PROTAC -An Outstanding Promising Technology in Drug Development and Cancer Therapy: a Review

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Abstract

PROTAC (Proteolysis targeting chimera) is an evolving technique that is being presented with a lot of recognition for therapeutic intervention. It is a bifunctional molecule that utilizes targeted protein degeneration to eliminate certain proteins from inside the cell. This novel approach has the power to put forward an abundant progression in the area of drug discovery and development. Over the last few years, several studies have broadened our perception of the utility and performance of PROTAC molecules. These chimeric molecules are distinct from classical pharmacology in their ability to induce the breakdown of the protein of interest and not just its inhibition. Nonetheless, there are also challenges like restricted structural data, advancement of computational tools, etc. Despite challenges, it is anticipated that PROTACs will soon develop into a new therapeutic class of pharmaceuticals. As a summary of the PROTAC's history, uses, state right now, and bright future prospects are given in this review.

Keywords:*Androgen receptor (AR), Ubiquitin-Proteasome Systems(UPS), E3 ligase, Protein of Interest (POI), chemical linker.*

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1. Introduction:

Protein degradation by the Ubiquitin-Proteasome System (UPS) is an important mechanism in the cell to maintain the intracellular protein homeostasis (Nalawansha & Crews, 2020). The fusion of protein with ubiquitin, a small protein modifier, is necessary for balanced protein degradation by the 26s proteasome. Although the ATP-dependant pathway of protein degradation was interpreted during the end of 1970s, thirty years later its utility in targeted protein degradation was made available(Paiva & Crews, 2019).

The Proteolysis Targeting Chimera (PROTAC) was developed as a strategy to prompt the degradation of a given protein by taking over the UPS. PROTACs are bifunctional hybrid molecules that consist of an E3 ligase amalgamated to a protein of interest (POI) with the help of an intervening chemical linker. As soon as the E3-PROTAC-POI ternary complex is formed, the E3 brings about ubiquitination. Afterwards, the proteasome degrades the POI (Figure) (Ishida & Ciulli, 2020)(Nalawansha & Crews, 2020). Considering the PROTAC is not degenerated in this process, it can contribute to ubiquitination and degradation of numerous POI counterparts, thus working in a sub-stoichiometric fashion (Paiva & Crews, 2019). The mode of action of PROTACs is event-driven, and not occupancy-driven. Occupancy driven modalities are a trait of classical receptor pharmacology and they need elevated drug concentrations to give a significant result. Nevertheless, high drug concentrations are also associated with off-target effects. This can be decreased by drugs having favourable pharmacokinetic properties and high specificity. On the other hand, PROTAC displays a much better efficacy at sub stoichiometric levels (Konstantinidou, et al., 2019). Thus, PROTAC can be proved to be a beneficial technology.

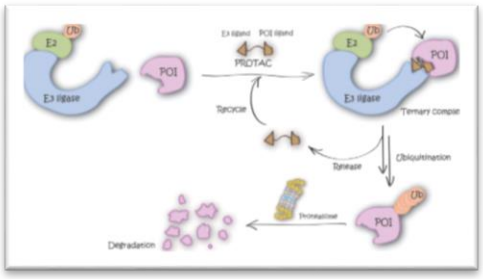

Figure1. An Illustration of a PROTAC's Degradation Process

(Ishida & Ciulli, 2020).

1.1 PROTAC's Precursor

In an effort to make alterations to the toxic effects of geldanamucin (a natural product benzoquinone ansamycin antibiotic) which is responsible for binding HSP90, a molecule chaperone for many proteins which includes estrogen receptor (ER), it was perceived by a number of groups that geldanamycin rapidly caused degradation of many proteins which include Raf-1, Brc-Abl, HER-2, IGFR1R, ER, p53 and mutated v-Src. So, linking geldanamycin to estradiol was a logical plan of action to decrease its toxicity so that ER

could be targeted specifically. Likewise, geldanamycin was contemplated to connect to testosterone in order to target androgen receptor (AR). Through these studies, the possible idea of a hybrid molecule being able to bring upon specific degradation of a protein of interest (POI) was put forward. On the other hand, efforts to use chimeric proteins from the SCF proteolytic machinery, which is a multimeric E3 ubiquitin ligase complex, were also made. Zhou et al (2000) designed the SCF E3 ubiquitin ligase complex by utilising a specific protein interaction domain to target pRb in human osteosarcoma SARS-2 as well as yeast cells. These attempts could be considered as the forerunner of PROTAC, which was subsequently evolved by Raymond J. Deshaires and Kathleen M. Sakamoto, in collaboration with Frank Mercurio, Craig M. Crews, and Kyungbo Kim in the year 2001 and 2003 (Zou, et al., 2018).

