




Targeted Protein Degradation: An Important Tool for Drug Discovery for “Undruggable” Tumor Transcription Factors

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Abstract

Conventional small-molecule drugs (SMDs) are compounds characterized by low molecular weight, high cell permeability, and high selectivity. In clinical translation, SMDs are regarded as good candidates for oral drug formulation. SMD inhibitors play an important role in cancer treatment; however, resistance and low effectiveness have been major bottlenecks in clinical application. Generally, only 20% of cell proteins can potentially be targeted and have been developed as SMDs; thus, some types of tumor targets are considered “undruggable.” Among these are transcription factors (TFs), an important class of proteins that regulate the occurrence, formation, and development of tumors. It is difficult for SMDs and macromolecular drugs to identify bioactive sites in TFs and hence for use as pharmacological inhibitors in targeting TF proteins. For this reason, technologies that enable targeted protein degradation, such as proteolysis-targeting chimera or molecular glues, could serve as a potential tool to solve these conundrums.

Keywords

PROTAC, transcription factors, protein of interest, targeted protein degradation, molecular glue

Abbreviations

BCL6, B-cell lymphoma 6; BET, bromodomain and extraterminal; BRD4, bromodomain-containing protein 4; C2H2-ZF, Cys2-His2 zinc finger; CRBN, cerblon; IAP, inhibitor of apoptosis proteins; DLBCL, diffuse large B-cell lymphoma; EGF, epidermal growth factor; FGFR1, fibroblast growth factor receptor 1; FLT3, Fms-related receptor tyrosine kinase 3; IκZF, Ikaros zinc finger; iMiD, immunomodulatory drugs; MDM2, murine double minute 2; MYC, myelocytomatosis; p53, tumor protein 53; PLZF/ZBTB16, promyelocytic leukemia zinc finger; POI, protein of interest; PROTAC, proteolysis-targeting chimera; RARA, retinoic acid receptor alpha; SMD, small-molecule drug; SNIPER, specific and nongenetic IAP-based protein eraser; STAT3, signal transducer and activator of transcription 3; TOI, transcription factor of interest; TF, transcription factor;

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TPD, targeted protein degradation; TRAFAC, transcription factor-targeting chimera; UPS, ubiquitin-proteasome system; VHL, von Hippel-Lindau; ZMYM2, zinc finger MYM-type containing 2

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Introduction

Conventional small-molecule drugs (SMDs) are compounds characterized by low molecular weight, high cell permeability, and high selectivity. In clinical translation, SMDs are regarded as good candidates for oral drug formulation. Two key features of SMD inhibitors are: (1) the ability to bind to the protein of interest (POI) and (2) the ability to display therapeutic activity. SMD inhibitors play an important role in cancer treatment; however, resistance and low effectiveness have been major bottlenecks in clinical application.¹ Generally, only 20% of cell proteins can potentially be targeted and have been developed as SMDs; thus, some types of tumor targets are considered “undruggable.”^{2,3} Among these are transcription factors (TFs), an important class of proteins that regulate the occurrence, formation, and development of tumors. TFs are distributed inside the cell and are generally present on flatter surfaces that form complex structures via protein-DNA or protein-protein interactions (PPIs) that lack binding pockets for small molecules. It is difficult for SMDs and macromolecular drugs to identify bioactive sites in TFs and hence for use as pharmacological inhibitors in targeting TF proteins.⁴ For this reason, technologies that enable targeted protein degradation (TPD), such as proteolysis-targeting chimera (PROTAC) or molecular glues, could serve as a potential tool to solve these conundrums.

