Unlocking Cellular Control: The Promise of PROTACs in Disease Intervention

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Abstract: The discovery of proteolysis-targeting chimeras (PROTACs)[6] is among the most exciting and promising avenues in cancer therapy. [14] These fascinating compounds signify a paradigm shift from traditional approaches to medication development, offering a new idea that leverages the complexities of biological mechanisms to accomplish highly focused degradation of particular proteins implicated in pathological processes.[16] This novel strategy has the potential to address a number of drawbacks with conventional therapy techniques, such as the development of drug resistance and unexpected adverse effects resulting from interactions that are not intended. [14] The fundamental attraction of PROTACs is their distinct mode of action, which is based on controlling the cell's own machinery for protein degradation.[11] This orchestrated degradation translates to a substantial reduction in the levels of disease-driving proteins, often leading to the disruption of critical pathways involved in cancer growth and progression.[9]

The in-depth principles underlying PROTAC technology are thoroughly explored in this review study, which also provides insight into the complex chemical mechanisms that enable these chimeric molecules to specifically degrade certain proteins while leaving others intact. Showcasing the potential of PROTACs as a revolutionary force in targeted cancer therapy, and focusing on its application in prostate and breast cancer especially, the article draws from a comprehensive compilation of preclinical and clinical studies, advancements, and breakthroughs in the field. [10]

The methods used to create and refine PROTACs for various cancer types will be examined throughout the review, along with the subtleties of the ligand and linker choices that are crucial to their effectiveness and selectivity.[6] The difficulties and possibilities of transferring this ground-breaking technology from the lab to clinical practice will also be thoroughly examined, with an emphasis on issues like bioavailability, administration strategies, and potential resistance mechanisms.[9]

Through the integration of perspectives from various studies, the objective is to present a thorough but succinct review of the state of ongoing PROTAC research, emphasizing both, noteworthy advancements and the important issues that still need to be resolved. In the end, our investigation into PROTACs aims to shed light on how they can change the face of cancer therapy by providing a preview of a day when targeted protein degradation of disease-causing proteins would lead the way in novel therapeutic approaches.[9]

Keywords: PROTAC, Ubiquitin-Proteasome System(UPS) E3 ubiquitin ligase, Ubiquitination, Targeted Protein Degradation(TPD) Proteolysis, Bioceramic Nanocarriers, Small molecule inhibitors ARV-771, Pan-BET degrader BET inhibition, Docetaxel Cabazitaxel, AR-mediated Transcription Thienotriazolodiazepine Birabresib, HER2, Vepdegestrant, ARV-471, Selective Estrogen Receptor Degraders (SERDs) Fulvestrant, Drug Delivery

Introduction

Millions of individuals worldwide are impacted by the terrible disease known as cancer, which not only has a detrimental effect on health but also shortens life expectancy and causes death. Thus, there is constant research and development going on in the global health care system to find new medicines and treatments or enhance those

that already exist. In the field of cancer treatment, numerous medicines and interventions have been created to tackle this intricate and varied illness. Surgical intervention continues to be a fundamental treatment option for localized tumor eradication; yet, its accessibility, invasiveness, and potential consequences can present obstacles. Chemotherapy works well for fast dividing cells, but because it damages healthy cells as well, it frequently has severe side effects such nausea, hair loss, and lowered immunity. Radiation therapy can target malignant tumors precisely, but it can additionally erroneously harm nearby normal cells, which can have adverse effects over time.[5] By employing the body's immune system, immunotherapy exhibits potential; nevertheless, patient efficacy varies greatly, and immune-related side effects are cause for concern. Specific molecular pathways are addressed by targeted therapy; however, resistance may eventually arise. Hormone-driven malignancies can be efficiently treated with hormone therapy, although only some subtypes can benefit from this treatment. Gene therapy is a promising field that needs further research to determine its long-term safety and effectiveness. In order to make the best therapeutic decisions, we must carefully analyze the benefits and drawbacks of each of these therapy options while taking the patient's features, cancer kind, and stage into account. In order to combat this disease, a treatment where the benefits greatly exceed the negatives and address the shortcomings of the already available and researched therapeutic techniques should be considered.[2][25]

Mechanism

PROTAC technology, at its core, represents a fundamental break from standard drug development methodologies. It takes advantage of the complexities of the animal cell's very own degradation machinery, particularly the ubiquitin-proteasome system(UPS), to achieve highly specific and favorable degradation of target proteins of interest implicated in disease processes, particularly in cancer therapy. [9] A PROTAC molecule is made up of namely three components: a target POI, an appropriate ligand for an ubiquitin ligase (the enzyme that attaches ubiquitin molecules to proteins), and a linker unit that binds the two ligands. These factors work together to speed up the degradation process. A PROTAC-mediated protein degradation event begins with the PROTAC molecule attaching to its target protein.[4] This binding happens concurrently with the PROTAC's interaction with an E3 ubiquitin ligase. The complex formed by the targeted protein, PROTAC molecule, and E3 ubiquitin ligase is critical to the process. Within this ternary complex, proximity plays a central role. Due to PROTAC binding, the E3 ubiquitin ligase is brought close to the target protein and assists in the transfer of ubiquitin(U1,U2) molecules to specific amino acid residues on the protein target. This is referred to as ubiquitination.[7]

