To overcome these limitations, Wang and colleagues have also turned to the functionalization of PROTACs with nanotechnology leading to nano-PROTACs. Some of these nanostructured PROTACs can improve issues concerning the delivery, stability, as well as the selectivity of PROTACs. However, the nano-PROTACs can be partly modified to enhance solubility and bioavailability to enable enhanced targeting of disease-protein. Thus, employing NPs as carriers helps to obtain targeted delivery of PROTACs into the body without causing multiple side effects and enhance the overall efficiency of the treatment [94]. These developments point to the possibilities of using nanoparticle based delivery systems in extending the potential of PROTAC bioavailability and its actual application in therapies. The application of NPs in the formulation of drugs has been well illustrated in the improvement of solubility and pharmacokinetics. Compared to drug molecules, NPs are relatively bigger in size, thus once the drugs are loaded into NPs the solubility of the drugs is solely determined by that of the NPs [97-99]. Dendritic targeted passive targeting, also known as enhanced permeability and retention (EPR), occurs in the case of NP within certain size ranges that are preferably taken up in tumor tissues because it lacks functional lymphatics and encompasses abnormally permeable vasculature [100–102].

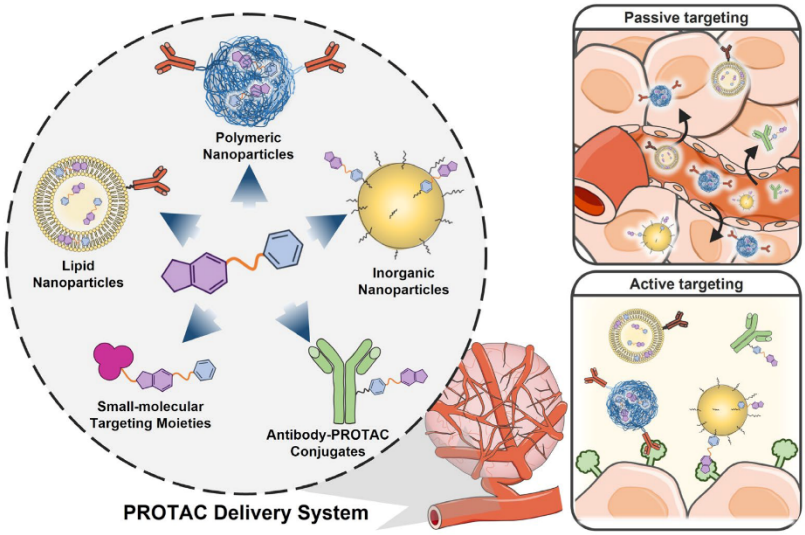

Figure 2. Carriers for PROTAC delivery. Through passive and active targeting approaches, they control the pharmacokinetic features of PROTACs while simultaneously raising their accumulation levels in tumours. Reprinted under the terms of the Creative Commons Attribution 4.0 International (CC BY) license [84].

The pharmaceutical NPs are involved in delivery of drugs as are other NPs in the pharmaceutical field. There are inorganic and organic forms of NPs; these are further sub-divided into lipid and polymeric forms. From among those, polymeric NPs can boast of being one of the most standard and efficient systems for the delivery of drugs. In these respects of, these NPs have the ability of presenting a broad spectrum of pharmacological/physicochemical properties through adjustment on monomers

and polymeric chains [103]. Among the polymeric based drug delivery systems, which has been approved by the FDA, the most common is, block copolymer of poly (ethylene glycol) (PEG) and poly (D, L-lactide-glycolide) (PLGA) [65]. The research work done by Sarawat et al. has, therefore, resulted in a significant success in the treatment of pancreatic cancer. They developed an inert delivery approach for lipophilic ARV-825, a PROTAC degrades bromodomain 4 (BRD4) that is nestled within PEG-PLGA NPs for parenteral administration. The outcome was dramatic and showed the usefulness of the ARV-Nanoparticle on pancreatic cancer cells. It provoked cytocidal effect, cell death, and an anti-clonogenic activity. The pro-apoptotic protein cleaved caspase-3 was also induced while the anti-apoptotic protein Bcl-2 was significantly reduced together with BRD4 and c-Myc. Probably, the most important proof of its efficiency was the decrease of cell viability of 3D pancreatic cancer tumor spheroids after treatment with ARV-NP. It is opening the way to a new approach to pancreatic cancer treatment that holds much promise [104].

