Drugging the undruggable

Exploring targeted protein degradation as therapies

In classical pharmacology, small-molecule drugs elicit their effects by binding to a receptor site on the target molecule, usually a protein. This typically has the effect of decreasing the biological activity of the protein, resulting in modulation of a particular cell function. Examples of such targets include many enzymes and ion channels.

This approach to drug design depends on there being a suitable site on the protein molecule to which the drug can bind. But how can the activity of the target protein be modulated if it does not have a suitable 'druggable' binding site? It has been estimated that around three-quarters of the human proteome does not display suitable binding sites, and thus is not amenable to this approach.[1] There are other limitations to this approach too. To elicit a therapeutic effect *in vivo*, for example, sustained high concentrations of the drug may be needed, which can lead to unwanted side-effects.

What if, instead of merely inhibiting the activity of the target protein, a drug had the ability to reduce the actual amount of active protein present in the cell, thereby reducing its activity indirectly? This approach to drug discovery is being adopted by a growing number of specialised biotechs, and is also attracting the attention of several big pharma companies, as well as investors. This article will discuss some of these companies and the targeted protein degradation technology they are using.

Theoretically at least, there are a number of ways in which intracellular levels of a particular protein could be modulated. For example, production of the protein could be inhibited using knock-out techniques such as antisense oligonucleotides or RNA interference, or even direct genome editing via CRISPR/Cas9 technology. An alternative approach would be to increase the rate at which the protein is removed from the cell. In recent years, a technique referred to as small-molecule induced protein degradation has generated considerable interest as it has the potential not only to address a much broader range of proteins than conventional strategies but also to retain the pharmaceutical advantages of using small molecules, such as predictable pharmacokinetics and relative ease of manufacture.

The focus of much research in this area is the ubiquitin-proteasome pathway, which is one of the two main mechanisms by which cells control the location and activities of individual intracellular proteins (the other is lysosomal proteolysis). Ubiquitin is a small regulatory protein present in almost all eukaryotic cells (hence its name), and is actually involved in a number of cellular processes. As far as protein degradation is concerned, proteins to be disposed of are 'marked' by the cell by attaching ubiquitin via a lysine residue on the protein molecule. This acts as a recognition signal for the proteasome, where the protein is broken down.

The protein degradation process occurs in two steps. First, enzymes perform the ubiquitinylation of the target protein: E1 (ubiquitin-activating enzyme) and E2s (ubiquitin-carrier or conjugating proteins) prepare ubiquitin for conjugation, and E3 (ubiquitin-protein ligase) catalyses the transfer of activated ubiquitin to the protein. In the second step, ubiquitinylated proteins are recognised by the proteasome and broken down via an ATP-dependent proteolytic mechanism. There are many ubiquitin ligases, each with different substrate binding properties. Differential regulation of these ligases allows the cell to control specific target protein levels individually.

PROTACs

A number of companies are now harnessing the ubiquitin-proteasome pathway to bring about the destruction of selected proteins associated with a particular disease. One of the first successful approaches to this was the use of small molecules known as PROTACs (PROteolysis-TArgeting Chimeras), and some of them have now advanced as far as clinical trials. PROTACs have three components: a ligand that selectively binds to the target protein, a ligand that binds to ubiquitin-protein ligase (E3), and a connector that joins the two ligands. When a protein-PROTAC-E3 complex is formed, one or more ubiquitin moieties are attached to the target protein, which marks it for degradation by the proteasome. Several companies have now developed proprietary technologies that improve on this basic process.

One of the companies furthest ahead in this area is New Haven, Connecticut-based Arvinas Inc, which has three PROTACs in clinical trials. Arvinas was founded in 2013 with a PROTAC technology platform based on research originally carried out at Yale University by the company's scientific founder and chief scientific advisor, Professor Craig Crews. In addition to the advantages already mentioned for small molecule protein degraders, Arvinas says it has engineered PROTACs that have successfully achieved blood brain barrier penetration in preclinical studies, suggesting a potential role for the technology in the treatment of neurodegenerative diseases.