Ubiquitination is the progressive attachment of ubiquitin (U1,U2) molecules to the target protein, resulting in a polymeric chain. This chain works as a molecular indicator that proteasome, the cellular machinery in charge of protein breakdown, recognizes. Following that, the proteasome engages the tagged target protein and commences its translocation into its central core. The target protein is proteolytically degraded within the proteasome, resulting in its fragmentation into smaller peptide fragments. As a consequence of this degradation process, the levels of the target protein within the cell diminish significantly. This reduction can disrupt critical pathways and functions associated with the target protein, particularly in the context of cancer, where the aberrant expression or activity of certain proteins drives tumorigenesis.[11] The inherent selectivity and specificity of PROTACs makes them highly effective and appealing as a treatment method. The choice of target protein ligand ensures that the PROTAC binds with high affinity to the specified target while sparing non-targeted proteins.[7] Furthermore, the specificity of degradation is further fine-tuned by the selection of the E3 ubiquitin ligand, as different ligases have varied substrate preferences.[8]

To summarize, PROTACs are a sophisticated and novel approach to protein degradation that uses the cell's natural protein turnover mechanism to selectively and efficiently destroy disease-associated proteins. This mechanism of action has the potential to transform cancer therapy and other fields where precise regulation of protein levels is critical. [4]

Parts of a PROTAC Molecule

Target-Binding Moiety: The fundamental element in the architecture of Protac molecules is the target-binding moiety. This moiety is tasked with detecting and binding to the POI with exquisite

specificity as well as high affinity. This component's strategic selection is a critical factor of Protac performance since it affects the degree of selectivity and efficacy obtained in the action of targeted protein degradation (TPD). Notably, this target-binding moiety is frequently a ligand or small molecule that has been carefully engineered to interact with the POI's particular structural and metabolic properties. Kinase inhibitors, hormone analogs, and custom ligands tailored for a specific POI are examples of such moieties.[16]

Linker: Within the PROTAC structure, the linker is an adjustable chemical conduit that connects this target-binding segment to the E3 ligase-recruiting moiety. It is critical in bringing the protein of choice and the E3 ubiquitin ligand close to each other, aiding the process of ubiquitination and, eventually, proteolysis. The design of the linker is a delicate art, with length and chemical composition considerations playing a critical role in maximizing the effectiveness of PROTAC-induced protein breakdown.[6] The length and flexibility of the linker can have a significant impact on the spatial relationship between the constituent components, and as such, they must be meticulously optimized.[3]

E3 Ligase-Recruiting Moiety: The E3 ligase-recruiting moiety is the executive element in charge of recruiting a specific E3 ubiquitin ligase, the key orchestrator of protein ubiquitination. This moiety is often composed of a ligand or peptide fragment with optimum binding affinity for the E3 ligase. The meticulous selection of the E3 ligase recruiting moiety is critical in providing selectivity and efficacy to the PROTAC, since different PROTAC constructions can be created to recruit distinct E3 ligases, allowing for the targeted destruction of specific POIs. [1]

Enhancements in Solubility and Cellular Permeability: In conjunction with the fundamental constituents delineated previously, Protac molecules commonly integrate modifications intended to improve solubility and cellular permeability. This augmentation aims to optimize their efficacy in both cellular and in vivo environments. Such supplementary alterations may involve the incorporation of distinct functional groups, prodrug moieties, or cell-penetrating peptides. Collectively, these modifications contribute to enhancing the stability, bioavailability, and intracellular uptake of the molecule.[11]

PROTAC Delivery mechanisms

Nanoparticle mediated drug delivery has been an impressive contender in the controlled release of therapeutic agents. The size of nanoparticles is within the range of 1-100 nm, which proves to be perfect for nanomedical applications as the preferred range is below
200 nm. This range aids the drugs in diffusing into cellular membranes and the circulatory system. One such example is the bioceramic nanoparticle, such as nano-hydroxyapatite (nHA) and nano-tricalcium phosphate (nTCP). These have been extensively researched and considered to help in bone regeneration.

The physicochemical and pharmacokinetic properties of mesoporous silica nanoparticles have been deeply investigated as drug nanocarriers. They have many impressive properties such as mechanical, thermal and chemical stability. Their affinity to adsorb many different types of molecules is what gives them the incredible loading capacity inside the porous system. Drug delivery employing nanosystems as well as nanosystems used for gene transfection, have been extensively used in diagnosis. [24]

Under optimum conditions and/or appropriate stimulation, cascade responsive nanocarriers can realize multi-stage trigger release of the therapeutic drugs in tumor cells as well as in some organelles, lessen side effects, and improve their specific bioavailability. [24]

Advantages of PROTAC Molecules Over Other Therapies

Within the field of oncology, proteolysis-targeting chimeras (PROTACs) have drawn a lot of attention from researchers developing approaches because of their distinct mechanism, which offers significant advantages over conventional cancer therapy. Future drugs aim to provide a therapeutic method where benefits exceed drawbacks and transcend the constraints inherent in current treatment options.[12]