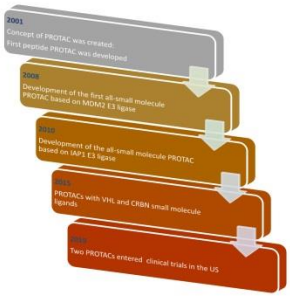

Figure 1. Timeline and the Major Milestones for the Development of PROTAC

2. Types of PROTACs:

2.1. VHL-based PROTACs:

Peptidic VHL PROTACs came into existence many years back but due to the lack of high affinity small molecule ligands to hijack the $CRL2^{VHL}$ complex, the evolution of such PROTACs in the form of therapeutic drugs has been restricted. PROTAC_ERRα is a VHL-based PROTAC which at a concentration of 100nM downregulates ERRα (estrogen-related receptor alpha) levels by 50%. ERRα is an orphan nuclear receptor which shows major activity in the management of cellular energy equilibrium by regulating the transcription of genes participating in fatty acid and glucose metabolism and mitochondrial biogenesis. Another VHL-based PROTAC is PROTAC_RIPK2 which targets the serine/threonine kinase RIPK2 for degradation. RIPK2 is responsible for activating the MAPK and NF-κB signalling pathways. It interacts with the bacterial sensors NOD1 and NOD2 and plays a critical role in the innate immune response. Another two VHL-based PROTACs are MZ1 and MZ2.These target BRD4 for

degradation with variable lengths of linkers. A VHL-based PROTAC which was recently developed is Compound 3iwhich targets another serine/threonine kinase, TANK-binding kinase 1 (TBK1). It has implications in tumorigenesis, innate immune response and development(Gu, et al., 2018) (Li & Song, 2020).

2.2. MDM2-based PROTACs:

MDM2 is the most important E3 ligase which targets tumor suppressor p53 and performs polyubiquitination and degradation. The first all-small-molecule PROTAC to be synthesized is a hybrid bi-functional molecule which is composed of a non-steroidal androgen receptor ligand (SARM) and a Nutlin, connected by a PEG-based linker. Androgen receptor belongs to the steroid nuclear receptor family. Nutlins, a class of potent, are selective small molecule antagonists of MDM2. The Nutlin regulates the stability and transcriptional activity of p53 by binding to the p53-binding pocket of MDM2. It disrupts the interaction of MDM2 with p53 but it doesn't affect the E3 ligase activity of MDM2 (Gu, et al., 2018).

2.3. cIAP1-based PROTACs:

The cIAP1 based PROTAC is capable of successfully degrading the intracellular CRABP-I and CRABP-II proteins via the UPS pathway. CRABP-I and CRABP-II are intracellular retinoic acid-binding proteins. This PROTAC has better permeability and stability in cells and thus it has been found to be beneficial in the inhibition of the migration of neuroblastoma IMR-32 (Gu, et al., 2018).

2.4. CRBN-based PROTACs:

$CRL4^{CRBN}$ is an E3 ubiquitin ligase complex which consists of cereblon (CRBN), damaged DNA-binding protein 1 (DDB1), cullin-4A/B, and regulator of cullins 1 (RBX1). It participates in the breakdown of numerous substrates in numerous biological processes (Gu, et al., 2018).

3. Current Status of PROTAC Technology:

A ligand that binds to the target protein, a ligand that binds to E3 ubiquitin ligase and a linker that binds these two ligands together make up a hetero bi-functional small molecule known as proteolysis targeting chimera. A chemical knockdown method called PROTAC uses the ubiquitin proteasome machinery to break down target proteins. Unlike traditional inhibitor-based, competitive, occupancy-based processes, PROTAC is catalytic in its mechanism of action and may accelerate the degradation of target proteins at low levels of exposure. PROTAC may degrade pathogen-targeted proteins and regulate associated signalling pathways. As a new approach, PROTAC has received a lot of attention not only in academia, but also in the pharmaceutical and biotechnology industries such as Kymera Therapeutics and AstraZeneca(Gao et al., 2020).

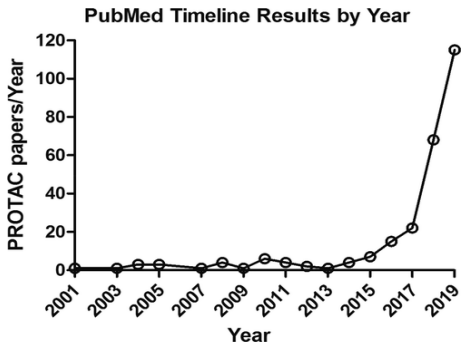

Figure 3. Graph representing number of research papers on PROTAC trending through years showing increase in activities and research on PROTAC.

(Zou et al., 2018).

Between 2001 and 2018, more than 30 review articles and 80 research papers were published, according to PubMed (Zou et al., 2018).

Currently PROTACs had been correctly hired within side the degradation of various kinds of goal proteins associated with diverse diseases, which include viral infection, cancer, neurodegenerative diseases, and immune disorders. Certain instances mentioned consist of PROTACs concentrated on B-mobileular lymphoma from AstraZeneca, P300/CBP-related issue in addition to well-known manipulate nonderepressible from GlaxoSmithKline, Bruton's tyrosine kinase from Pfizer and Interleukin-1 receptor-related kinase from GSK. Furthermore, obstruction due of PROTACs becomes illustrated through researchers from Abbvie, and Promega mentioned the quantifiable live-mobileular kinematic degradation and systematic outlining (Gao et al., 2020).