Arvinas's two lead product candidates are ARV-110 and ARV-471, which are in Phase 2 trials; a third candidate is in Phase 1. ARV-110 targets the androgen receptor protein and is being developed for the treatment of men with metastatic castration-resistant prostate cancer. It was granted Fast Track designation by the US Food and Drug Administration for this indication in May 2019. A Phase 1 trial of ARV-110 initiated in March 2019 includes measures of antitumour activity as secondary endpoints, including reduction in prostate specific antigen. ARDENT, the Phase 2 expansion portion of the trial, commenced in October 2020.[2]

ARV-471 meanwhile targets the estrogen receptor (ER) protein and is being developed for the treatment of patients with locally advanced or metastatic ER-positive/HER2-negative breast cancer. A Phase 1 trial which includes measures of antitumour activity as secondary endpoints was started in August 2019. The protocol for the trial was amended in 2020 to include a Phase 1b cohort expansion of

ARV-471 in combination with Pfizer Inc's cyclin dependent kinase 4/6 inhibitor palbociclib (Ibrance). The Phase 2 expansion portion of the trial began in February 2021.[3]

ARV-766, an oral PROTAC that targets the androgen receptor but with a different profile from that of ARV-110, is in Phase 1 trials. In addition, Arvinas has preclinical programmes in other oncology and neuroscience areas.

While retaining full development and commercialisation rights for product candidates developed using its technology across a wide range of diseases, Arvinas's plan is to bring them to market via strategic collaborations with leading biopharmaceutical companies. In human therapeutics, the company has collaborations with Bayer AG, Genentech Inc (and its parent F Hoffmann-La Roche Ltd) and Pfizer Inc, and has established a joint venture, Oerth Bio LLC, with Bayer to pursue agricultural applications of the technology.

Chaperones

A different approach is being taken by the privately-held biopharmaceutical company Ranok Therapeutics Co Ltd, which has offices in Hangzhou, China and Waltham, Massachusetts, US. Ranok's focus is on chaperones, proteins that facilitate the proper folding, stability and/or activation states of their substrate proteins. While the cell normally uses this mechanism to its advantage, it has been reported that in some circumstances chaperones can stabilise aberrant oncoproteins in tumours and prevent their degradation, thus promoting the survival and growth of cancer cells.

Some chaperones interact with the ubiquitin-proteasome system by recognising misfolded proteins and directing them towards the cell's ubiquitinylation machinery. Ranok has developed a proprietary, next-generation approach to targeted protein degradation known as CHAperone-Mediated Protein Degradation (CHAMP). Besides exploiting different underlying biological processes from those used by PROTACs, the CHAMP technology has a number of advantages, Ranok claims, such as improved safety due to selective targeting of the tumours.[4]

Ranok says its development pipeline includes both well-validated and novel drug targets implicated in cancer and other diseases with significant unmet medical needs. However, at the time of writing (September 2021) it had yet to release any details about its pipeline.

In August 2021, Ranok announced that it had secured a $40 million Series B funding round denominated in both US dollars and Chinese yuan. The financing was led by Lapam Capital and Shanghai Healthcare Capital, with additional participation from Wu Capital, Zhongguancun Kaiyuan Capital and existing investors Med-Find Capital and LC Ventures. The transaction brought Ranok's total funding to date to more than $50 million.

Another company exploring alternatives to PROTACs as a way of recruiting the ubiquitin-proteasome system is the Scottish company Amphista Therapeutics, founded in 2018 with seed funding by venture capital firm Advent Life Sciences. Amphista's technology is built on research previously carried out by Professor Alessio Ciulli at the University of Dundee.

According to Amphista, clinical studies with targeted protein degradation-based therapies up to the present time have identified several potential limitations that could slow their continued growth and success. Their scope may be limited, for example, because they do not work equally well for all drug targets in all cells and tissues. Secondly, there is a potential risk that applying targeted protein degradation approaches to antitumour therapies will lead to the emergence of resistance, limiting the durability of clinical response. In addition, Amphista says that the chemical complexity of many current targeted protein degradation-based approaches could lead to limitations in their drug-like properties such as incompatibility with oral dosing regimens and ability to cross the blood-brain barrier.

More robust effect

Amphista has used proprietary medicinal chemistry techniques to optimise ligands to proteins of interest and incorporate them into modular degrading molecules, which it calls Amphistas. These molecules are independent of existing mechanisms that rely on the E3 ligases. The company claims that Amphista degraders give a more robust cellular antitumor effect across a broader range of cells relative to other protein degradation strategies. In addition, Amphista says its mechanisms will be more challenging for tumour cells to develop resistance against, and also introduce favourable drug-like properties more readily than existing approaches.