A PROTAC drug is a dual-functional molecule with 2 moieties, one that binds to POIs and another that recruits the E3 ligase. These 2 moieties are connected by a chemical linker. PROTAC drugs degrade POIs via the ubiquitin-proteasome pathway.⁵ The ubiquitin-proteasome system (UPS) consists of ubiquitin (Ub), the 26S proteasome, substrates, and 3 Ub cascade enzymes, namely Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub ligase (E3).⁶ Ubs are a class of small proteins that exist in most eukaryotes and are conjugated to other target proteins at their lysine residues. Protein ubiquitination may lead to degradation, localization, or modulation of signal transduction.⁷ Ub can be activated by E1 with the energy provided by ATP, Ub, and ATP, which are composed of ubiquitin adenylate complexes, after which it is transferred to the cysteine residue of E1.⁵⁻⁷ E1 then transfers activated Ub to E2 via a transthioesterification reaction. In the last step of the ubiquitination cascade, E3 catalyzes the formation of an amide bond between Ub and the lysine residue of the POI.⁶⁻⁸ Several Ub molecules are then added to form a polyubiquitin chain in the POI that is recognized by the 26S proteasome, which finally degrades the POI into small peptides.⁹⁻¹¹

Earlier versions of PROTAC compounds depended on peptides to target POIs but had unsatisfactory efficiency, limiting

their potential for further application. With the development of new ligands for E3, high-efficiency E3 ligases such as cereblon (CRBN), von Hippel-Lindau (VHL), and inhibitor of apoptosis proteins (IAPs), have been successfully used for designing PROTAC compounds, and PROTAC drugs consisting of small molecules have been developed.¹² Some immunomodulatory drugs (IMiDs), such as thalidomide and its derivatives, lenalidomide, and pomalidomide, were found to have the ability to bind the CUL2 (CRBN domain of unknown activity, binding cellular ligands, and thalidomide) domain at the C-terminal of CRBN, and CRBN formed an E3 ubiquitin ligase complex with damaged DNA-binding protein 1 (DDB1), Cullin 4A (Cul4A), and ROC1 (also known as CRL4^{CRBN} E3 ubiquitin ligase).^{13,14} Ligands for CRL4^{CRBN} have been widely used in the design of PROTAC compounds.¹⁵

VHL is a tumor suppressor protein that can be combined with elongin B (ELOB) and C (ELOC), cullin-2 (Cul2), and a new gene (RING) box protein 1 (VBCCR complex) to form the so-called CRL2^{VHL} E3 ubiquitin ligase. Multiple ligand structures binding with VHL have been applied in PROTAC research and were found to be effective at inducing target degradation.¹²

IAP can inhibit apoptosis by blocking caspase, and a RING finger domain is present at the C-terminal, which can bind to the E2 Ub-conjugating enzymes to function as E3 ubiquitin ligases. Based on this principle, specific and nongenetic IAP-based protein erasers (SNIPERs) have been designed to degrade POIs and consist of POI ligands, linker structures, and E3 ligase ligands. SNIPER drugs have been developed for some “undruggable” targets.^{12,16} The 2 requirements for conventional pharmacological strategies are: (1) it needs to bind with substrates and (2) it needs to exert a bioactive function on POIs.¹⁷ However, PROTAC drugs can degrade POIs only through a relatively weak binding ability without requiring adhesion to active sites because they work as molecular ligands to attract the recruitment of E3, which triggers the ubiquitination cascade reaction. This feature of PROTAC is especially suitable for solving the challenges with the “untargetable” or “undruggable” targets in tumors, such as TFs.^{3,5,8,10} PROTAC technology has gradually become an important potential solution for these “untargetable” or “undruggable” tumor targets.¹⁸ In general, PROTACs have a high molecular weight, which affects their practical and pharmaceutical properties.

In recent years, monomer molecules such as molecular glues, which can induce the degradation of POIs, have been developed. Molecular glues are a class of monofunctional small-molecule compounds that are different from bifunctional PROTAC. Molecular glue compounds have been applied to induce the degradation of some TFs in hematological cancer.¹⁹ Here, we

Table 1. TPD Drugs for Tumor TFs.