Gao et al. engineered a polymeric PROTAC (POLY-PROTAC) nano platform for tumor-targeted degradation of the bromodomain and extra terminal (BET) protein BRD4. First, they synthesized four von Hipel-Lindau (VHL)-based small molecular PROTACs. Then, they designed a series of reduction-activatable POLY-PROTACs and self-assembled them into micellar NPs for systemic PROTAC delivery (Figure 3a). Then, a dibenzocyclooctyne (DBCO)-loaded pre-targeted NP was engineered to enhance intratumoral accumulation and retention of azide-modified POLY-PROTAC NPs via in situ click reaction. Upon internalization into the tumor cells, the POLY-PROTAC NPs release the PROTAC payload via glutathione (GSH)-mediated reduction of the disulfide bond (Figure 3b). Outcomes demonstrated that the POLY-PROTAC NPs synergistically induce apoptosis of tumor cells when combined with photodynamic therapy (PDT) in a mouse model of MDA-MB-231 breast cancer (Figure 3c). This study might provide a generalizable nano platform for tumor-specific PROTAC delivery and potentiated cancer therapy [105].

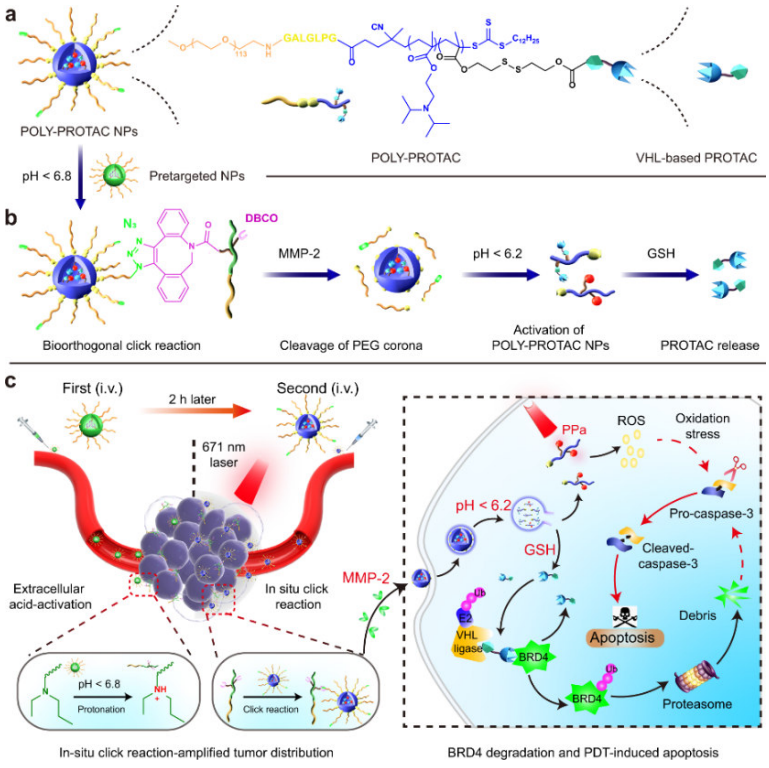

Figure 3. Schematic illustration of the 9iorthogonal POLY-PROTAC NPs for tumour-specific protein degradation and precise cancer therapy. [a Cartoon illustration of the azide-functionalized biorthogonal POLY-PROTAC NPs. POLY-PROTAC was engineered by integrating an MMP-2-liable PEG chain, an acid-activatable DPA moiety and a reduction-sensitive disulfide spacer. B Schematic illustration of the extracellular acidity-triggered click reaction between POLY-PROTAC and DBCO-loaded pretargeted NPs and sequential activation of POLY-PROTAC in response to the extracellular enzyme and intracellular acidic/reductive microenvironment. C In situ click reaction-promoted protein degradation and combinatorial cancer therapy with POLY-PROTAC NPs. The POLY-PROTAC NPs showed tumour-specific accumulation and retention via a biorthogonal click reaction with the pretargeted NPs and cleavage of the PEG corona in the tumour mass. The POLY-PROTAC NPs were then internalised into the tumour cells for BRD4 degradation and combination therapy with PDT]. Reprinted under the terms of the Creative Commons Attribution 4.0 International (CC BY) license [105].

In another study, Xu et al. created an RCNprotac, a nanomicelle that responds to X-ray radiation to combat cancer. A 141.80 ± 5.66 nm nano micelle was self-assembled after a previously reported small molecule PROTAC (MZ1) was covalently coupled to hydrophilic PEG via a carbon chain that contained diselenide bonds. Due to its improved permeability and retention effect, RCNprotac—which at first showed no bioactivity in circulation because the hydroxyl group on the E3 ubiquitin ligand component was occupied-could accumulate at the tumor site. By breaking the radiation-sensitive diselenide

linkages, X-ray radiation released MZ1 for the targeted degradation of the tumor BRD4 protein. A decrease in BRD4 protein level occurred, making the tumour more radiosensitive. Synergistic increases in anticancer effects were demonstrated in vitro and in vivo by RCNprotac. Finally, this X-ray-responsive PROTAC nano micelle may give an innovative approach to X-ray-activated spatiotemporally controlled protein degradation and BRD4 proteolysis improved tumour radiosensitivity, which anticipates the eventual success of cancer research [106].

