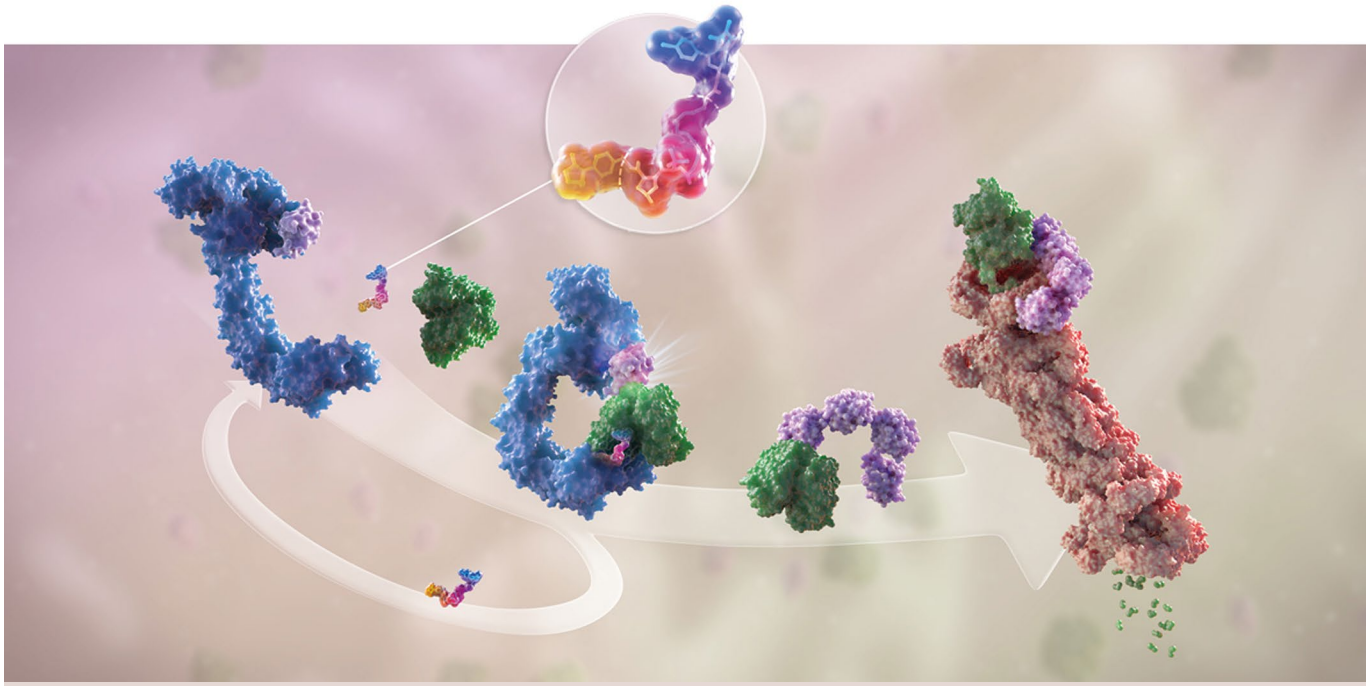

A targeted degrader (multicoloured) binds a target (green) and an E3 ligase (blue), to drive ubiquitylation (purple) and degradation by the proteasome (orange). Credit: Arvinas.

First targeted protein degrader hits the clinic

New therapeutics that harness cellular machinery to degrade targets are entering clinical trials, led by a PROTAC anticancer candidate developed by Arvinas.

Asher Mullard

As soon as Ian Taylor read a story in his local newspaper about how a young biotech firm was focused on developing targeted protein degraders, he was hooked. Whereas most small molecules inhibit a protein's activity by blocking its active site, the company Arvinas was on the hunt for small molecules dubbed 'PROTACs' that could co-opt the cell's degradation machinery to break the proteins down entirely. "I remember thinking to myself: wow, that would be awesome. You could really hit undruggable targets with this," recalls Taylor, who was then at Pfizer Oncology. Now he is overseeing the first clinical test for this emergent modality as senior vice president of biology at Arvinas.

Arvinas is set to start recruiting patients into a phase I trial of ARV-110 in prostate cancer. Like other drugs in this modality, ARV-110 is a bifunctional molecule that uses one arm to bind a target — in this case the androgen receptor (AR) — and the other to bind an E3 ubiquitin ligase. The ligase then tags the target with ubiquitin, marking it for disposal by the cell's proteasomal machinery. Later this year, Arvinas also plans to launch a breast cancer trial of their ARV-471, a targeted degrader that acts in the same way on the oestrogen receptor (ER).