Name	Derivative	Target TF	E3 ligase	Cancer	Ref
ARV-825	OTX015	BRD4	CRBN	AML	32,33
ARV-771	Triazolo-diazepine acetamide	BRD4	VHL	AML, prostate cancer	32,34
dBET1	JQ1	BRD4	CRBN	Microglia, colorectal cancer	35,36
SD-36	SI-109	STAT3	CRBN	Blood cancer	45–47
XD2-149	Napabucasin	STAT3	CRBN	Pancreatic cancer	48
MD-224	MI-1061	MDM2	CRBN	Leukemia	55
WB214	Nutlin	MDM2/p53	CRBN	Leukemia	56
11a-1	Nutlin	MDM2	VHL	Lung cancer	57
CC-122	Thalidomide	IKZF1, IKZF 3	CRBN	Blood and solid tumors	62
CC-220	Thalidomide	IKZF1, IKZF 3	CRBN	Blood cancers	62
CC-885	Thalidomide	IKZF1	CRBN	AML	62
BI-3802	Monomer molecule	BCL-6	SIAH1	DLCL	77

Abbreviations: TPD, targeted protein degradation; TF, transcription factor; AML, acute myeloid leukemia.

summarize the progress of drug development for TPD of tumor TFs in research and clinical experiments.

TPD Drug Discovery on Tumor TFs

c-MYC and BET

In Table 1, the myelocytomatosis oncogene (*MYC*) family comprises *c-MYC*, *n-MYC*, and *l-MYC*, which are crucial TFs that play an important role in cell cycle regulation, metabolism, and tumorigenesis. Nearly 30% of malignant tumors are driven by the *c-MYC* gene, especially in lung, breast, myeloid leukemia, cervical, and colon cancers. *c-MYC* is a 62-kDa basic-helix-loop-helix-leucine zipper (bHLH-ZIP) TF, and is relatively unstructured until it binds with another bHLH-ZIP protein, due to the absence of the pockets applicable to SMDs.^{20–26} Owing to this special conformation, *c-MYC* is often believed to be an “undruggable” cancer target.² Currently, the main inhibition strategy for *c-MYC* focuses on blocking the PPI with its downstream target, *MYC*-associated protein X (*MAX*), which functions by forming a heterodimer with *c-MYC*. This heterodimerization is usually driven by bromodomain and extraterminal (*BET*) proteins, the most important of which is bromodomain-containing protein 4 (*BRD4*).^{27–31} The IMiDs—thalidomide and its analogs, such as lenalidomide—were shown to bind specifically to the E3 ligase of *CRBN* and were designed as PROTAC drugs for degrading *BRD4* that showed persistent inhibition of *c-MYC* even in the picomolar concentration range. Based on these results, the PROTAC drug ARV-825 was developed by Arvinas and was applied to acute myeloid leukemia (AML).^{32,33} The backbone of another highly efficient E3 ligase binder, *VHL*, was used to design a PROTAC that degrades *BRD4* proteins in prostate cancer cells (ARV-771).^{32,34} The *BRD4* inhibitor JQ1 has also been converted into the PROTAC drug dBET1.^{35,36} These drugs are more efficient than inhibitors in suppressing the function of *c-MYC* in neuroblastoma, prostate cancer, and leukemia. The PROTAC molecule for the broad *BET* family, BETd-260, was tested in vitro and in vivo for osteosarcoma tumors and hepatocellular carcinoma.

BETd-260 induced a robust suppressive effect on *BET* and *c-MYC* in these diseases.^{37–39} The degradation function of PROTACs is affected by the binding valence numbers. A trivalent PROTAC molecule named SIM1 was designed with PROTACs of 2 bivalent *BET* inhibitors and one E3 ligand connected by a branched linker and targets *BET* proteins. Compared to bivalent PROTACs for the same targeting, the efficiency of SIM1 for protein degradation was 300-fold higher and was more effective at inducing cancer cell apoptosis.⁴⁰