The primary finding on the cancer-fighting potential of the HSP90 degrader BP3, which was synthesized using the PROTACs principle, was conducted by Jiang et al. [107]. Unfortunately, its insolubility in water and high molecular weight limited its practical use. This research aimed to enhance these features of HSP90-PROTAC BP3 by incorporating it into NPs made of human serum albumin (BP3@HSA NPs). The findings revealed that BP3@HSA NPs had a consistent spherical form, a size of 141.01 ± 1.07 nm, and a polydispersity index of less than 0.2. Moreover, compared to free BP3, BP3@HSA NPs were more or less taken by breast cancer cells and exhibited a stronger inhibitory impact in laboratory tests. It was also shown that BP3@HSA NPs may break down HSP90. From a mechanistic standpoint, BP3@HSA NPs' enhanced capacity to induce cell cycle arrest and apoptosis was associated with their enhanced inhibitory effect on breast cancer cells. Additionally, BP3@HSA NPs demonstrated enhanced pharmacokinetic characteristics and demonstrated more robust tumor reduction in mice. These results highlight the potential of encapsulating hydrophobic HSP90-PROTAC BP3 NPs in human serum albumin to enhance BP3's antitumor activity and safety significantly.

Amphiphilic lipids are the building blocks of liposomes and lipid NPs, which form nanostructures through self-assembly in water. Their increased biocompatibility and potential for mass manufacture make them an appealing alternative to drug carriers based on polymeric or inorganic NPs [108]. The loading process for lipid NPs is straightforward, and they can accommodate substantial amounts of various medicinal chemical compounds. The size and shape of lipid NPs are influenced by several variables, including lipid chemical content and structure and development techniques [109]. However, the morphological parameters of lipid NPs are not the only aspect of interest. They are meticulously controlled by the properties of the payloads [110-113], a factor that underscores the significance of the work in the pharmaceutical and medical fields. These properties profoundly impact the drug loading/releasing patterns, physiological stability, and pharmacokinetics.

Research led by Rathod's team has strongly emphasized delivering lipid-based PROTACs [87]. After studying the anticancer activity of a protein degrader known as the Bromodomain and Extra-Terminal motif (BET) in both sensitive and vemurafenib-resistant melanoma, they conducted pre-formulation studies and formulation development. Rather than inhibiting the BRD4 protein, the ARV-825 (ARV) molecule, developed using PROTAC technology, degrades it. The development of ARV-SNEP, or ARV-loaded self-nano emulsifying preconcentrate, was contingent upon thorough pre-formulation experiments. ARV exhibited hydrolytic breakdown dependent on pH and extremely low water solubility (<7 μg/ml). ARV is a substrate of CYP3A4 but not of the P-gp efflux pump, according to investigations using human liver microsomes and a CaCO-2 cell uptake assay. Enhanced ARV solubility in a range of water- and bio-relevant environments was accompanied by the formation of 45.02 nm nano globules by optimized ARV-SNEP, which exhibited a zeta potential of -3.78 mV. Crucially, when tested on vemurafenib-resistant melanoma cells, ARV demonstrated promising cytotoxicity, anti-migration, and apoptotic effects. The development of ARV-SNEP, with its unique properties and demonstrated efficacy, may herald a new therapeutic paradigm for drug-resistant melanoma.

An emerging class of anticancer compounds, PROTACs, has substantial solubility problems. Much research has been conducted into colloidal systems based on lipids, such as nanostructured lipid carriers, for these highly lipophilic compounds. Scientists used the melt emulsification method to create an ARV-825 loaded PEGylated nanostructured lipid carrier (AP-NLC) of BRD4 protein degrading PROTAC that targets non-small cell lung carcinoma. The ARV-825 was stabilized with the use of Precirol® ATO5 as the solid lipid and Captex® 300 EP/NF as the liquid lipid. The results showed a hydrodynamic diameter of 56.33 ± 0.42 nm, a polydispersity index of 0.16, and a zeta potential of -21 ± 1.24 mV. ARV-825 and AP-NLC demonstrated antitumor efficacy in cultured cell migration and colony

formation assays. Researchers observed an almost 38% and 50% apoptotic cell population following ARV-825 and AP-NLC therapy, respectively. An immunoblotting experiment showed evidence of complete inhibition of BRD4 and c-Myc protein expression for AP-NLC. To conclude it all, BRD4 PROTAC and its lipid nanoparticle were proven efficient against non-small cell lung cancer (NSCLC) when they significantly reduced the growth of multicellular 3D spheroid of A549 cells. The increased red fluorescence observed across the spheroid surface provides more evidence of AP-NLC's enhanced penetrating and cell-killing capabilities, highlighting the promising potential of this new delivery system in clinical applications [114].