Amphista says its research is currently proceeding through preclinical phases, and that it expects to enter clinical studies in 2024. Although the company's initial focus is oncology, it says its overall strategy is to address areas of high unmet clinical need, including neurological and neurodegenerative conditions and immunological disorders. Amphista completed a $53 million Series B financing round in March 2021, when lead investors Forbion Capital Partners and Gilde Healthcare were joined by Novartis Venture Fund and Eli Lilly & Co among others.

A third company in the protein degradation space to attract significant investment this year is the US-based Cullgen Inc. In February, the company closed a $50 million Series B financing led by 3E Bioventures Capital. Cullgen's approach is predicated on the observation that human cells possess at least 600 different E3 ligases, each specific for particular protein targets. This gives rise to the possibility of developing novel E3 ligands that target the degradation of proteins of interest in a selective tissue, tumour type and subcellular compartment. The technology, which Cullgen refers to as ubiquitin-mediated, Small Molecule-Induced Target Elimination technology (uSMITE), is focusing on various difficult- to-treat diseases, including inflammation, autoimmune diseases, neurodegenerative disease and cancer.[6]

At the time of the Series B financing, the company said the proceeds would be used to help advance the company's lead product, CG001419, for which an IND filing was scheduled for later in the year. Cullgen did not respond to enquiries about the current status of this product, although it is believed to target tropomyosin receptor kinase (TRK): TRK fusion proteins are associated with a number of human malignancies.

Molecular glue

One of the newest companies in the protein degradation space is Monte Rosa Therapeutics Inc, which completed a $255.6 million IPO in June 2021. The Boston, US-based company, which also has research facilities in Basel, Switzerland, is developing molecular glue degraders (MGDs), compounds that induce interactions between an E3 ubiquitin ligase and target proteins (referred to as neosubstrates). These interactions lead to ubiquitinylation and subsequent degradation of the protein. MGDs differ from PROTACs in that the latter are bifunctional molecules (target protein and E3 ligase ligands joined by a linker) whereas MGDs are small molecules that act as direct adhesives between two separate protein molecules. The best-known example is thalidomide, which binds to an E3 ubiquitin ligase called cereblon and the target protein (a transcription factor called Ikaros), thus leading ultimately to the protein's degradation.

Monte Rosa's proprietary QuEEN (Quantitative and Engineered Elimination of Neosubstrates) platform is designed to support the identification of therapeutically relevant protein targets that may be amenable to MGD-induced degradation, and the rational design of molecules that can be optimised towards high potency and selectivity.[7]

Central to this technology is the concept of degrons, structural features on the surface of therapeutically relevant proteins. Using artificial intelligence, Monte Rosa is developing a catalogue of degrons for therapeutically relevant proteins, together with a growing library of proprietary, drug-like MGDs. Currently the company has programmes in oncology, inflammation, immunology and genetic diseases such as sickle cell disease. According to a Registration Statement filed with the US Securities and Exchange Commission in June 2021, the most advanced of these is the development of an oral MGD that targets GSPT1, a translational termination factor and degron-containing protein, for the treatment of lung cancers that overexpress one of the *Myc* family of genes. The high level of protein translation in these cancers is driven by GSPT1: selective GSPT1 degradation by Monte Rosa's MGD in these cells leads to cell death. Separately, a programme targeting NEK7, an inflammatory mediator in conditions such as Crohn's disease, is entering the lead optimisation stage.[8]

Monte Rosa has said it would consider strategic collaborations in select therapeutic areas to fully realize the potential of the QuEEN platform.

Big pharma

As well as specialised biotechs, big pharma companies have recognised the potential of targeted protein degradation as a therapeutic modality. One of the earliest in the field was GlaxoSmithKline Plc, which established a collaboration with Yale University in 2012 to combine GSK's expertise in medicinal chemistry with Yale's work on PROTACs.