A few of the highlighted advances successfully made in the field of academia and industry with the help of PROTAC are:

- **Overcoming drug resistance for cancer -** In addition to conventional chemotherapy, kinase inhibitors have advanced quickly over the past 20 years. Although they are quite effective at treating cancer, people frequently develop medication resistance and the disease subsequently returns (Gao et al., 2020).
- **Elimination of enzymatic and non-enzymatic function of kinase -**Traditional small molecule inhibitors typically block the enzymatic activity of the target, however PROTAC affects both the enzymatic and non-enzymatic activities of the protein by degrading the whole protein. PROTAC can regulate proteins that are difficult to control with conventional small molecule inhibitors and expand the drug-effective space of already existing targets(Gao et al., 2020).
- **Degradation of proteins that could not be targeted pharmacologically -**The majority of known protein targets, such as kinases, G protein-coupled receptors, nuclear hormone receptors, and iron channels, can currently be targeted by standard drug discovery techniques in roughly 20–25% of cases. Despite having catalytically inactive or independent activity, proteins are nonetheless referred to be "incurable" targets. Because it participates in numerous signal transductions, the signal

transduction and transcriptional activator is a desirable therapeutic target. The creation of STAT3 inhibitors is constrained by the lack of obvious pharmacological locations on the surface of STAT3(Gao et al., 2020).

PROTAC technology has a variety of challenges, some of which are outlined below, despite its bright potential in drug discovery:

- Until date, only one example of PROTAC has been reported for a "undruggable" target; more examples are required in the future to demonstrate the benefits of PROTAC in "undruggable" targets. In nature, "molecular glue" reflects the mechanism of stable protein connections via small molecule modulators of E3 ligases (Gao et al., 2020).
- Finding quick and effective ways to screen for target protein ligands that can be used in PROTACs, especially those that target protein-protein interactions, is another issue. It is unclear how to logically assemble PROTAC, and there are still questions regarding the degradative activity, selectivity, and potential off-target consequences (based on a variety of targets, cell lines, and animal models). The human genome contains the genetic code for more than 600 E3s ubiquitin ligases. But hardly any E3 Ligase is used in the creation of PROTAC (Gao et al., 2020).

4. Peptide Based PROTAC Technology:

From earlier experiments, the group of Kathlene M. Sakamoto decided that MetAP-2 was incharge SCF complex. This result was working to design chimeric spies to mark E stragon Receptor (ER) and Androgen Receptor (AR). They combined a 10-amino acid IκB-α peptide covalently accountable estradiol (E2) or dihydrotestosterone (DHT) besides habitual that two
together hybrid fragments function intracellularly artificial and in vivo. The experts proposed to start a poly-arginine container-harsh peptide to create PROTAC container-permeable. They even determined designative results that homo peptide-located PROTAC ruined X-proteins in HepG2 containers. Another group of scientists created PROTACs accompanying FKBP12 ligand and dihydrotestosterone for target FKBP12 and Androgen Receptor in a container model. At the same time, a various group of chemists devised a PROTAC established a peptide from HIF-1, restrain report, the interaction method middle from two points pVHL and HIF 1 (hypoxia inducible determinant 1). They synthesised estradiol HIF 1 octapeptide (Met Leu Ala ProOH Tyr Ile Pro Met) to favorably mark ER in living containers, and confirmed that the synthesised PROTAC guide Estrogen Receptor and was smart to prevent the distinction of endothelial containers in a three spatial angiogenic sprouting assay, as well (Zou et al., 2018).

Simultaneously, the first group of chemists follow seven amino acids from HIF1 that makes VHL, proposed to overcome the impediment of membrane permeability utilizing a poly D arginine tag arisen HIV TAT used to meld to the carboxyl end of the peptide to admit the mixture macromolecule to award cell permeability and hamper slack proteolysis. In addition to the use of E3 ubiquitin ligase by way of ubiquitin depravity by the proteasome, chaperone-intervened autophagy apiece combining of pentapeptides link two various HSP70 binding logos straightforwardly to the mutant Huntington protein that breaks below. After that, the fans reliable to lease a complete peptide autophagy structure for proteolysis. Their design contained pentapeptide CMA address a pattern that admits

the autophagy plan, a linker holding cells transmembrane rule and acknowledgment peptides of the aim protein. Obviously, these primary PROTAC sciences were based on the short peptide series to identify an E3 ubiquitin ligase (Zou et al., 2018).

5. Small Molecule based PROTAC:

The MDM2 ligand nutlin is a group of imidazoline derivatives that bind to MDM2 and prevent it from interacting with p53. They then used the inhibitor vandetanib and the hydroxyproline moiety with a 12 atom linker to create a PROTAC against the serine threonine kinase RIPK2. ARV-825 is a PROTAC that binds a BRD4 binding moiety of the triazolo diazepine acetamide family to pomalidomide, a cereblon binding moiety with a flexible polyethyleneglycol linker, to activate the E3 ubiquitin ligase cereblon. Pomalidomide is another tiny chemical that can be utilised to activate the ubiquitin E3 ligase employed in the PROTAC design.