In order to combine a synergistic cytotoxic ratio, a chimaera targeting BRD4 proteolysis (ARV-825) and nintedanib co-loaded PEGylated nanoliposomes (ARNIPL) were produced. Neither of the molecules is soluble in water at all. A modified hydration technique containing citric acid was employed to enhance the loading of both compounds into liposomes. At 4 °C, ARNIPL showed physical stability for one month and demonstrated an encapsulation efficiency of over 90% for both medicines, with an average particle size of 111.1 ± 6.55 nm. A375R, a vemurafenib-resistant cell line, demonstrated increased cytotoxicity, apoptosis, and down-regulation of target proteins BRD4 and c-Myc in response to both drugs and ARNIPL. ARNIPL considerably reduced the clonogenic potential and vasculogenic mimicry of A375R. With ARNIPL, tumor growth suppression and a significant decrease in TGF-β1, a key regulator of immune response and tumor progression, were observed in 3D spheroids. The results showed that ARNIPL has the potential to be a successful treatment for vemurafenib-resistant melanoma, with the added benefit of modulating the immune response [115].

Inorganic NPs, such as silica, gold, iron oxide, and quantum dots, are potential candidates for PROTAC delivery, offering unique properties distinct from organic NPs. The meticulous control over their morphology and size distribution, coupled with their rigid structure that minimizes the risk of drug leakage at off-target sites [116], presents a promising avenue for drug delivery. Gold NPs (GNP), in particular, hold great promise as drug carriers thanks to their bio inertness, well-established surface modification method, and versatility in providing additional functionalities, instilling optimism in their potential. A drug delivery system based on gold nanoparticles (GNPs) was developed in the study by Y. Wang et al. to deliver PROTACs to target Anaplastic lymphoma kinase (ALK). The Cer/Pom-PEG@GNPs, which are pegylated GNPs loaded with ceritinib and pomalidomide molecules, demonstrated excellent stability in various mediums as tested. There was a dose- and time-dependent reduction in ALK fusion protein levels and particular inhibition of NCI-H2228 proliferation caused by the GNP conjugates. Researchers found that Cer/Pom-PEG@GNPs could break down intracellular ALK fusion proteins with only a few side effects and could be used to treat patients not responding to ALK inhibitors. The Cer/Pom-PEG@GNPs nano-drug carrier can deliver medications to tumor areas in vivo with high precision, allowing for sustained circulation [117]. According to another study, a supramolecular gold(I)-thiol-peptide complex (Nano-MP) was designed to incorporate proteolysis recalcitrance, cellular internalization, and glutathione-triggered release. The complex was nanoengineered to target a tumor-driving protein, MDMX, for degradation. To enhance tumor targeting, nano-MP was coated with a pH-responsive macromolecule called polyacryl sulfydryl imidazole (PSI). Results demonstrated that Nano-MP@PSI induced the MDMX degradation by ubiquitination and subsequently restored the anti-cancer function of p53 and p73 [118]. Further investigation is essential to evaluate the effectiveness of different inorganic NPs for PROTAC delivery beyond gold, which has not been explored previously.

Targeting moiety-functionalized NPs

Thus, targeting moiety-functionalized NPs is a current state of the art in the targeted drug delivery, proving to be especially valuable in the case of PROTACs. These NPs are designed to be functionalized with specific ligands or antibodies at the surface, which can specifically react with receptors that are overexpressed on the surface of target cells, including tumour cells. This functionalization improves the targeting ability and the specificity in which drug loaded-NPs lodge in the required site, reducing the unwanted interaction with other tissues, and subsequently improves on the effectiveness of the

administered drug [95,96]. The exposure of PROTACs to tumor sites and the proper management of their undesired pharmacokinetic profile have been enhanced by linking PROTAC molecules to antibodies or aptamers. However, the loading capacity of mAb-PROTAC is restricted. Due to the tendency of antibodies to cluster or to be rapidly cleared in physiological circumstances if there are a large number of drug molecules conjugated to the antibodies, traditional ADCs allow no more than four drug/antibody ratios to ensure the optimal potency of the conjugate [101,119,120]. Since PROTACs, generally possess a higher molecular weight and are less soluble in water compared to chemo-drugs, the proportion of PROTACs to antibodies should be kept to not more than three. In turn, owing to their chemical structure, NPs can incorporate a rather large number of PROTACs in their structure. This is because they are comparatively less sensitive to the loading amounts than the antibodies and, therefore, make suitable PROTAC carriers.