STAT3

Signal transducer and activator of transcription 3 (*STAT3*) was first discovered as an oncogene with 3 isoforms, namely *STAT3 α* , *STAT3 β* , and *STAT3 γ* . Some cytokines, such as IL-5/IL-6 and the epidermal growth factor (EGF), can induce the phosphorylation of tyrosine 705 (pY705) in *STAT3*, which is then activated by receptor-associated Janus kinases (*JAKs*).^{41,42} The bioactive proteins form homo- or heterodimers mediated by Src-homology 2 (*SH2*) domains and translocate to the cell nucleus as a transcription activator. In addition, *STAT3* phosphorylation may occur at serine 727 via mitogen-activated protein kinases.⁴³ With the stimulation of oncogenic signaling, *STAT3* is persistently over-activated and is constantly present in the nucleus, resulting in the activation of downstream genes and the stimulation of tumor cell growth. Given that there are few bioactive domains in the *STAT3* protein, an inhibitory drug design that involves *STAT3* binding may be difficult.⁴⁴ The small molecule compound SD-36 has an inhibitory effect on the *SH2* domain of *STAT3*. In this instance, SD-36 could be a potential candidate for a *STAT3*-specific PROTAC for inducing *STAT3* degradation. Furthermore, this degradation works on both wild-type and mutant *STAT3* and does not affect *STAT1*, *STAT2*, *STAT4*, *STAT5A/B*, and *STAT6*. SD-36 is an efficacious drug (*KD* \approx 50 nM) and has high specificity for *STAT3*, which shows good druggability.^{45–47} The most common way to design PROTAC drugs is to conjugate the inhibitor of a POI with ligands of E3 using linkers. Recent research has also modified napabucasin, a

STAT3 inhibitor, linking it with the ligands of CRBN and VHL, and the resulting PROTAC decreased STAT3 proteins in some solid tumors. However, the mechanism remains unclear and seems independent of the UBS degradation pathway. Further studies are needed to validate these findings.⁴⁸

p53 and MDM2

Tumor suppressor protein p53 (*p53*) is a tumor suppressor gene, and 50% of malignant cancers contain mutations in this gene. p53 is a TF that regulates the initiation of the cell cycle.^{49,50} p53 can induce apoptosis in unreparable damaged cells under the negative regulation of the downstream protein murine double minute 2 (MDM2). In the case of malignant mutation of *p53*, the regulatory function is disrupted due to the conformational transition and structural stability changes that contribute to carcinoma tumorigenesis.⁵¹ In recent years, there have been initiatives to develop inhibitors targeting MDM2. However, they have not shown significant therapeutic benefits, especially in clinical trials.^{52–54} For this reason, PROTAC drugs based on these inhibitors are considered a potential way to obtain such compounds. The PROTAC drug MD-224, which was designed based on the MDM2 inhibitor MI-1061, not only induced strong degradation of MDM2 at the nanomolar level in leukemia cells but also durable tumor regression in a mouse model.^{55,56} Another PROTAC drug, which was designed using the MDM2 inhibitor nutlin, also exhibited a promising effect compared to inhibitors. PROTAC combined with the derivative of nutlin and the CRBN-binding molecule lenalidomide can induce the depletion of MDM2 in some leukemia cell lines, causing an indirect increase in wild-type p53.⁵⁶ The ligand of MDM2, WB214, can directly induce p53 degradation via the UPS. However, it induces the ternary complex formation and subsequently degrades POIs via a molecular glue mechanism.^{56,57} Furthermore, the PROTAC drug combining nutlin with a VHL ligand showed progressive effects by mediating MDM2 degradation in an in vivo lung cancer model with a satisfactory drug safety profile.⁵⁷

IKZF1 and IKZF3

The Ikaros zinc finger (*IkZF*) gene family encodes a series of IkZF proteins and includes the isoforms IKZF1 (Ikaros), IKZF2 (Helios), IKZF3 (Aiolos), IKZF4 (Eos), and IKZF5 (Pegasus), which are TFs involved in the regulation of blood cancer cell differentiation.^{58,59} The next-generation IMiDs, which are new derivatives of thalidomide, including CC-122 (avadomide), CC-220 (iberdomide), CC-99282, and CC-885, showed improved efficacy in the degradation of some IKZF factors, such as IKZF1 and IKZF3, in preclinical or clinical trials for different types of hematological cancers. These molecules, similar to thalidomide, also serve as targets for future PROTAC design. All these compounds exert degradation activity by working with CRBN.^{60,61} Among these drugs, CC-122 exerts degradation effects in multiple blood cancers, including myeloma, diffuse large B-cell lymphoma (DLBCL),