Other companies are also nearing the clinic, including Novartis, which is on track to advance a targeted degrader into the clinic this year against an as yet undisclosed target.

"Everyone in the industry is waiting with bated breath on those first trial results," says Jason Imbriglio, who works on targeted degraders at Merck & Co. "It has the potential to really change the way people think about this technology."

Reflecting this potential, a host of biotechs including Arvinas, C4 Therapeutics and Kymera Therapeutics are wholly focused on honing targeted degrader chemistries. Big pharma groups are also investing heavily in this science.

"Every big pharma company and even every medium-sized biotech has either a collaboration in this space or internal efforts," says Nello Mainolfi, CTO of Kymera.

While most of the focus is on bifunctional molecules that recruit an E3 ligase, a few firms are also exploring alternative degradation strategies (BOX 1).

"2019 through 2021 will be big years for this space," says Andrew Phillips, CEO of C4 Therapeutics.

Making degraders

Targeted degraders first entered the patent literature in 1999, when researchers from the biotech Proteinix submitted a patent for small-molecule compounds that could co-opt the ubiquitin machinery to degrade proteins of interest. Just 2 years later, Yale University's Craig Crews and California Institute of Technology's Raymond Deshaies published on a similar strategy, using a peptide-based approach to induce the ubiquitylation and degradation of the target methionine aminopeptidase 2.

While Proteinix never pursued the promise of this patent, Crews and colleagues kept tinkering away at their protein degraders, named proteolysis-targeting chimaeras (PROTACs), gradually turning chemical curiosities into a medicinal modality. By 2008 they had dropped the peptidic component of their molecules, designing a wholly small-molecule degrader that could bind and degrade the AR by

Box 1 | **Alternative degradation strategies**

Beyond the bifunctional targeted degraders, other firms are working at harnessing other components of the cell's proteasomal machinery to achieve targeted degradation of once undruggable targets.

Scientists at Cedilla Therapeutics, for example, are taking a broad approach. "There are mechanisms that control the abundance of any given protein, and that regulate the abundance of aberrant or misfolded or misformed variants of those proteins," explains CSO Brian Jones. "So our starting point was this notion that we don't want to artificially recruit anything; we want to co-opt the endogenous machinery to do what it is already doing."

In some cases this might mean finding small molecules that directly destabilize a protein, initiating protein quality control mechanisms. In others, they are working to identify and modulate the activity of upstream factors — such as post-translational modification machinery — that can control protein stability or abundance. Alternatively, the disruption of protein–protein interactions and multi-target complexes might make it possible to stoichiometrically 'orphan' a target, driving its degradation.

"Right now we are looking at a range of different target types across these axes to build a sense of where the richest pool of tractable targets are. And then later on we'll prioritize or expand based on where the successes are," says Jones.

"One of the overarching goals of our company is to really also try to define some of the rules that govern protein degradation," adds Cedilla CEO Alexandra Glucksmann.

Researchers at Mission Therapeutics, Forma Therapeutics and elsewhere are meanwhile focusing on deubiquitylating (DUB) inhibitors. Rather than using E3 ligases to boost the ubiquitylation of a protein, their strategy is to block the machinery that otherwise deubiquitylates and saves proteins from destruction. As of September 2017, at least 15 DUB inhibitors were in preclinical development.

bringing it into proximity with the E3 ligase MDM2. In 2013, Crews founded Arvinas to advance this technology to the clinic.

Around that same time, researchers were unravelling the biology of the immunomodulatory imide drugs, including thalidomide, lenalidomide and pomalidomide. After realizing that these engage the E3 ligase cereblon, it became clear that these might have utility as targeted degraders as well. In 2015, Jay Bradner, then at the Dana–Farber Cancer Institute and now president of the Novartis Institutes for BioMedical Research (NIBR), showed with colleagues that they could harness this activity to drive the targeted degradation of the BET family of proteins. Bradner and colleagues dubbed their degraders degronimids, and co-founded C4 Therapeutics that same year.

Whether drug hunters call their candidates PROTACs or degronimids, the leading targeted degradation strategies all operate somewhat like molecular glues: a bifunctional small molecule combines a target-binding warhead, a linker and an E3 ligase recruiter to bring a target protein into contact with an E3 ligase, enabling selective target ubiquitylation and subsequent protein degradation.

It wasn't always clear that this approach could make it to the clinic, however. From the start, researchers worried that these large and potentially floppy compounds would be hard to optimize into bioavailable, selective, effective and tolerable drug-like compounds. With the first compounds headed to the clinic, things are looking up.