Targeted Protein Degradation: One of PROTACs' most important benefits is their ability to selectively and highly target the degradation of disease-associated proteins inside cancer cells. This degree of specificity is typically lacking in

conventional treatments like radiation and chemotherapy, which leads to off-target effects and collateral tissue damage.[16] However, PROTACs are made to identify and draw in target proteins for ubiquitination and subsequent proteasomal breakdown, which causes less harm to non-cancerous cells.[12]

Overcoming Drug Resistance: It is commonly known that cancer cells can develop resistance to therapeutic medications, which over time can make many treatments useless. By employing a novel course of action, PROTACs might offer a workable resolution to this confusing circumstance.[13] Because PROTACs promote the degradation of target proteins, they may be less susceptible to resistance mechanisms than kinase inhibitors and other small molecule inhibitors.[12]

Expanded Target Range: Protein targets formerly considered "undruggable" by conventional small molecule inhibitors can now be drugged thanks to PROTAC technology.[13] By utilizing the cell's own degradation mechanism, PROTACs can target a wider variety of proteins, including those with poorly defined binding pockets or druggable sites, expanding the pool of possible therapeutic targets.[14]

Reduced Off-Target Effects: Arguably the most important objective in cancer therapy is to reduce off-target effects, which frequently occur as a side effect of treatment. By selectively attracting target proteins, PROTACs show promise in lowering off-target interactions and improving the safety profile of cancer treatments.[14]

Synergy with Combination Therapy: The flexibility of PROTACs allows them to be employed in conjunction with a range of therapeutic modalities, including immunotherapy, chemotherapy, and targeted therapies. This provides access to synergistic approaches that could enhance treatment effectiveness while tackling cancer heterogeneity, which is typically challenging to treat with single-agent approaches.[15]

Personalization Potential: A growing idea in oncology is to customize cancer treatment to a patient's unique cancer features.[14] By programming PROTACs to target particular dysregulated proteins or pathways within a patient's tumor, personalized treatment approaches that optimize their efficacy and minimize side effects may be possible.[14]

In summary, PROTAC drugs offer advantages such tailored protein degradation, resistance mitigation, increased target range, decreased off-target effects, synergy with combination therapies, and personalization potential, hence posing an opportunity for new direction in cancer therapy. Although additional research and clinical trials are necessary to fully achieve the therapeutic potential of PROTACs, their unique mode of operation renders them a desirable addition to the oncologist's toolkit in the ongoing fight against cancer. [16]

Prostate and Breast Cancer

Prostate Cancer: The prostate, a tiny, walnut-shaped organ in the male reproductive system, is prone to cancer. Men with older age groups are more likely to have it. Age, family history, and certain genetic factors are risk factors for prostate cancer. Other symptoms may include difficulty in urination, frequent micturition, presence of blood in the urine or semen, and pain in the pelvic region. The diagnosis is done by performing a digital rectal test, an antigen blood test specific to the prostate and a biopsy for the same. Depending on the progression and aggressiveness of the cancer, treatment for prostate cancer includes constant observation, surgery (prostatectomy), radiation, hormone therapy and immunotherapy. [18]

ARV 771 is a potent bromodomain PROTAC® degradation agent (DC50 = 1nM), that is used as a therapeutic strategy against prostate cancer. A BRD4-binding component is linked to a ligand for the Von Hippel-Lindau (VHL) protein by a linker. This degrades BRD2/3/4 in CRPC (Castration-Resistant Prostate Cancer) cell lines.

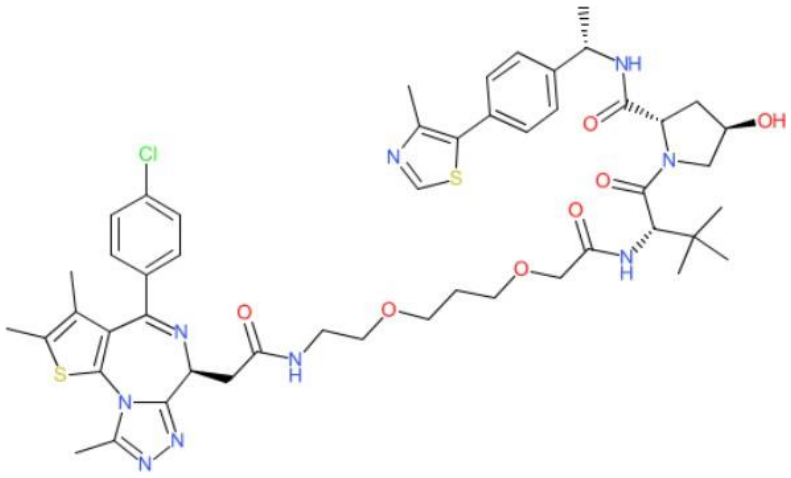

Figure 1: *ARV-771 [27]*

ARV-771, a pan-BET degrader based on proteolysis-targeting chimera (PROTAC) concept, shows significantly improved efficiency in cellular models of CRPC compared to BET inhibition. ARV-771 causes suppression of both AR signaling and its levels, in turn leads to tumor regression in a CRPC mouse xenograft model. Until very recently, the approved treatments for metastasized CRPC were taxanes that disrupt microtubules such as docetaxel and cabazitaxel, that provided only a modest survival benefit. An epigenetic method to dealing with CRPC has been proposed, which involves the inhibition of the bromodomain and extra-terminal (BET) family of proteins. In tumor models of CRPC, BET inhibitors inhibit growth. BET proteins 2, 3, and 4 (BRD2/3/4) bind to the androgen receptor directly, whose action is disrupted by BET inhibitors. By disturbing AR-mediated transcription, BET proteins have become a very desirable target for CRPC. A trimeric molecule that allows ubiquitination and the degradation of the target protein is formed by treatment of the cells. [19]