Winter et al. employed phthalimide as a radical to hijack the cereblon E3 ubiquitin ligase and destroy BET family members almost simultaneously. PROTAC was created to work both in vitro and in vivo in a leukaemia model. A PROTAC against the oncogenic BCR ABL kinase has been created using 48 small molecule PROTACs (Zou et al., 2018).

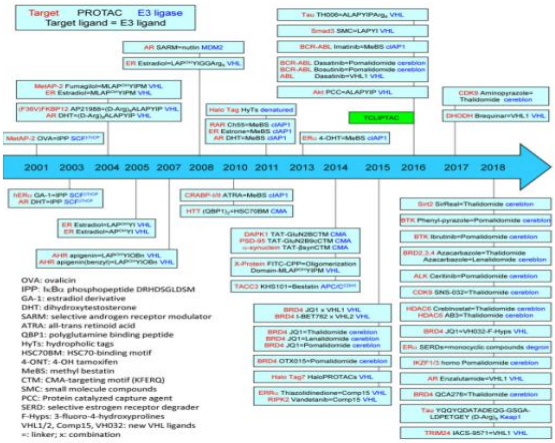

Figure 4. A Schematic Illustration of the Molecular Design for Proteolytic-Targeted Chimaeras (PROTAC) – (Ubiquitin Ligase is Coloured Blue, and Target Protein is Coloured Red).

(Zou et al., 2018).

6. PROTAC-Mediated Degradation of Epigenetic Erasers:

PROTACs have been developed to degrade nuclear proteins like histone deacetylases.SirReal3b, a strong Sirt2 inhibitor, was added to thalidomide via a triazole-based linker in order to target Sirtuin2 (Sirt2), a NAD+-dependent class III HDAC, for degradation. This produced a Sirt2-selective PROTAC with micromolar activity in HeLa cells. While promoting Sirt2 degradation in a CRBN- and proteasome-dependent manner and inducing downstream -tubulin hyperacetylation, this PROTAC kept its inhibitory efficacy against Sirt2. A PROTAC that included pomalidomide as its E3RE and an HDAC6 inhibitor based on hydroxamic acid was used to degrade HDAC6, a class IIB Zn

$^{2+}$-dependent HDAC. Degradation may aid in defining the non-catalytic activities of HDACs in the development and progression of disease because the majority of HDAC inhibitors bind the active site and/or chelate Zn^{2+} (Paiva et al.,2019).

7. Challenging Localization, Affinity, and Resistance Mechanisms of PROTAC:

ARCC-4 is an enzalutamide-based PROTAC causing androgen receptor (AR) degradation in VCaP cells (Paiva et al., 2019). It was demonstrated on membrane bound receptor tyrosine kinase (RTKs) that PROTAC mediated degradation is better than inhibition. (Paiva et al., 2019).FDA authorized 47 kinase inhibitors as a significant therapeutic target group (Paiva et al., 2019). Some inhibitors were effective recruiting elements for PROTACs that degrade serine/threonine and tyrosine kinases (Paiva et al.,2019). Nonetheless, kinome rewiring is frequently observed as a resistance mechanism. Degradation can circumvent resistance mechanisms, as when targeting other POIs (e.g. AR), (Paiva et al.,2019), (Gao et al.,2020). By incorporating RTK inhibitors into the VHL-recruiting E3RE, PROTACs that destroy membrane-bound WT EGFR as well as EGFR mutants that are disease-relevant were produced (Gao et al.,2020). Furthermore, longer-lasting reduction of RTK-downstream signalling was seen, in contrast to the kinome reconfiguration that results from inhibition alone, highlighting the scope of PROTAC target space and the benefits of degradation over inhibition, respectively (Paiva et al., 2019).Promiscuous kinase ligands have been used in PROTACs, which has offered insight into the foundation for PROTAC-mediated POI selectivity (Paiva et al.,2019), (Gao et al.,2020). Depending on the E3RE utilised, non-overlapping degradation trends were discovered using foretinib, a kinase inhibitor that binds over 130 kinases at 10 M. (i.e., VHL vs CRBN) (Paiva et al., 2019). Additionally, degradation was seen even with some weak-binding kinases, such p38, probably as a result of p38 and VHL's positive cooperation in the ternary complex through protein-protein interactions (PPIs) (Paiva et al.,2019). In comparison to the typical VHL E3RE, the affinity of PROTACs for VHL ligands decreased, suggesting that POI and/or E3 engagement alone do not dictate a PROTAC's efficacy (Paiva et al., 2019),(Gao et al.,2020).

8. Targeting Different Proteins for Anticancer Drug Development

Targeting Nuclear Receptors

Androgens have been under study for therapeutic efficacy in Prostate Cancer for several decades but not long ago, numerous AR (Androgen Receptor) PROTACs have been designed as SARDs (Selective AR Degraders). This study has been found to be of great importance by the prostate cancer community. The first AR PROTAC which went into clinical trials is ARV-110 (ClinicalTrials.gov: NCT03888612xlviii) (Nieto-Jiménez, et al., 2022). It is currently in a Phase I/II clinical trial and is found to be useful in patients suffering from metastatic CRPC. Several other AR PROTACs is also coming into view. To give an example, ARD-61, a newly developed AR PROTAC can bring upon on-target AR degradation and also shows antiproliferative and proapoptotic effects in prostate cancer cells (Yang, et al., 2020).