"These are unusual looking molecules — some might even say funny-looking molecules," says Phillips. But work over the past few years has shown that "they have surprisingly normal pharmaceutical properties," he adds. As yet confidential internal analyses, from multiple companies, suggest these compounds can solubilize well, can slip into cells, can be orally available, can resist metabolic processes and in some cases can even cross the blood–brain barrier.

"The big surprise for me — and this shouldn't really be a surprise — is that our ability to predict chemical properties for new classes of molecules based simply on chemical structures is no better now than it was 5 years ago, and arguably no better than it was 30 years ago," says Phillips.

Early hopes that targeted degraders might offer a sort of modular platform — in which any target-binding ligand can be paired with an off-the-shelf linker and E3 recruiter to generate a drug — meanwhile have not yet borne out.

Instead, there is growing appreciation that the properties of the ternary structure that forms between a target, a degrader and an E3 ligase are key. Even the slightest shifts in this structure can affect how the drugs work. In some cases, this can explain how promiscuous target-binding warheads can achieve super selectivity when transformed into a degrader. In others, minute changes to any component of the degrader can wipe out its activity entirely.

"It is not a plug and play system," says Phillips. "Chemists like to pull systems apart to their constituent parts for reasons related to synthesis strategies. But what we have learned over the past hundred years again and again is that a system is not simply the sum of the parts. This is very much on point for degraders; they are not simply the sum of the parts," says Phillips.

C4 Therapeutics' strategy, as laid out in a review article last year, is consequently to optimize its compounds to activate the ubiquitin system, not just to bring the target and the ligase into proximity with one another. "You have to bring things together, but that alone is clearly not sufficient. You have to activate the process as well," says Phillips. "There really is a need for a more holistic understanding of how degraders work."

Mainolfi similarly adds that focusing only on ternary complex formation "is probably short sighted". In some cases, even successful protein ubiquitylation doesn't guarantee that a target is delivered to the proteasomal system for degradation. "There is a lot that we don't know, or at least that is not disclosed out there," he says.

This is true of the ligase landscape as well, in which an estimated 600 E3 ligases have unique activity profiles and distribution patterns throughout the body. Picking the right ligase to recruit to your target protein can make or break a programme, says Mainolfi. If a target can be knocked out across the body without adverse events — as perhaps shown by human knockout data — then Kymera's team might choose to work with an ubiquitously expressed E3 ligase, he explains. But if they need a wider therapeutic window, they may focus on ligases that are preferentially expressed in a specific tissue type or cancer cell type. The E3 ligases also have different patterns of subcellular distribution, providing the opportunity to add other layers of selectivity into the mix.

Only five or six E3 ligases have been publicly validated for use in targeted degraders so far, says Mainolfi, but Kymera and others are working on validating other E3 ligases to add to their tool boxes.

Testing targets

One of the biggest theoretical benefits of targeted degraders is their ability to make once undruggable targets druggable. Whereas small-molecule inhibitors have to block catalytic sites or bind in well-defined pockets that impact protein function, targeted degraders can in theory bind any nook or cranny to drive degradation. "It opens up a subset of human biology that's yet to be effectively targeted by drugs," says Phillips.

But a limitation of this approach may have got lost in the noise, he cautions. "There are still a huge number of targets where there is just no ligand. And if there's no ligand, I can't build you a degrader," says Phillips. Long-time appealing targets like

MYC, despite hopes to the contrary, remain for now as intractable as ever, he says.

"Right now the most impactful parameter that I think the industry hasn't solved for is: what are the ligandable targets?" says Mainolfi.

Bradner is optimistic that the field will open up most targets, eventually. "My instinct is that most proteins will prove amenable to small-molecule discovery chemistry, either by direct engagement with a small molecule or through a molecular-glue-like activity of a molecule crosslinking a protein of interest," he says. "This is admittedly a hunch, but I think the high-hanging fruit is reachable with this chemistry." Researchers at NIBR have already induced targeted degradation of over 40 targets, he points out, many of which would have otherwise been classed as undruggable.

Given the competitive nature of this field, companies are being tight-lipped about which targets they are working on, especially when it comes to previously undruggable proteins that might do the most to open up new disease spaces. But a few publicly disclosed degrader programmes — working on validated or at least known ligandable targets — highlight some of the lower-hanging opportunities these drugs can offer.

Arvinas's two lead candidates act on the AR and the ER, respectively, both of which are clinically validated targets of approved drugs. This conservative strategy is by design, notes company CEO John Houston, and stands to ultimately simplify the initial development of the modality. Arvinas's founders thought about testing their degraders against unvalidated targets from the get go, but worried that with a failure it would be unclear whether the target or the technology was at fault. With validated targets, by contrast, the compounds alone have the opportunity to shine or resign. "That was the thinking I inherited when I joined, and I'm very glad they did that," says Houston. And there is still plenty of room for these lead targeted degraders to go above and beyond the competition, he points out.