non-Hodgkin's lymphoma, chronic lymphocytic leukemia, small lymphocytic lymphoma, and follicular lymphoma. Most of these diseases along with some solid tumors have been applied in the clinical trials of CC-122. Generally, CC-122 has a good safety profile and has shown anticancer activity in the clinic, despite some acceptable side effects. In some phase 2 clinical trials of CC-122, the monotherapy effect was better than that of standard anticancer therapy, and the more pronounced effect of CC-122 in combination with other conventional therapies showed a more promising lead.⁶⁰ In a phase 1/2 study of CC-122 combined with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapy (NCT03283202), the overall response rate was 88% among 34 patients with DLBCL, the complete response was 79%, and one-year progression-free survival was 80%, which showed that CC-122 had promising effects in this phase.⁶² The anticancer mechanisms of CC-220 and CC-99282 are similar to that of CC-122, and multiple clinical trials on both monotherapy and combination therapy with chemotherapy are in progress. Among these trials, a multicenter, open-label, phase 1 clinical trial (NCT03930953) aims to test the clinical activity of CC-220 in combination with R-CHOP and CC-99282 in combination with R-CHOP in patients with B-cell lymphoma (BCL). CC-885, based on the degradation of IKZF1 proteins, has shown good therapeutic effects in preclinical experiments on AML.⁶⁰ In contrast to other IMiDs, CC-885 also induces the degradation of the translation termination factor GSPT1, suggesting its broad spectrum of application.^{60,63}

C2H2-ZF

A Cys2-His2 zinc finger (C2H2-ZF) domain exists in some TFs, such as IKZF1 and IKZF3. IMiDs, such as thalidomide and its analogs, can recruit these proteins to CRBN, leading to the induction of ubiquitination and proteasomal degradation.⁶⁴ IMiDs can promote the binding of CRBN and substrates because the conserved degromotif CXXCG exists in C2H2-ZF proteins.^{64,65} Human genes can be fused by chromosomal rearrangement, and the resulting proteins are frequently used as biomarkers of some cancer cells.⁶⁶ The zinc finger MYM-type containing 2 (*ZMYM2*) gene encodes a TF that translocates with fibroblast growth factor receptor 1 (*FGFR1*) and Fms-related receptor tyrosine kinase 3 (*FLT3*) genes to form fusion genes that express the fusion proteins *ZMYM2-FGFR1* and *ZMYM2-FLT3*.⁶⁵ *ZMYM2-FGFR1* is associated with approximately half of patients with *FGFR1*-rearranged myeloid/lymphoid neoplasms.⁶⁷ *ZMYM2-FLT3* was also confirmed to be expressed in myeloproliferative neoplasms with eosinophilia.⁶⁸ The CXXCG motif was detected and identified in the second MYM domain of *ZMYM2*, indicating that the thalidomide analog CC-122 (avadomide) can recognize this site and induce fusion protein degradation. Through drug application, CC-122 induced the degradation of *ZMYM2-FGFR1* and *ZMYM2-FLT3* both in vitro and in vivo.⁶⁵ Promyelocytic leukemia zinc finger (PLZF), also known as ZBTB16, is a C2H2-ZF protein that was confirmed to be a neosubstrate of CRBN.^{69,70} In patients with acute promyelocytic