8.1. PROTACs Targeting Bromodomain-Containing Protein 4 (BRD4):

Many researches have been carried out to utilize PROTACs for the degradation of BRD4 to treat cancers. Not long ago, BRD4-targeted PROTACs mainly designed based on thalidomide derivatives were reported. They were found to be small ligands of the cereblon (CRBN), which is a component of the $CRL4^{CRBN}$ E3 ligases, and VH-032and VHL-2, two high-affinity ligands of $CRL2^{VHL}$ E3 complex (Duan, et al., 2018).

8.2. PROTACs Targeting Bruton's Tyrosine Kinase (BTK):

BTK is a non-receptor tyrosine kinase that is expressed mostly in hematopoietic cells and plays an important function. Its function in B cells is to promote development, differentiation, and signal transduction. Through B cell receptor signalling, BTK is linked to the survival and proliferation of B cell neoplasms. BTK is translocated from the cytosol to the plasma membrane in response to BCR-triggered antigen stimulation. BTK is phosphorylated and so activated. BTK activates a variety of pro-survival pathways, including AKT, ERK, and NFB. Gray and colleagues created the first BTK target PROTAC D04015, which is made up of pomalidomide and the BTK ligand RN486. It destroys BTK effectively and selectively and suppresses B-cell lymphoma TMD8 cells (Li et al., 2020).

8.3. PROTACs Targeting BCR-ABL:

The fusion oncoprotein BCR-ABL, which is the result of chromosomal translocation t, is a constitutively active tyrosine kinase whose activation contributes to the development of chronic myeloid leukaemia. Small molecule BCR-ABL inhibitors, such as imatinib and dasatinib, are effectively used to treat cancer. BCR-ABL mutations, on the other hand, can result in medication resistance and treatment failure. PROTAC was created to combat medication resistance or as an alternate therapy. Using pomalidomide and dasatinib, the Crews group created the first BCRABL that targeted PROTAC DAS6226CRBN. It showed one highly effective in degrading BCRABL and inhibiting the growth of CMLK562 cells. GMB475 contains BCRABL inhibitor GNF5 may induce BCRABL degradation in CML K562 cells. Compared to imatinib, the antiproliferative activity of GMB475 was less affected cells with imatinib-resistant BCRABL mutants. In addition, GMB475 induced the degradation of BCRABL sub-micromolar concentration of primary CML patient cells(Li et al., 2020).

8.4. PROTACs Targeting MCL1:

MCL1 is a member of the family of pro-survival proteins known as B-cell lymphoma 2 (BCL2), and is targeted by PROTACs. It interacts with proteins having BH3 domains, such as Bad, Noxa, Bak, Bim, and Bcl-2 associated protein X (Bax), thanks to its three BH domains. When MCL1 interacts with these proteins, Bak/Bax conformational activation is suppressed, and the caspase cascade that results in cell death is avoided. It is now known that MCL1 overexpression is a critical component of lymphoma, leukaemia, and multiple myeloma survival. MCL1 degradation therefore presents a novel therapeutic approach for these cancers. The first MCL1-targeting PROTAC dMCL1-2 consists of thalidomide and the MCL1 inhibitor A-1210477, which can degrade MCL1 in multiple myeloma OPM2 cells at nM doses (Li et al., 2020).

8.5. PROTACs Targeting FMS-like Tyrosine Kinase 3 (FLT-3):

The early stages of hematopoiesis are regulated by the tyrosine kinase receptor FLT-3. Common FLT-3 mutations in acute myeloid leukaemia (AML) activate FLT-3 constitutively and downstream signalling pathways include STATs, RAS, MAPKs, and PI3K/AKT. These events suppress apoptosis and differentiation. Pomalidomide and the FLT-3 inhibitor quizartinib were used in the development of the first FLT-3-targeting PROTACs, TL13-117 and TL13-149, by Gray and colleagues. These probes reduced cellular FLT-3 levels in FLT3 ITD-mutant leukaemia MOLM-14 cells. However, they showed less efficiency against leukaemia cell proliferation than their parent inhibitor. It is more efficient to use a FLT-3 PROTAC that contains VHL1 and quizartinib (Li et al., 2020).

8.6. RNA Targeted PROTAC:

In 2018, Matthew D. Disney expanded PROTAC's sphere of influence to encompass RNA. The ribonucleases (RNase) L and MicroRNA (miR)-96 hairpin precursor were the targets for recruitment. They combined a tiny chemical that targets miR-96 with a 2′-5′A4 oligonucleotide, an RNase L ligand, to develop ribonuclease targeting chimaeras (RIBOTACs) 34. RIBOTAC 34 was capable of catalytic and sub stoichiometric cleavage of the target RNA. In addition, the RIBOTAC was able to induce apoptosis in MDA-MB-231 cells and increase the amount of FOXO1, which is regulated by the microRNA -96, without harming healthy cells. This is the first instance where bifunctional molecules have been utilised to target RNA, proving that they can do so as well (Zhou et al.,2020).