Moreover, the incorporation of active targeting moieties on the nanoparticle surface can enhance tumor accumulation of PROTACs, suggesting a potential strategy for improving the efficacy of cancer treatment [84]. In one study, The nanoprecipitation approach was used to load the BRD4-degrading PROTAC (MZ1), which consists of a BRD4-binding ligand and a VHL E3 ligase recruiter, into poly(D, L-lactide) (PLA) NPs. The polyethyleneimine (PEI) layer was conjugated with trastuzumab, and the particles were developed with a size of around 114 nm. The nanoparticle was found to have a loading amount of 0.5% MZ1, and the loaded MZ1 was released gradually in solution. Endocytosed MZ1 induced death in HER2-expressing cancer cells linked with BRD4 deficiency, and trastuzumab on the particle surface greatly improved nanoparticle internalization [121].

Subsequently, He et al. suggested a therapy strategy that combines doxorubicin (DOX) with the BRD4 PROTAC degrader ARV-825 (ARV) using a self-assembly process and a nanoparticle modified with Cyclo (Arg-Gly-Asp-d-Phe-Lys) (cRGDfk) peptides, cRGD-P. Conformational changes made possible by molecular dynamics simulations allowed cRGD-P to offer interaction sites for optimal co-loading of DOX and ARV. On average, the cRGD-P/ARV-DOX was 39.95 nm in size and had a zeta potential of -0.25 mV. After being stimulated with cRGD-P/DOX, glioma cells showed increased BRD4 expression, which supports one of the potential mechanisms of DOX resistance and the synergistic tumor inhibitory impact of BRD4 degrading ARV coupled with DOX. Through glioma cell cycle arrest in the G2/M phase and activation of tumor cell apoptosis-related pathways, such as triggering a cascade of caspases, downregulating Bcl-2, and upregulating Bax, the study found that DOX and ARV combined in the cRGD-P nanoparticle system synergistically suppressed tumor growth. By enhancing tumor apoptosis, reducing tumor proliferation, and decreasing tumor angiogenesis in vivo, the cRGD-P/ARV-DOX system successfully limited gliomas' heterotopic and orthotopic growth. More effective and safer combination therapy for glioma may be possible with the cRGD-modified nanoparticle that co-delivers DOX and ARV [122].

Another type of PROTAC carrier that can be used for active cancer targeting is lipid NPs. In an effort to combat hepatocellular cancer, A. Saraswat et al. investigated the use of galactose-decorated liposomes to transport PROTAC [123]. In order to study the anticancer effectiveness of GALARV for targeted delivery in hepatocellular carcinoma, they created asialoglycoprotein receptors (ASGPR) directed nanoliposomes that included a new BRD4 protein-targeted PROTAC, ARV-825 (ARV) (GALARV). In vitro studies on hepatocellular carcinoma cells demonstrated that ARV and GALARV (with a size of 93.83 ± 10.05 nm) caused cytotoxicity and apoptosis. Compared to non-targeted nanoliposomes (~3 fold) and ARV alone (~4.5 fold), GALARV had a significantly greater intracellular concentration of ARV, exhibited good physical stability, and exhibited almost no hemolysis. There was a significant decrease in the levels of target BRD4, oncogenic c-Myc, apoptotic Bcl-2, and survivin proteins, as shown by immunoblotting. Notably, 3D hepatocellular carcinoma tumour spheroids treated with GALARV showed markedly reduced cell viability and death. Based on these findings, GALARV appears to be an innovative nanotherapeutic strategy for hepatocellular carcinoma that actively targets PROTACs.

PROTACs in RNA viral infections

Viruses are minute, non-cellular microorganisms that severely threaten human health and the global economy [124]. Viruses can have a whole genome and a variety of proteins, or they can have infectious RNA (viroid and virusoid) [125]. Several viruses have recently produced major epidemics, accounting for more than 70% of infectious disease cases. The current standard of care for preventing and treating human viral infections combines antiviral drugs and vaccines [126,127]. Unfortunately, existing antiviral therapy techniques face increasing resistance from newly emerged viruses, and vaccines are not always effective against new or modified viruses [128]. This highlights the urgent need for innovation in targeting or vaccination techniques and the identification of new pharmacological targets to develop effective antiviral therapeutic approaches. Above, we covered how PROTAC technology has been studied extensively for cancer-related targeted protein degradation of POI. Recently, however, much more information has been available about the function of PROTACs as antiviral medicines. Antiviral treatment techniques based on PROTAC have lately been investigated in various ways to improve resistance profiles. A potent antiviral medication that can withstand the present and future threats of new and re-emerging viral pandemics may be conceivable with the help of these innovative methods [129]. These advancements in antiviral therapy hold great promise but also come with challenges, such as safety and efficacy. Researchers and healthcare professionals must stay updated on these developments and contribute to the ongoing discussions and research in this field.