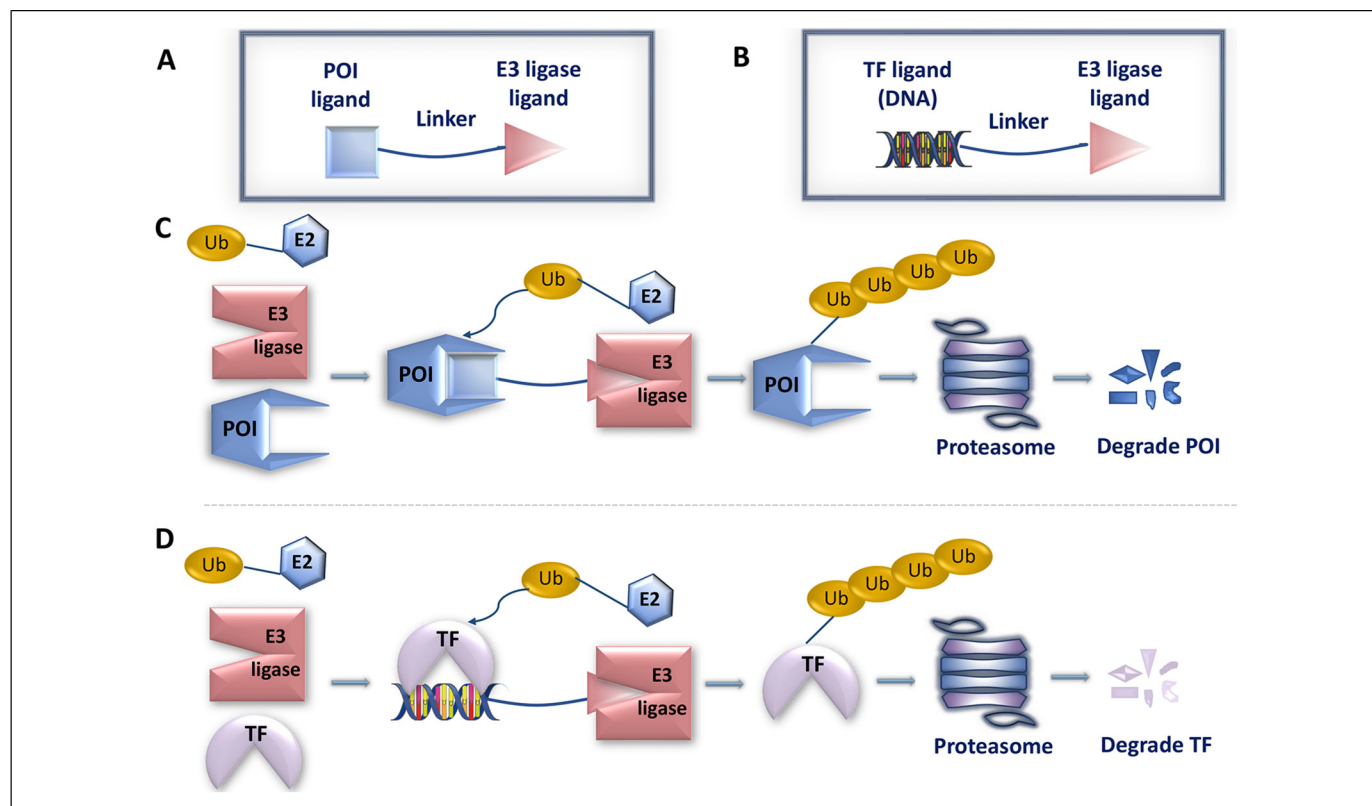


Figure 1. Targeted protein degradation. (A) The small-molecule ligands for POIs used in conventional PROTAC. (B) The DNA ligands for TF binding are used in TF-PROTAC. Ubiquitination of the POI/TF is triggered (C), and the POI is degraded by the proteasome (D). Abbreviations: POIs, proteins of interest; PROTAC, proteolysis-targeting chimera; TF, transcription factor.

leukemia (APL), *ZBTB16* can translocate with the retinoic acid receptor alpha (*RARA*) gene, forming the fusion proteins ZBTB16-*RAR* α or *RAR* α -ZBTB16, which play an important role in the deterioration of APL.^{71,72} CC-3060 is a novel CRBN modulator with a structure similar to that of pomalidomide.⁷⁰ To test whether CC-3060 can induce ubiquitination and proteasomal degradation, it was applied in ZBTB16-*RAR* α - and *RAR* α -ZBTB16-overexpressing cell lines and was found to strongly decrease the levels of fusion proteins, more effectively than pomalidomide.⁷⁰

BCL6

The *BCL6* gene encodes the BCL6 protein, which is a sequence-specific repressor of zinc finger TFs and modulates the function of B cells. The *BCL6* gene has been associated with the development and pathogenesis of DLBCL.^{73,74} The inhibitor of BCL6 could be synthesized into a PROTAC, aiming to achieve degradation of BCL6. However, this PROTAC lacked specificity and no conspicuous phenotypic response was induced.⁷⁵ Recently, a molecular glue, BI-3802, with a molecular weight under 500 Da, which differs from the relatively high molecular weight of conventional PROTACs (generally more than 800-1000 Da), was found to cause rapid ubiquitination of BCL6 resulting in significant protein depletion.