8.7. PROTACs Targeting STAT3:

STAT3 is a factor that mediates the passage of transcriptional signal from a cell surface receptor to the nucleus (Li et al.,2020). Recently, Bai and associates revealed the development of the tiny drug PROTAC, which specifically targets STAT3. This PROTAC, known as SD-36, caused complete tumour regression in AML and ALCL animal xenograft models and destroyed STAT3 protein potently and selectively in AML and ALCL cells. (Li et al.,2020), (Shiah et al.,2021). The chemical SI-109, a STAT3 SH2 domain inhibitor with a high affinity for STAT3 (Ki = 14 9 nmol/L), served as the warhead for SD-36. To make the SD-36 PROTAC, SI-109 was joined to a lenalidomide analogue with a six-carbon ligand that attracts the CRBN E3 ligase. In addition, the lenalidomide analogue moiety was changed to form SD-36Me, a compound that served as a negative control because it was unable to attract CRBN. It appears that SD-36 can break down STAT3 when it is activated by non-canonical pathways that do not require phosphorylation of Y705 in any way. Since PROTACs effectively lower the total amount of cellular STAT3 protein, it is increasingly clear from a variety of sources that these additional, noncanonical pathways work in concert with canonical STAT3 signalling to support tumour growth. Given the significance of STAT3 in the emergence of both solid and hematologic malignancies, it is anticipated that in the near future, major effort will be required to develop and enhance novel STAT3-targeting PROTAC drugs. (Shiah et al.,2021).

8.8. PROTACs Targeting Brg/Brahma-Associated Factors(BAF complex):

The BAF complex, which belongs to the ATP dependent chromatin remodelling complex family, is involved in gene expression and differentiation regulation, up to 15 component proteins make up this protein complex, several of which can be substituted by paralogs. The BAF complex is formed in leukaemia around the Brg ATPase, which is required for leukaemia progression, Inactivation or knockdown of the Brg ATPase has therapeutic benefits in AML. The logical and efficient optimization of ACBI1 is a powerful and coordinated decomposition agent of SMARCA2, SMARCA4, and PBRM1 was guided by high resolution ternary composite crystal structure and biophysical studies. (Farnaby et al.,2019), (Li et al.,2020).

8.9. Targeting Transcriptional Regulators BET Family Proteins:

It is the second-leading cause of cancer-related death among men worldwide, particularly in the US. Although castration-resistant prostate cancer (CRPC) is still dependent on androgen receptor signalling, which has been related to a poor prognosis, androgen deprivation may lead to the cure of this illness. Growth inhibition by BET family proteins has been shown in CRPC preclinical models. They demonstrated that BET inhibition is much less effective than ARV-771, a small-molecule pan-BET degrader based on PROTAC technology, in CRPC cells models. In contrast to BET inhibitors, ARV-771 suppresses both AR signalling and AR levels to produce tumour regression in a CRPC mice xenograft model (Raina et al.,2016).

BRD4 inhibitor OTX015 inhibits the E3 ligase cereblon binding moiety pomalidominde. According to the authors, ARV825 enhanced the rapid, effective, and long-lasting degradation of BRD4 in all cell lines examined. Further research was done on ARV825's potential to increase apoptosis in CD34+ post-MPN sAML cells. Treatment with ARV825 caused significant and long-lasting decreases in the levels of BRD4 downstream genes like cMyc, CDK4/6, JAK2, pSTAT3/5, PIM1, and BclxL, but greater increases in the levels of p21 and p27. Surprisingly, in addition to the quick, efficient, and prolonged degradation of the BET family, the PROTAC produced alterations in MYC, p21, and AREG downstream of BRD4. Because BET proteins are necessary for the generation of NF-kB activated genes, PROTACs targeting BET proteins reduced the proinflammatory response in microglia after Lipopolysaccharide exposure. When compared to BET inhibition, Raina and colleagues demonstrated that ARV771, a small molecule-based PROTAC that uses panBET inhibitors, greatly boosted efficacy in CRPC cellular models. Recent research has looked into the effects of PROTACs built on BETi, ARV825, and ARV771 in MCL cells (Zhou et al.,2018).

The results showed that BET PROTACs induced more apoptosis in MCL cells than BETi. In MCL cell engrafted nude mice, ARV771 therapy markedly reduced tumour growth and improved survival when compared to OTX015. Finally, those researchers discovered that there was a synergistic effect on MCL cell death when ARV771 was combined with other drugs like ibrutinib, venetoclax, and palbociclib. Later research confirmed Qin's discovery that QCA570 is a potent PROTAC against BET proteins (Zhou et al.,2018).

The development of a new PROTAC by Zhou and colleagues against BET family proteins led to the creation of an intriguing chemical that can degrade BRD4 at a concentration of 30 pM. In 2018, Chong Qin developed a PROTAC using oxazepines, a novel class of

BET inhibitors. According to the researchers, PROTACs decreased the expression of cMYC and NMYC, two downstream genes of BRD4. They showed that bortezomib, dexamethasone, lenalidomide, and pomalidomide drug resistance may be overcome by PROTACs. Finally, they showed that PROTACs might rapidly reduce the viability of patient-derived primary cells from myeloma and prevent the development of MM1.PROTACs with over 100 times the activity of a typical one was produced by altering the hydroxylation of proline, which may also improve PROTACs against BRD4. (Zhou et al.,2018).