PROTAC: novel vaccine strategy

Developing effective antiviral vaccines is not without its challenges. Preventing the transmission and infection of viral infections by triggering the host antiviral innate immune response is a vital function of these vaccines, which are expected to work similarly to antiviral drugs [130,131]. Various strategies can be used to generate live-attenuated virus vaccines; these include cold-adapted live-attenuated influenza vaccines, codon-deoptimized viruses, premature termination codon-harboring viruses, hyper-interferon-sensitive viruses, and viral-protein-altered viruses. Live-attenuated virus vaccines are among the most effective and proven preventative measures. However, most of these existing methods result in live-attenuated vaccines, which are linked to a considerable or even entire loss in safety and effectiveness [132]. Vaccines are developed by using microorganisms' toxins or surface receptors, which mimic their identity within the host body. These vaccines, when administered, stimulate an immune response upon the detection of a new foreign item in the body, thereby assisting in the development of humoral immunity. The classification of vaccines includes attenuated, inactivated, toxoid, subunit, conjugate, heterotypic, and genetic types [132,133].

Researchers have demonstrated the effectiveness of a novel PROTAC-based approach in degrading viral proteins. This approach involves fusing a removable proteasome-targeting domain (PTD) to eight viral proteins (M1, PB2, PB1, PA, NP, M2, NEP, and NS1) to create PROTAC viruses. The PTD, containing a proteasome-targeting peptide, ALAPYIP, and a tobacco etch virus cleavage site (TEVcs) linker, ENLYFQG, were individually used to generate PROTAC viruses in co-cultured HEK293T-TEVp/MDCK-TEVp cells. The putative PROTAC viruses were then amplified in MDCK-TEVp cells, and their production and infectivity were verified by the cytopathic effects (CPEs) caused by viral infection. M1-PTD caused evident CPE in MDCK-TEVp cells, whereas no CPE was detected for PB2-PTD, PB1-PTD, PA-PTD, NP-PTD, M2-PTD, NEP-PTD or NS1-PTD. Notably, the CPE caused by M1-PTD was observed only in MDCK-TEVp cells and not in the conventional MDCK.2 cells, providing strong evidence for the effectiveness of TEVp-dependent M1-PTD. Additionally, the group examined the ability of M1-PTD to elicit an immune response in ferrets and mice. They found that the titers for HI (Hemagglutinin Inhibition), NT (Neutralisation), HA (Hemagglutinin), and internally conserved nucleoprotein antibodies were significantly higher than those of individuals who had been immunized with inactivated influenza vaccine (IIV) or cold-adapted influenza vaccine (CAIV). As a result of the improved presentation of degraded viral peptide antigens by MHC molecules, it was additionally observed that the T-cell immune response was robust and competent [134].

PROTAC viruses could be an excellent candidate for a vaccination. An ideal vaccination can achieve a level of attenuation in the host that is suitable for safety while at the same time maintaining a robust immunogenicity in cell lines [135,136]. By utilizing the degraded viral peptides produced by the proteasomal degradation pathway, PROTAC can stimulate an effective immune response, which is in contrast to conventional methods of vaccine manufacture. For this reason, PROTAC technology has emerged as a significant choice for producing safer and more effective vaccinations.

Prodrug approaches

Prodrugs are bioreversible, inactive drug substances that become active and can be metabolized to functional parent drugs in the human body [137]. Thus to optimize the oral bioavailability and therapeutic effect lipophilic prodrugs are used to overcome biopharmaceutical, pharmacokinetic and pharmacodynamics problems like chemical stability, poor solubility, non-site specificity, extensive metabolism, crossing biological barriers, utilizing enzymes of the body, toxicity, and compliance problems due to un-acceptable taste or odour [154]. The prodrug technique can be employed to optimize new chemical entities, and also to improve the qualities of already marketed drugs. Though the prodrug approach was a late stage optimization strategy, it has been tried right from the onset of research and development process from Discovery stage. There is the use of a new chemical entity when producing a prodrug, but it is cheaper than developing a new drug molecule from scratch. The higher efficiency (as compared to the parent medicine) tends to reduce the time taken for drug development, which may lead to saving costs, time and effort [139-141].