BI-3802 induces BCL6 degradation by binding to its Broad-Complex, Tramtrack, and Bric-à-Brac (BTB) domain. This binding leads to the formation of a supramolecular structure with BCL-6 homodimers, making it easier to ubiquitinate BCL6 and induce degradation.^{76,77} In contrast to IMiD-induced degradation, BI-3802 triggers endogenous degradation via the 7 in absentia homolog 1 (SIAH1) pathway and provides a novel rationale for future degrader drug design.⁷⁷ BI-3802 had an antiproliferative effect on DLBCL models both in vitro and in vivo, and the effect was comparable to what was observed in a BCL6 knockout.⁷⁷

New PROTAC Technology Targeting TFs

In Figure 1, generally, the design of conventional PROTAC drugs is based on the structure of POI inhibitors. Inhibitors connected to ligands of E3 ligase by molecular linkers constitute the fundamental structure of PROTAC drugs. However, TFs are intracellular proteins and mostly have complex structures, and designing a molecular inhibitor of a TF may be challenging. To target TFs, a new degradation technology called TF-PROTAC has been developed. TFs are a class of proteins that bind to specific sites on genomic DNA through a DNA-binding domain (DBD). The DBD initiates transcription by binding the specific nucleotide sequence with high affinity and specificity, which provides a good potential tool for

designing PROTAC molecules for TFs. In TF-PROTAC synthesis, the VHL E3 ligase binder is modified by bicyclooctyne (BCN) and is then conjugated with azide-modified DNA oligomers (N3-ODN) via the copper-free strain-promoted azide-alkyne cycloaddition (SPAAC) reaction. TF-PROTAC compounds can recruit target TFs and induce their degradation. Two tumor-related TFs, nuclear factor- κ B (NF- κ B) and eukaryotic transcription factor (E2F), were used as POIs. In HeLa cells, the compounds of VHL-BCN reacted with N3-NF- κ B-ODN via click reaction, and the resulting TF-PROTAC was able to degrade P65, the primary constituent of NF- κ B. A similar reaction was also observed between the compounds VHL-BCN and N3-E2F-ODN, of which the resulting TF-PROTAC significantly suppressed HeLa cell proliferation. TF-PROTAC is free from the limitations of ternary small-molecule PROTACs and directly induces the degradation of TFs.⁷⁸

Another technology, transcription factor-targeting chimeras (TRAFTACs), has shown good effects on some oncogenic TFs, such as NF- κ B and brachyury, both in vitro and in vivo. In TRAFTACs, the designed oligo sequence is composed of TF-binding double-stranded DNA (dsDNA) linked with Cas9 clustered regularly interspaced short palindromic repeats (CRISPR)-binding RNA and then combined with the E3 ligase-recruiting moiety to form the PROTAC. The function of dsDNA is to bind the TF of interest (TOI), and the CRISPR-RNA binds to the fusion protein dCas9HT7, which is incubated with a small molecule, haloPROTAC. haloPROTAC recruits VHL E3 ligase, which triggers the degradation of TOIs. This new PROTAC technology provides a strategy different from previous studies but may depend on a reliable delivery system to confirm its practical effects.⁷⁹

Discussion

Although the development of the current TPD technologies provides potential solutions for “undruggable” tumor TF targets, some problems remain that need to be considered. Tumor TFs are intracellular and intranuclear, and the bulky size of PROTACs poses a problem with cell penetration compared with SMDs. Furthermore, drug resistance also occurs in mutant E3 ligases, which undoubtedly affects the druggability of TPD drugs.^{80,81} In addition, off-target toxicity,⁸² linker selection,⁶ and lack of highly efficient E3 ligases¹² are current problems in TPD drug development.

In summary, with the development of PROTAC drugs, an increasing number of tumor TFs, which were previously believed to be “undruggable” targets, are susceptible to targeted degradation methods in both research and clinical trials.

Declaration of Conflicting Interests


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