9. PROTAC Efficacy Determination:

The development of novel drugs and chemical knockdown tools has begun a new chapter thanks to PROTACs, which have unmatched potential for both business and academia as can be observed in the following ways: overcoming drug resistance in cancer patients, eliminating the enzymatic and nonenzymatic activities of kinase. Destroy the protein target that is "undruggable." In contrast to conventional inhibitor medications, PROTACs do not require a deep hydrophobic binding pocket or active site. This makes it possible to develop novel ligands that can bind to various regions of the target protein.

In vivo, a fast and reversible chemical knockdown strategy: Furthermore, typical genetic methods cannot be employed to investigate the function of embryonic-lethal genes in vivo. The dTAG technology may now be used to examine and validate the biological ramifications of PROTAC-mediated POI degradation without the necessity for POI ligand production by tracking the degradation of $FKBP^{F36V}$ POI fusion proteins. This method has been utilised to: selectively induce POI degradation that distinguishes the target from similar isoforms; validate preclinical treatment targets; and validate previously discovered disease progression targets. (Paiva et al.,2019).

Using CRBN-recruiting and VHL-recruiting PROTACs, this innovative method can track the formation, ubiquitination, and degradation of HiBiT-BRD2, BRD3, and BRD4 ternary complexes. In contrast to ternary complex formation rate and stability, there was a direct association between degradation rates and ubiquitination rates. (Paiva et al.,2019).

10. PROTACs in clinical trials:

ARV-110 and ARV-471, two PROTAC probes made by Arvinas LLC, are presently undergoing phase I clinical studies for prostate and breast cancer, respectively (NCT03888612 and NCT04072952 on clinicaltrials.gov). In a castrated mouse model of VCaP prostate cancer, treatment with ARV-110 at 1 mg/kg, p.o., once daily for three days led to AR degradation of more than 90% at 16 hours. ARV-471-targeting ER has been shown to effectively degrade both clinically significant ER mutants and wild-type ER in a number of ER-positive breast cancer cell lines with DC50 values under 2 nM. In MCF7 breast cancer mice models, treatment with ARV-471 at a dose as low as 3 mg/kg, p.o., daily led to tumour regression and > 90% ER decrease in tumour tissues. In PDX models of hormone independent breast cancer with ER mutations, treatment with ARV-471 at a dose of 10 mg/kg completely prevented tumour formation and significantly reduced mutant ER levels.(Song et al.,2020).

11. Future of PROTAC Technology:

The documented changes in POI degradation depending on which E3 ligase is employed, the researcher should have looked into the 'PROTACability' of multiple E3 ligases in addition to diverse protein types. Only a small percentage of the putative E3s in the human proteome—primarily those in the RING family—have been effectively hijacked. Without going through the bother of optimising E3RE affinity, different E3s might be targeted, potentially in a tissue-specific manner, because attenuating E3 ligase affinity with minor changes to the E3RE has little impact on degradation efficiency. The identification of new E3 modulators may be facilitated by methods for categorising and blocking active E2/E3 pairs. For instance, the GPCR-driven activation of c-Src kinase activity is what drives the activity of NEDD4-2, a HECT-type E3 ligase. In addition to figuring out the kinetics of the fundamental events needed for an effective PROTAC, researchers should also look into what occurs between ubiquitination and destruction. Should the PROTAC design further characterise this function? Should this role be better characterised in PROTAC design? For instance, p97 prepares protein transport from E3 ligase machinery to the 26S proteasome, which includes CRBN neosubstrates. (Paiva et al.,2019).

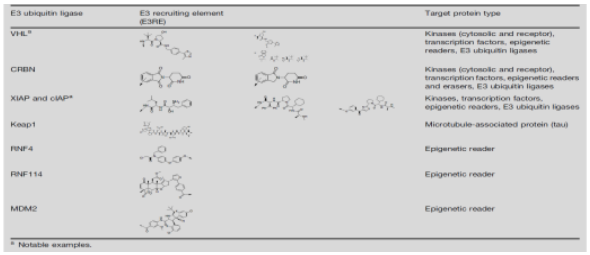

Figure 5. Examples of E3 recruiting Components and Associated E3 Ubiquitin Ligases Used Recently for PROTAC-Development. Vectors for Linker Attachmentin PROTAC Synthesis are Represented by Dashed Arrows.
(Paiva et al.,2019).

Conclusion:

Additionally, macrocyclization enables a PROTAC molecule to be constrained in its bioactive conformation, biassing it to adopt or discriminate against a desired ternary complex, which the researchers showed can help degradation potency and selectivity among homologous targets in macrocyclization PROTAC design strategies. (Testa et al.,2020). The molecule Fak, which also functions as a kinase and a scaffold for numerous signalling proteins simultaneously, is an important player in tumour invasion and metastasis. (Cromm et al, 2018). Numerous investigations have revealed that PROTAC-3, a clinical contender and selective and effective Fak degrader, outperforms defactinib in terms of activating Fak and preventing Fak-mediated cell migration and invasion. (Cromm et al, 2018). PROTACs from the BET family, which have shown to be more successful than BET inhibitors and have significant potential for the treatment of a range of disorders, including cancer and inflammation, are also known from numerous studies. (Riching et al.,2018). These results demonstrate the potential of PROTACs to increase the druggable area and modulate protein functions that are challenging to regulate with conventional small molecule treatments (Riching et al.,2018).