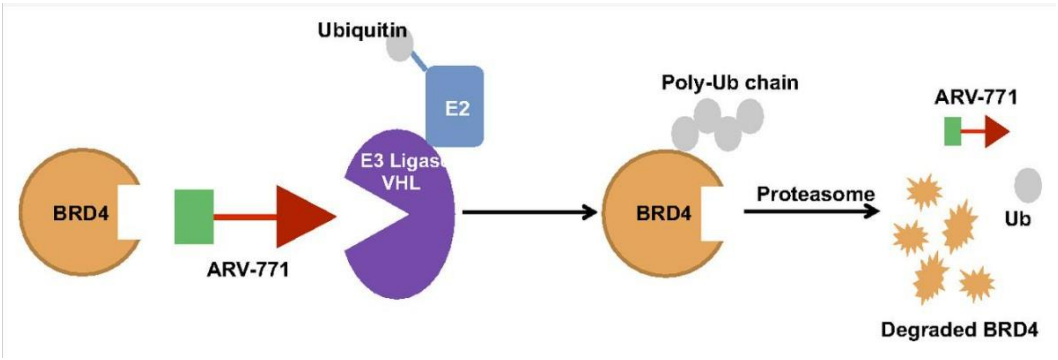

Figure 2: *Working of ARV-771 [27]*

None of these BET-based PROTACs have reportedly exhibited in-vivo activity in a solid tumor malignancy. The physicochemical properties of the first-generation BET PROTAC shown to be efficient in a xenograft mouse model of intraperitoneal delivery, which is not a frequent route of administration. [27] [30]

The superiority of a BET-PROTAC compared to BET inhibitor is shown by the observation that ARV-771 induces apoptosis in CRPC cells grown in-vitro, whereas JQ-1(Thienotriazolodiazepine and a potent inhibitor of the BET family) and OTX015 (Birabresib, an experimental small molecule inhibitor of BRD2, BRD3, and BRD4) have a minor effect. The 80% tumor growth inhibition that occurs in

mice treated with OTX015 is induced by ARV-771. BET degraders, although efficacious in vivo, without inhibitors, still gives rise to progression of disease. [30]

Breast Cancer: Breast cancer is a disease that affects women, but also men, although less frequently. The disease is one of the most common cancers in women. Genetic changes, lifestyle preferences and hormonal factors are some of the risk factors for breast cancer. Changes in skin, breast size or shape changes, nipple discharges are some telltale signs of breast cancer. Mammography, breast imaging, breast MRI, and a biopsy are some of the methods used to establish the presence of cancer. Treatment options depend on factors like stage of progression, hormone receptor status, and HER2 status. Treatment options include surgery (lumpectomy, mastectomy), radiation therapy, chemotherapy, hormonal therapy, targeted therapy, and immunotherapy. [25]

Vepdegestrant (ARV-471) is an orally available estrogen-receptor protein degrader for breast cancer. Vepdegestrant is a hetero molecule that is also bifunctional that aids interactions between the estrogen receptor and an intracellular E3 ligase complex. The degradation of estrogen receptor through the proteasome can be caused by Vepdegestrant. An E3 ubiquitin ligase and ER are bound by ARV- 471 to cause the ubiquitination of the estrogen receptor and in turn its proteasomal degradation. In contrast to this, selective estrogen receptor degraders (SERDs) indirectly conscript the ubiquitin-proteasome system through modifications done to the conformations and/or the immobilization of ER2. The intramuscular route of administration and only about half of ER protein degradation are some of the significant limitations of the SERD fulvestrant. In xenograft models, treatment with ARV-471 resulted in significantly higher ER degradation and TGI than fulvestrant. [20] [27]

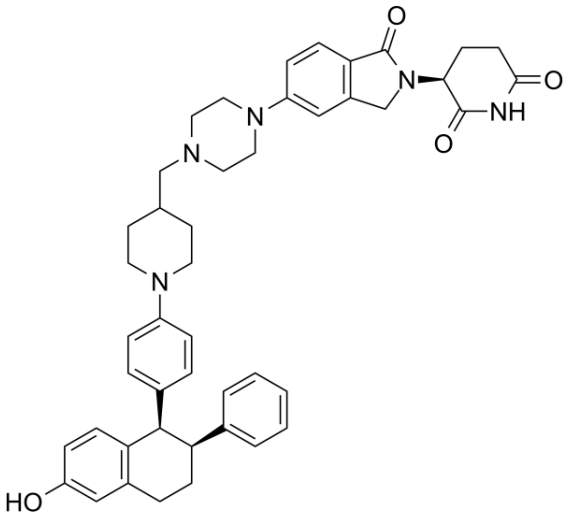

Figure 3: *ARV 471 [27]*

In the ongoing clinical trials, half dose escalation and the safety, tolerability, and physicochemical activity of ARV-471 alone as well as in combination with palbociclib have been evaluated in patients with ER+, advanced or metastatic breast cancer who were given chemotherapy. The initial phase of this study employed the traditional three-plus-three dose progression along with ARV-471, which was orally administered once a day, daily for 28 consecutive days. The beginning dosage for ARV-471 was 30 milligrams. The main objective of the initial phase was to determine the MTD (maximum tolerated dosage) and the recommended second dose. Adverse side effects, pharmacokinetic and pharmacodynamic parameters and markers, such as ER expression in biopsy samples, were included in the secondary outcomes. Clinical benefit rate was defined as complete response. Stable disease longer than 24 weeks, as determined by the RECIST criteria.