There are significant concerns over the possible toxicity of the method due to the uncontrolled degradation of proteins and undesired ligase-mediated off-target effects, even though PROTACs have become viable therapeutic approaches. The potential for toxicity and adverse effects could be reduced through the precise manipulation of the degrading activity of PROTACs. As a consequence of this, a significant amount of work has been put into the creation of cancer biomarker-activating prodrugs of PROTACs [142]. Using a bioorthogonal prodrug technique (called click-release "crPROTACs"), Chang et al. could activate PROTAC prodrugs on-target and selectively release them into cancer cells. Two inactive PROTAC prodrugs, TCO-ARV-771 and TCO-DT2216, were rationally developed by attaching a bioorthogonal trans-cyclooctenes (TCO) group to the ligand of the VHL E3 ubiquitin ligase. The tetrazine-modified RGD peptide, c(RGDyK)-Tz, activates the PROTAC prodrugs for click-release. This enables the targeted degradation of proteins of interest (POIs) in cancer cells, as opposed to noncancerous normal cells, by targeting the integrin $\alpha v\beta 3$ biomarker in cancer cells. The results demonstrated that this technique proves to be viable by selectively activating PROTAC prodrugs in an integrin $\alpha v\beta 3$-dependent way. This activation process then generates PROTACs, which break down POIs in cancer cells. An all-encompassing, abiotic method of targeting cancer cells via the ubiquitin-proteasome pathway could be the crPROTAC strategy [142].

The polymer-conjugated PROTAC prodrug platform, as suggested by Zou et al., offers a safe and effective approach for tumor-targeted administration of the most prevalent von Hippel-Lindau (VHL)- and cereblon (CRBN)-based PROTACs, specific codelivery of a degrader, and conventional small-molecule drugs. The activated, self-assembling PROTAC prodrug NPs demonstrate their ability to target tumor cells and release free PROTAC for targeted protein degradation. In a mouse model, the PROTAC prodrug NPs effectively degraded bromodomain-containing protein 4 (BRD4) or cyclin-dependent kinase 9 (CDK9), resulting in a more efficient regression of MDA-MB-231 breast tumors with lower systemic toxicity. These findings underscore the versatility of PROTAC prodrug NPs as a promising approach for the codelivery of chemotherapeutics and PROTACs, thereby enhancing anticancer efficacy and combination advantages [143].

In order to make HNSCC tumors more sensitive to radiation therapy (RT), Zhang et al. have created a nanosensitizer (RPB7H) by combining NPs of hafnium dioxide (HfO2) with a PROTAC prodrug (BPA771). When administered intravenously, RPB7H NPs bind to neuropilin-1, which is overexpressed in tumors, and then they aggregate in tumor tissue, where they internalize into tumor cells. By increasing oxidative stress, DNA damage, and X-ray deposition, HfO2 NPs make RT more effective. At

the same time, RT-induced H2O2 secretion can activate BPA771, which degrades bromodomain-containing protein 4 (BRD4) and inactivates RAD51-associated protein 1 (RAD51AP1), stopping RT-induced DNA damage repair. The results showed that in a mouse model of head and neck squamous cell carcinoma (HNSCC), the tumor growth might be efficiently regressed by combining these nanosensitizers with X-ray irradiation. The results suggest that targeting the BRD4-RAD51AP1 axis with a PROTAC prodrug-based radiosensitization strategy could be an excellent way to improve the efficacy of radiation therapy (RT) and other treatments for head and neck squamous cell carcinomas [144].

There are some potential advantages when applying prodrug and PROTAC technologies collectively. This approach can be applied to improve the stability and delivery of PROTACs. For example, one can modify PROTAC into a prodrug that is more soluble and less prone to degradation. That is why, developing PROTAC prodrugs that are activated only in the desired tissues or cells provides for rather selective protein degradation without side effects. It can be highly effective especially in oncolytics, where this approach can minimise systemic side-effects. Therefore, applying prodrug strategies to PROTACs can improve their pharmacokinetics profile that will allow them to exhibit adequate bioavailability and prolonged efficiency in the target tissue.

Miscellaneous approaches

Conjugates were synthesized by Banik et al. to bind to target proteins' extracellular domains as well as cell surface lysosome targeting receptors. An antibody is linked to agonist glycopeptide ligands for the cation-independent mannose-6-phosphate receptor (CI-M6PR) in these lysosomes targeting chimaeras ("LYTACs"). A CRISPRi knockdown screen, made possible by LYTACs, uncovered the molecular mechanism for cargo internalization mediated by CI-M6PR. As proven, apolipoprotein-E4, EGFR, CD71, and programmed death-ligand 1 (PD-L1) are efficiently degraded by LYTACs. LYTACs provide a modular approach to targeting secreted and membrane proteins for destruction in therapeutic and fundamental research settings [15].