References:

[1] Cromm, Philipp M., et al. "Addressing kinase-independent functions of Fak via PROTAC-mediated degradation." *Journal of the American Chemical Society* 140.49 (2018): 17019-17026. doi : 10.1021/jacs.8b08008.PMID: 30444612.

[2] Duan, Y., Guan, Y., Qin, W., Zhai, X., Yu, B., & Liu, H. (2018). Targeting Brd4 for Cancer therapy: Inhibitors and Degraders. *MedChemComm*. doi:10.1039/C8MD00198G. PMID: 30542529;PMCID: PMC6238758.

[3]Farnaby, William, et al. "BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design." *Nature chemical biology* 15.7 (2019): 672-680. doi: https://doi.org/10.1038/s41589-019-0294-6.PMID: 31178587;PMCID: PMC6600871.

[4]Gao, H., Sun, X., & Rao, Y. (2020). PROTAC Technology: Opportunities and Challenges. *ACS Medicinal Chemistry Letters*, *11*(3), 237–240. doi:https://doi.org/10.1021/acsmedchemlett.9b00597. PMID: 32184950**;**PMCID: PMC7073876 .

[5] Gu, S., Cui, D., Chen, X., Xiong, X., & Zhao, Y. (2018). PROTACs: An Emerging Targeting Technique for Protein Degradation in Drug Discovery. *BioEssays*. doi:10.1002/bies.201700247.PMID: 29473971.

[6]Ishida, T., &Ciulli, A. (2020). E3 Ligase Ligands for PROTACs: How They Were Found and How to Discover New Ones. *Sage*. doi:https://doi.org/10.1177/2472555220965528. PMID: 33143537 **;**PMCID: PMC8013866.

[7]Konstantinidou, M., Li, J., Zhang, B., Wang, Z., Shaabani, S., Brake, F. T., . . . Dömling, A. (2019). PROTACs– a game-changing technology. *Expert Opinion on Drug Discovery*. doi:10.1080/17460441.2019.1659242. PMID: 31538491; PMCID: PMC7008130.

[8] Li, X., & Song, Y. (2020). Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *Journal of Hematology& Oncology*. doi:https://doi.org/10.1186/s13045-020-00885-3. PMID**:** 32404196**;** PMCID: PMC7218526

[9] Liu, Jing, et al. "Light-induced control of protein destruction by opto-PROTAC." *Science advances* 6.8 (2020): eaay5154. doi: 10.1126/sciadv.aay5154.PMID: 32128407**;**PMCID: PMC7034987 .

[10] Nieto-Jiménez, C., Morafraile, E. C., Alonso-Moreno, C., &Ocaña, A. (2022). Clinical considerations for the design of PROTACs in cancer. *Molecular Cancer*. doi: https://doi.org/10.1186/s12943-022-01535-7. PMID:3524;PMCID: PMC8900451.

[11] Nalawansha, D. A., & Crews, C. M. (2020). PROTACs: An Emerging Therapeutic. *Cell Chemical Biology*. Retrieved from https://doi.org/10.1016/j.chembiol.2020.07.020.PMID: 32404196;PMCID: PMC7218526.

[12] Paiva, S.-L., & Crews, C. M. (2019). Targeted protein degradation: elements of PROTAC. (P. J. Hergenrother, & Y. Aye, Eds.) *Current Opinion in Chemical Biology, Elsevier Ltd.*(50), 111-119. doi:https://doi.org/10.1016/j.cbpa.2019.02.022. PMID: 32795419.

[13]Raina, Kanak, et al. "PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer." *Proceedings of the National Academy of Sciences* 113.26 (2016): 7124-7129. doi: www.pnas.org/cgi/doi/10.1073/pnas.1521738113.PMID: 27274052;PMCID: PMC4932933.

[14]Yang, Z., Gimple, R. C., Zhou, N., Zhao, L., Gustafsson, J.-A., & Zhou, S. (2020). Targeting Nuclear Receptors for Cancer Therapy: Premises, Promises, and Challenges. *CellPress : Trends in Cancer*.doi: https://doi.org/10.1016/j.trecan.2020.11.007. PMID: 33341430.

[15] Zhou, Xin, et al. "PROTAC: A promising technology for cancer treatment." *European journal of medicinal chemistry* 203 (2020): 112539. doi:https://doi.org/10.1016/j.ejmech.2020.112539.PMID**:** 32698111**.**

[16] Zou, Y., Danhui, M., &Yinyin, W. (2018). The PROTAC technology in drug development. *Wiley Cell Biochemistry & Function.* doi:10.1002/cbf.3369. PMID**:** 30604499;PMCID: PMC6590639.