More recently, Bristol Myers Squibb Co has been investing heavily in protein degradation, partly as a result of its $74 billion acquisition of Celgene Corp in 2019. The company is investigating two different methods of targeted protein degradation: molecular glues known as CELMoDs (Cereblon E3 Ligase MoDulators,) and heterobifunctional agents referred to as ligand-directed degraders, or LDDs. A sub-category of CELMoD agents, the immunomodulatory imide drugs (IMiD agents), includes thalidomide, pomalidomide (Pomalyst) and lenalidomide (Revlimid), which are approved for the treatment of multiple myeloma and certain other conditions. Although pomalidomide and lenalidomide were developed (originally by Celgene) before their actions as molecular glues were fully appreciated, investigation of this pharmacological mechanism is now leading to the identification of new drug candidates.

CELMoDs are currently being investigated in different types of blood cancers such as multiple myeloma and acute myeloid leukaemia, as well as solid tumours and immune-mediated diseases like lupus.

Also arising from the acquisition of Celgene, BMS is partnering with Evotec SE to exploit the German company's PanOmics platform, which combines enhanced throughput proteomics, high-throughput transcriptomics and cell imaging with the integrated data analysis platform PanHunter. This research has led to the discovery of novel first-in-class targeted protein degradation projects, two of which have reached the lead optimisation stage.

Some of the other big pharma companies active in this field are shown in the table above.

As mentioned earlier, ubiquitinylation is not the only route to targeted protein degradation under study. Other approaches include selective hormone receptor degraders, such as AstraZeneca PLC's oestrogen receptor degrader fulvestrant (Faslodex), which is used in the treatment of hormone receptor-positive metastatic breast cancer. Fulvestrant is a derivative of 17β-estradiol: it not only acts as an antiestrogen but, on binding to the oestrogen receptor, it induces a structural change that leads to increased surface hydrophobicity, in turn destabilising the receptor and accelerating its subsequent degradation. Other selective oestrogen receptor degraders are in development: selective

Company	Partner	Area(s) of interest
Amgen Inc	-	PROTACs, molecular glues, oncology
AstraZeneca Plc	-	Oncology, neuroscience
Biogen Inc	C4 Therapeutics Inc	Alzheimer's, Parkinson's
CH Boehringer Sohn AG & Co KG	Proxygen GmbH	Molecular glue degraders, oncology
Eisai Co Ltd	University of Dundee	Oncology
Eli Lilly and Company	Lycia Therapeutics	Lysosomal-targeting chimeras (LYTACs), immunology, pain
Merck & Co Inc	Arvinas Inc	PROTACs
Novartis AG	University of California, Berkeley	-
Pfizer Inc	Arvinas Inc	Estrogen receptor protein degrader
F Hoffmann-La Roche Ltd	Vividion Therapeutics Inc	Oncology, immunology
Sanofi Company	Nurix Therapeutics Inc	Oncology

androgen receptor degraders have also been investigated.

A related technology is hydrophobic tagging, in which a ligand against the target protein is conjugated to a hydrophobic tag: binding of the conjugate to the surface of the target protein results in localised conformational instability or unfolding of the target protein, which is thus marked for degradation. Researchers at the Mount Sinai Center for Therapeutics Discovery in New York, US, led by Professor Jian Jin (who incidentally is a co-founder of Cullgen), have used this approach to produce a degrader of EZH2, an enzyme overexpressed in multiple types of cancer. The degrader, known as MS1943, is reported to display a profound cytotoxic effect in multiple triple-negative breast cancer cells *in vitro*, while sparing normal cells, and to be efficacious in mouse models of breast cancer *in vivo*. The researchers suggest that pharmacologic degradation of EZH2 can be advantageous for treating cancers that are dependent on EZH2.

Although targeted protein degradation was virtually unknown as a therapeutic modality 20 years ago, today it is a hot topic in the pharma sector. While only a handful of drugs based on the technology have so far reached the market, there is widespread agreement that the opportunity it represents is huge. This view is reflected in the size of some of the financings reported already this year.

This article has highlighted just a few of the companies active in the protein degradation area. The majority use technology based on the ubiquitin proteasome pathway, although the way in which it is manipulated varies from company to company. Other pathways to selective protein degradation are just beginning to be explored. Whichever approach comes out on top, there are numerous therapeutic opportunities for this type of drug, and it will be fascinating to watch as targeted protein degradation technology evolves.

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