In the case of the AR target, for example, approved small molecules rely on occupancy-driven pharmacology to prevent activity, and so they lose their activity when they are flushed from the body or overwhelmed by replacement proteins. And most patients will acquire resistance to the AR antagonist enzalutamide, because cancer cells can increase their androgen or AR production levels or can pick up AR mutations such that they no longer respond to treatment.

ARV-110 — like many other targeted degraders — by contrast makes the most of an event-driven activity in which each compound efficiently catalyses the complete degradation of multiple protein constructs. Instead of needing more drug than there is target, which is the case with traditional inhibitors, Arvinas can dose less drug than target. And this lower dosing could translate into a better side effect profile, explains Taylor.

Another benefit of event-driven pharmacology is that targeted degraders promise sustained activity even after they are gone, for as long as it takes for a cell to resynthesize degraded proteins. "We have data from a number of programmes where PROTACs, because of their catalytic activity, can take care of all the additional protein that a cell is trying to make as part of a resistance mechanism," says Taylor. In some cases, these drugs might as a result be able to delay the rise of resistance.

ARV-471 addresses other shortfalls of the approved ER-targeting drugs as well. The approved selective ER degrader fulvestrant — which drives degradation by making the ER more hydrophobic and therefore unstable, rather than by recruiting an E3 ligase — is not orally bioavailable, has poor systemic exposure and does not fully deplete its target.

When these trials wrap up, people will be watching closely for insights into how unconventional first-in-modality degrader compounds are absorbed, distributed, metabolized and excreted from the body, and whether they can live up to the expectations of oral dosing.

The field is also keen to see how low these drugs can knock protein levels down, how that compares with protein re-synthesis rates and whether those levels are in line with expectations from animal models.

"The promise with targeted degrader molecules is to dissociate pharmacodynamics from pharmacokinetics, meaning that brief exposure to a degrader can result in durable impact on a pathway," says Bradner. "And so the pharmacokinetic and pharmacodynamic relationships will be very exciting to watch," adds Bradner.

The team at Arvinas is particularly excited. "This year will tell us a lot about whether we truly have cracked the code for turning these things into small molecules with drug-like properties, and whether that will pan out through the rest of the platform," says Houston.

And when the leaders in this space disclose the as-yet confidential structures of their drugs, researchers elsewhere will be keen to take lessons back to their own programmes. "I'm curious to see what the key parameters were that they optimized for in order to translate their preclinical programmes into the clinic and what their compounds look like, which to be honest I assume is quite different from what our molecules look like," says Mainolfi.

"We hope the first clinical investigations of the first few compounds go really well," he adds.

Other opportunities

Kymera's targeted protein degrader of IRAK4 demonstrates another set of near-term opportunities for the targeted degraders. IRAK4 is a kinase with a key role in the innate immune system, and has been implicated in various cancers. But while small-molecule inhibitors can block IRAK4's kinase capabilities, the protein also has a kinase-independent role as a scaffolding protein, enabling the assembly of the innate immune system's myddosome protein complex.

"Based on genetic knockdown and knockout experiments, we hypothesize that we will be able to achieve completely unique and superior phenotypes than are possible with inhibitors," says Mainolfi.

Kymera presented preclinical data for an IRAK4 inhibitor at the American Society of Hematology meeting last year, and plans to advance this programme into the clinic in the first half of 2020.

Similar principles could be applied to other scaffolding proteins, including RIP kinases that some degrader groups have been working on, as well as to other non-enzymatic proteins and transcription factors that can otherwise be hard to target.

Central nervous system targets are also increasingly on the table. Drug developers are already working on antibody, antisense and gene-therapy-based approaches to knock down tau and α-synuclein, for example, but given concerns about the brain-penetrating capabilities and administration profiles of these modalities, targeted degrader advocates hope that their drugs will offer a better way forward. Arvinas is already making progress with a tau-targeted compound that can cross the blood–brain barrier in in vivo models of disease, and the company is optimistic that they will be able to advance an oral or intravenous formulation of such a drug into the clinic. They are also working on an α-synuclein targeted degrader for the treatment of Parkinson disease.

C4 Therapeutics and Biogen partnered in January on Alzheimer and Parkinson disease as well, but they have yet to disclose targets.

And the broader opportunity is bigger still, says Mainolfi. "We have an opportunity to achieve broadly applicable body-wide knockdown in a way that oligonucleotide and CRISPR therapeutics cannot yet do. And we can do it with the flexibility and the scalability of small molecules. This really has the potential to be the biggest game changer in drug discovery."