By November 2020, twenty-one patients were enrolled in phase 1 who were heavily pre-treated and so had a comparatively poorer prognosis. Among them, 48% had visceral metastatic disease in the liver and lung, 100% had previous CDK4/6 inhibitors, 71% had fulvestrant, 38% had chemotherapy, and 24% had other selective ER degraders. They had a median of 5 previous lines of therapy. No grade 3 or 4 adverse events occurred with ARV-471, even at the highest dose level of 360 mg. The maximum tolerated dosage has not yet been reached, and no dose limiting toxicity has been reported. The pharmacokinetic study showed that administration of ARV-471 at 60 mg per day or higher, surpassed the threshold in preclinical models with a half-life of 28 hrs. Quantitative immunofluorescence was found to be efficient in ER degradation as the mean value of degradation was 62% at all dosage levels. Patients with either wild-type or mutant ER Y537S, Y537N, and D538G have shown to have ER degradation. Five out of the 12 evaluated patients had clinical benefit, with one PR and four stable conditions lasting more than 24 weeks. The highest average ER degradation and overall clinical benefit rate were provided by ARV-471 in comparison to other selective ER degraders that are in active early phase clinical trials. [31]

Existing PROTAC molecules:

Some examples of PROTAC molecules include:

ARV-110: A PROTAC developed to target and degrade the specific androgen receptor (AR) to treat prostate cancer.

ARV-471: Another PROTAC developed by Arvinas, which targets and breaks down the estrogen receptor (ER) in the treatment of ER-positive(ER+) breast cancer.

dBET1: A PROTAC molecule that targets and degrades the bromodomain-containing protein BRD4, which is involved in various cancers.

ARV-825: Developed to target and degrade BRD4, this PROTAC has been investigated for its potential in cancer therapy.

MZ1: A PROTAC molecule designed to target the protein von Hippel-Lindau (VHL), which is involved in regulating the stability of hypoxia-inducible factors (HIFs).

Table 1 | **Heterobifunctional PROTAC targeted protein degraders in clinical development**

Company	Degrader	Target	Indications	E3 ligase	ROA	Highest phase	Clinical trial no. (if applicable)
Arvinas	ARV-110	AR	Prostate cancer	CRBN	Oral	Phase II	NCT03888612
Arvinas/Pfizer	ARV-471	ER	Breast cancer	CRBN	Oral	Phase II	NCT04072952
Accutar Biotech	AC682	ER	Breast cancer	CRBN	Oral	Phase I	NCT05080842
Arvinas	ARV-766	AR	Prostate cancer	Undisclosed	Oral	Phase I	NCT05067140
Bristol Myers Squibb	CC-94676	AR	Prostate cancer	CRBN	Oral	Phase I	NCT04428788
Dialectic Therapeutics	DT2216	BCL-x_L	Liquid and solid tumours	VHL	I.v.	Phase I	NCT04886622
Foghorn Therapeutics	FHD-609	BRD9	Synovial sarcoma	Undisclosed	I.v.	Phase I	NCT04965753
Kymera/Sanofi	KT-474	IRAK4	Autoimmune diseases (e.g., AD, HS, RA)	Undisclosed	Oral	Phase I	NCT04772885
Kymera	KT-413	IRAK4	Diffuse large B cell lymphoma (MYD88-mutant)	CRBN	I.v.	Phase I	
Kymera	KT-333	STAT3	Liquid and solid tumours	Undisclosed	Undisclosed	Phase I	
Nurix Therapeutics	NX-2127	BTK	B cell malignancies	CRBN	Oral	Phase I	NCT04830137
Nurix Therapeutics	NX-5948	BTK	B cell malignancies and autoimmune diseases	CRBN	Oral	Phase I	NCT05131022
C4 Therapeutics	CFT8634	BRD9	Synovial sarcoma	CRBN	Oral	IND-e	
C4 Therapeutics	CFT8919	EGFR-L858R	Non-small-cell lung cancer	CRBN	Oral	IND-e	
Cullgen	CG001419	TRK	Cancer and other indications	CRBN	Oral	IND-e	

AD, atopic dermatitis; AR, androgen receptor; BCL-x_L, B cell lymphoma-extra large; BRD9, bromodomain-containing protein 9; BTK, Bruton's tyrosine kinase; CRBN, cereblon; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; HS, hidradenitis suppurativa; IND-e, in IND-enabling preclinical studies; IRAK4, interleukin-1 receptor-associated kinase 4; i.v., intravenous; PROTAC, proteolysis-targeting chimera; RA, rheumatoid arthritis; ROA, route of administration; STAT3, signal transducer and activator of transcription 3; TRK, tropomyosin receptor kinase; VHL, von Hippel–Lindau.