Challenges to PROTACs

Research institutions and pharmaceutical companies alike are keenly interested in PROTAC because of its status as a cutting-edge technology. Like any new technology, PROTAC has potential and obstacles as it develops. Prospects for PROTAC, both positive and negative, will aid in the study and creation of tailored protein-degrading medications. Despite PROTAC's distinct benefits over competing drug development paradigms, it has its share of drawbacks, which pose severe problems Like Resistance, Off target, and Target selection [43]. Because of their unique designs, PROTACs often exhibit poor pharmacokinetic performance and high molecular weight. Despite several reports of PROTAC activity in vivo and their current clinical investigation owing to their unique structures, these compounds often have a significant molecular weight and perform poorly in pharmacokinetic and druggability studies [32]. The potential toxicity and side effects of PROTACs raise significant concerns regarding their clinical application. Unlike traditional inhibitors that only suppress activity without affecting protein expression, PROTACs trigger targeted protein degradation, potentially preventing drug resistance and possibly leading to higher irreversible toxicity [145]. On the flip side, the unintended impact of PROTACs on normal cells or organs could significantly affect medical care. Therefore, it is essential to monitor and assess the toxicological effects of PROTACs rigorously, just as we do for clinical investigations and in the later stages of drug development, and further reduce both on-target and off-target toxicities associated with PROTACs. Generally, the molecular weight of PROTACs falls somewhere between 700 and 1200 Da. Also, compared to standard small-molecule antivirals, PROTACs have more hydrogen bond donors and a more extensive polar region on their surface. These factors can influence the permeability of PROTAC cells for oral administration [146]. Because antiviral PROTACs have to pass across the host cell or even the nuclear membrane to have a biological impact, transmembrane permeability is a potential disadvantage.

Moreover, due to their poor bioavailability and weak pharmacokinetic characteristics, PROTACs do not follow Lipinski's conventional rule of five [147,148]. In clinical treatment, greater dosages of oral

PROTAC medicines are required owing to low oral bioavailability resulting from poor permeability and solubility. This could lead to an increase in the risk of side effects and manufacturing costs. In order to better understand the druggability of antiviral PROTACs, it is essential to create credible and systematic guidelines for optimizing their physicochemical attributes and oral bioavailability.

Conclusion and perspectives

In tumors, immunological illnesses, neurological diseases, cardiovascular diseases and in viral infections the therapeutic potential of PROTAC was explained through the 20 years of PROTAC technology, where some molecules already have come close to clinical stage. Among the new insights there is the fact that PROTACs appear to be sensitive to drug-resistance targets. This method can address the problem of the existing drugs which the current therapies may be encountering such as drug resistance. PROTACs degrade the complete target protein, which affects protein function, which maybe enzymatic or otherwise. The second is 'undruggable targets' as a potential approach to PROTACs. Most researchers agree that greater than 80 percent of proteins in human cells, where drug targets can reside, do not have active sites of binding enzymes or receptors, a significant problem for most small-molecule drugs or large-molecule antibodies. Despite their discovery as enzyme-tagging proteins, distinct functions of PROTACs are possible. Interference with enzymes is known in pharmacology as the traditional type of small-molecule drugs' action. It indicates that PROTACs can regulate not only proteases and other enzymes but also other non-enzyme processes and increase the "drug-target" interface. Several of the issues elicited by classical small molecular inhibitors can, however, be worked around. From this perspective, nanotechnology presents an innovative route to optimise the delivery, performance, and selectivity of PROTACs. The augmentation of PROTACs with nanotechnology can address some of the challenges, which includes solubility, stability and bioavailability can therefore open up more opportunities of therapeutic strategies. One of the advantages of using nanoparticle-based delivery systems is that PROTACs can be included in those nanoparticles to shield it from breakdown in the gastrointestinal tract or the bloodstream. This encapsulation increases the solubility and the stability of PROTACs so that a greater amount of the reagent gets to the target site. They are capable to improve the BBB permeability thus helping in delivery of PROTACs to the affected areas in the body such as the brain in the case of neurodegenerative diseases. Using engineered NPs these barriers can be crossed and PROTACs can be delivered to the central nervous system thereby moving recreational proteins that are linked with neurodegenerative processes. PROTACs carry the potential of future combinations with nanotechnology as ongoing studies include work on better nanoparticle design, better targeting, and far better safety assessments of the delivery systems used. Future evolution of nanotechnology will escalate to the generation of advanced NPs, which would be ligand for PROTACs besides other drugs enhancing the treatment of diseases. Moreover, PROTAC prodrugs do not exist yet in the real-world scenario, and the preliminary findings suggest their effectiveness. For instance, the PROTAC versions of prodrugs can be developed based on the activation by the enzymes that exist in cancer cells, thus providing the direct degradation of oncogenic proteins. Combined application of prodrug and PROTAC technologies seems to have enormous potential to create the new generation of therapeutics with enhanced activity, reduced toxicity and targeting.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

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