Figure 4: *PROTACs in Clinical Trials [27]*

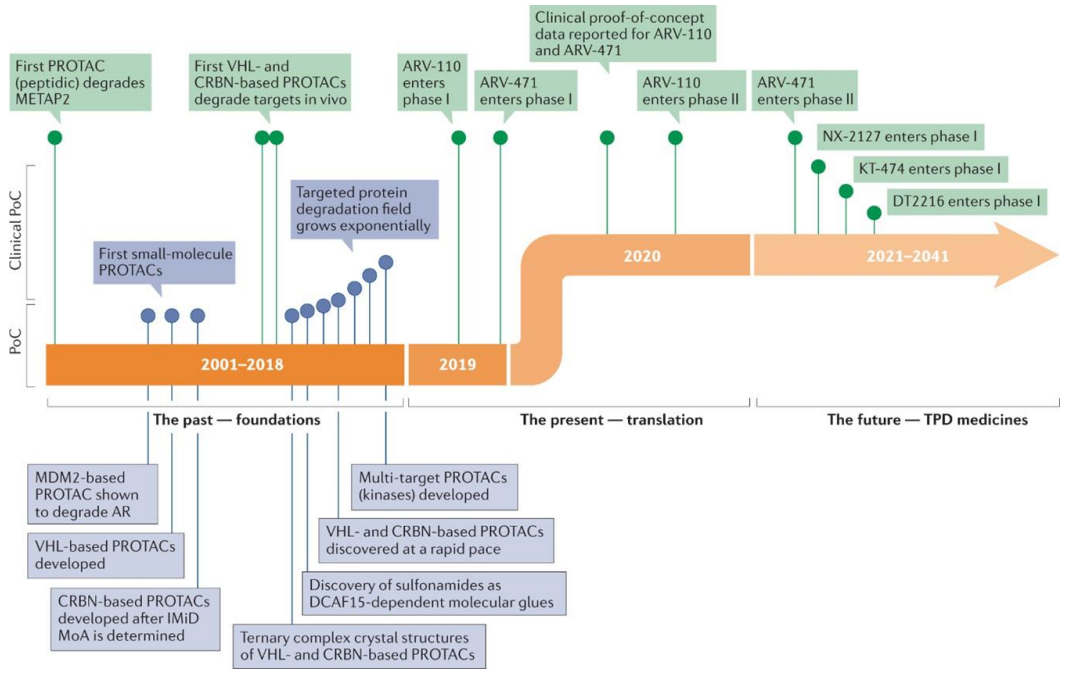

Figure 5: *Timeline of PROTACs [27]*

Drug Delivery Strategies

Currently, there is a limited number of prospects for drug delivery due to a number of hurdles while using the PROTAC technology. Blood brain barrier (BBB) penetration, binding site inhibition, bioavailability, lipophilicity, solubility and many more. When it comes to the miniscule size of the molecules we manufacture, traversing across capillaries, cell membranes also proves to be a problem. Rate of metabolism, drug-drug interaction, hydrophobicity, all prove to be factors that the pharmaceutical industry must overcome periodically. [23]

There are various parameters that affect the pharmacokinetics and pharmacodynamics of drugs that affect effective delivery to the site of impact. One of the ways to look at the many processes involved in the journey of a drug from its site of administration to its site of action or impact is to consider every potential barrier along the path of delivery as a "location" of possible variation in drug response where they may be one or more factors/processes affecting the delivery. [22]

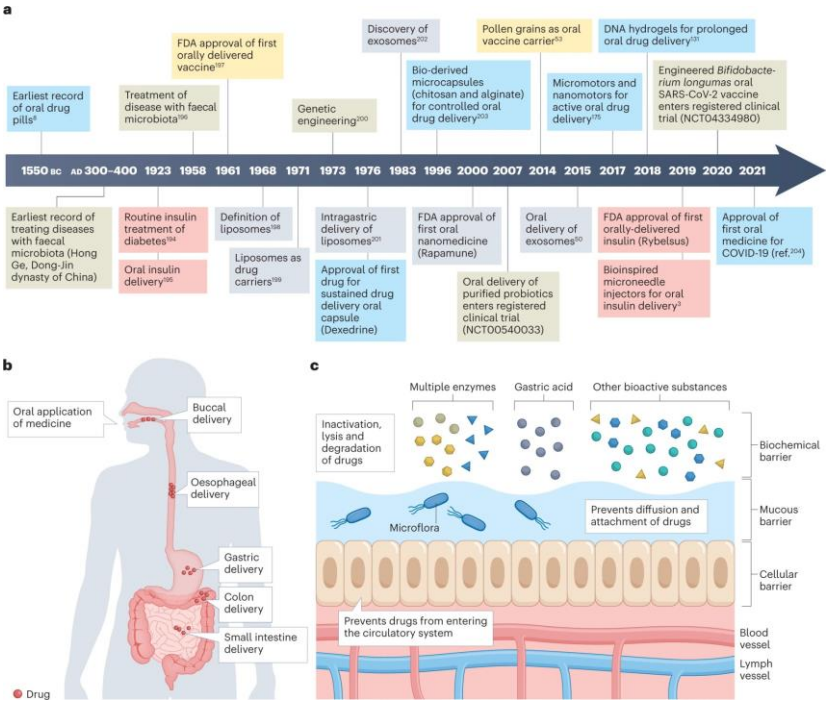

Figure 6: *The timeline of key advancements in oral administration and bioinspired oral delivery device development.*

General drug delivery mechanisms

The oral administration and delivery of polypeptides has been a long ongoing challenge. Polypeptides have been explored in almost every oral form used to deliver small molecule drugs. [21] [22] [26]

Enzyme inhibitors: The use of enzyme inhibitors is suggested to slow down the degradation of polypeptides in the gastrointestinal tract, according to research. The proposed theory was that a slow rate of breakdown would allow for a higher amount of polypeptide drug available for absorption, which would allow for a slower rate of degradation.[22]

Absorption enhancers: These moieties enhance the absorption of polypeptides by improving their paracellular as well as transcellular transport. The closed off junctions of the cells are modulated to better paracellular transport and the fluidity and flexibility of the cell membrane is connected to an increase in transcellular transport. [21][26]

Nanoparticles: The nanoparticle method is backed by literature, which states that nanosized particles have more chances to reach the site of action because they are less likely to be metabolized by the body's enzymes. Contrary to the statement made, nanoparticle absorption is erratic in nature. The percentage of intact particles reaching the circulatory system, not metabolized, was estimated to be in the ballpark of 5%. Nanoparticle drug delivery is also not economically feasible. [24][28]

Emulsions: Emulsions theoretically protect the drug from chemical and enzymatic metabolism in the intestine. The mixture of oil and water in many successful emulsions results in tiny, uniformly and irregularly shaped oil droplets dispersed in the water phase (oil in water) or water droplets dispersed in the oil phase (water in oil). Lipophilic proteins can be shielded from enzymatic degradation in the intestinal tract by the oil part of the emulsion. The water phase microemulsion preparations have been made for the delivery of oral insulin. [21][22]

Liposomes: The acidic nature of the stomach, bile salts, and lipase secreted by the pancreas can degrade liposomes upon oral delivery. There are even fewer attempts to formulate oral preparations to deliver the polypeptides through a liposome system than there are for the parenteral route. The increased bioavailability of the encapsulated agents within liposomes is unclear, whether the liposome was absorbed fully intact or if the lipid triggered

the penetration of the released agent at the site of absorption. The stability of liposomes has been enhanced by including polymers at their surface or employing GI-resistant lipids. [28]

Small molecule PROTAC conjugates: The authors selected small molecular folate groups as ligands for cancer-specific PROTAC delivery in 2021. Folate groups, highly expressed in various cancers, are perfect targets for folate binding PROTACs, as they form ester groups which are then cleaved by intracellular enzymes.

Conclusion and perspectives

Although the PROTAC field is an up and coming one, there are many problems concerning bioavailability, delivery, formulation, stability and economic feasibility. To prepare such drugs in-silico, in-vivo would be a tremendous feat. The concept is definitely promising, considering there are many of them in clinical trials. The main advantage of PROTACs is that it is less invasive. The main selling point would be that PROTACs do not add to the chemicals administered to the body, but manipulate the body's natural degradation machinery to degrade tumor cells. This would be a long yet encouraging path for pharmaceutical companies to invest resources into. More research is definitely required, but the therapeutic potential of small molecule inhibitors or degraders is extremely hopeful.

References

1. Girardini, M., Maniaci, C., Hughes, S. J., Testa, A., & Ciulli, A. (2019). Cereblon versus VHL: Hijacking E3 ligases against each other using PROTACs. Bioorganic & Medicinal Chemistry, 27(12), 2466–2479. doi:10.1016/j.bmc.2019.02.048

2. Khodavirdipour, Amir, et al. "Apoptosis detection methods in diagnosis of cancer and their potential role in treatment: advantages and disadvantages: a review." Journal of Gastrointestinal Cancer 52 (2021): 422-430

3. Troup, Robert I., Charlene Fallan, and Matthias GJ Baud. "Current strategies for the design of PROTAC linkers: a critical review." Exploration of Targeted Anti-tumor Therapy 1.5 (2020): 273.

4. Wang, Y., Jiang, X., Feng, F., Liu, W., & Sun, H. (2020). Degradation of proteins by PROTACs and other strategies. Acta Pharmaceutica Sinica. B, 10(2), 207–238. doi:10.1016/j.apsb.2019.08.001

5. Rutqvist, L. E. "Radiation therapies for breast cancer: current knowledge on advantages and disadvantages." Adjuvant Therapy of Breast Cancer IV (1993): 119-127.

6. Reddy Chichili, V. P., Kumar, V., & Sivaraman, J. (2013). Linkers in the structural biology of protein–protein interactions. Protein Science: A Publication of the Protein Society, 22(2), 153–167. doi:10.1002/pro.2206

7. Sang, Y., Yan, F., & Ren, X. (2015). The role and mechanism of CRL4 E3 ubiquitin ligase in cancer and its potential therapy implications. Oncotarget, 6(40), 42590–42602. doi:10.18632/oncotarget.6052

8. Vittal, V., Stewart, M., Brzović, P. S., & Klevit, R. E. (2015). Regulating the regulators: Recent revelations in the control of E3 ubiquitin ligases. Journal of Biological Chemistry, 290(35), 21244–21251. https://doi.org/10.1074/jbc.r115.675165

9. Garber, Ken. "The PROTAC gold rush." Nat. Biotechnol 40.1 (2022): 12-16.

10. Békés, Miklós, David R. Langley, and Craig M. Crews. "PROTAC targeted protein degraders: the past is prologue." Nature Reviews Drug Discovery 21.3 (2022): 181-200.

11. Paiva, Stacey-Lynn, and Craig M. Crews. "Targeted protein degradation: elements of PROTAC design." Current opinion in chemical biology 50 (2019): 111-119.

12. Nalawansha, Dhanusha A., and Craig M. Crews. "PROTACs: an emerging therapeutic modality in precision medicine." Cell chemical biology 27.8 (2020): 998-1014.

13. Li, Ke, and Craig M. Crews. "PROTACs: past, present and future." Chemical Society Reviews 51.12 (2022): 5214-5236

14. Liu, Jing, et al. "PROTACs: A novel strategy for cancer therapy." Seminars in Cancer Biology. Vol. 67. Academic Press, 2020.

15. Li, Xintong, et al. "c-Myc-targeting PROTAC based on a TNA-DNA bivalent binder for combination therapy of triple-negative breast cancer." Journal of the American Chemical Society 145.16 (2023): 9334-9342.

16. Toure, Momar, and Craig M. Crews. "Small-molecule PROTACS: new approaches to protein degradation." Angewandte Chemie International Edition 55.6 (2016): 1966-1973.

17. Ebrahimi, Mehdi, et al. "Synthesis and characterization of biomimetic bioceramic nanoparticles with optimized physicochemical properties for bone tissue engineering." Journal of Biomedical Materials Research Part A 107.8 (2019): 1654-1666.

18. Neklesa, Taavi, et al. "ARV-110: An oral androgen receptor PROTAC degrader for prostate cancer." J. Clin. Oncol 37.259 (2019): 10-1200.

19. 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.

20. Snyder, Lawrence B., et al. "The discovery of ARV-471, an orally bioavailable estrogen receptor degrading PROTAC for the treatment of patients with breast cancer." Cancer Res 81.13 (2021): 44.

21. Pöstges, Florian, et al. "Solubility enhanced formulation approaches to overcome oral delivery obstacles of PROTACs." Pharmaceutics 15.1 (2023): 156.

22. Benowitz, Andrew B., Paul T. Scott-Stevens, and John D. Harling. "Challenges and opportunities for in vivo PROTAC delivery." Future Medicinal Chemistry 14.3 (2022): 119-121.

23. Chen, Yu, et al. "Proteolysis-targeting chimera (PROTAC) delivery system: Advancing protein degraders towards clinical translation." Chemical Society Reviews 51.13 (2022): 5330-5350.

24. Ebrahimi, M., Botelho, M., Lu, W., & Monmaturapoj, N. (2019). Synthesis and characterization of biomimetic bioceramic nanoparticles with optimized physicochemical properties for bone tissue engineering. Journal of Biomedical Materials Research Part A, 107(7), 1461-1472. https://doi.org/10.1002/jbm.a.36681

25. Moon, Y.; Jeon, S.I.; Shim, M.K.; Kim, K. Cancer-Specific Delivery of Proteolysis-Targeting Chimeras (PROTACs) and Their Application to Cancer Immunotherapy. Pharmaceutics 2023, 15, 411.https://doi.org/10.3390/pharmaceutics1502041

26. Zhang, X., Chen, G., Zhang, H., Shang, L., & Zhao, Y. (2023). Bioinspired oral delivery devices. Nature Reviews Bioengineering, 1, 208–225.

27. Békés, M., Langley, D.R. & Crews, C.M. PROTAC targeted protein degraders: the past is prologue. Nat Rev Drug Discov 21, 181–200 (2022). https://doi.org/10.1038/s41573-021-00371-6

28. Kou, Ponien, et al. "Development of Liposome Systems for Enhancing the PK Properties of Bivalent PROTACs." Pharmaceutics 15.8 (2023): 2098.

29. Juan, Alberto, et al. "Options to improve the action of PROTACs in cancer: development of controlled delivery nanoparticles." Frontiers in Cell and Developmental Biology 9 (2022): 805336.

30. Deng, Yuanfei, et al. "ARV-771 acts as an inducer of cell cycle arrest and apoptosis to suppress hepatocellular carcinoma progression." Frontiers in Pharmacology 13 (2022): 858901.

31. Schott, Anne F., et al. "Abstract GS3-03: GS3-03 ARV-471, a PROTAC® estrogen receptor (ER) degrader in advanced ER-positive/human epidermal growth factor receptor 2 (HER2)-negative

breast cancer: phase 2 expansion (VERITAC) of a phase 1/2 study." Cancer Research 83.5_Supplement (2023): GS3-03.

Conflict of Interest Statement

The authors of this article declare that they have no financial or personal relationships with other people or organizations that could inappropriately influence their work within the manuscript.