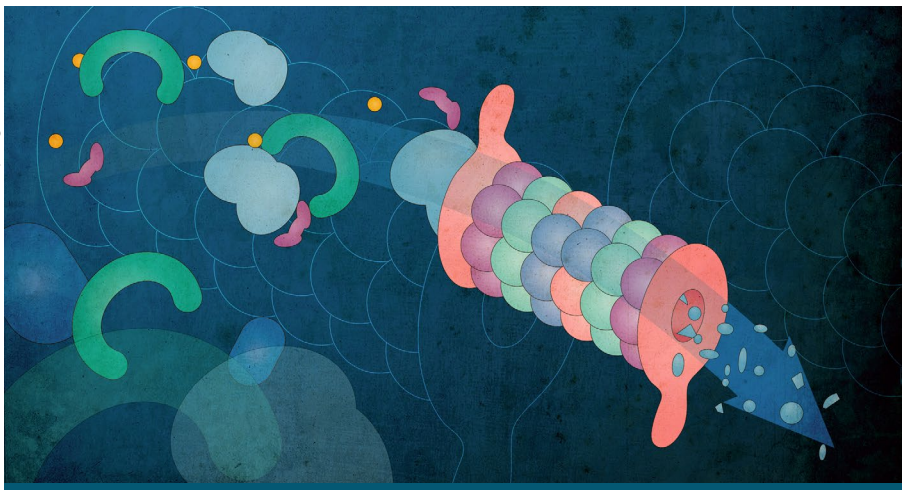

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Targeted protein degraders crowd into the clinic

At least 15 targeted degraders — from heterobifunctional PROTACs to molecular glues — should be in patients by the end of the year.

Asher Mullard

In 2019, Arvinas Therapeutics broke ground with a first-in-man trial of a heterobifunctional targeted degrader — an emerging class of small-molecule drugs that destroys rather than inhibits protein targets. Now, Arvinas is getting company. At least 15 deliberately designed degraders should be in the clinic by the end of 2021.

How times have changed. Ian Churcher remembers the pushback he faced in 2012 when GlaxoSmithKline set up its Protein Degradation team. "People were very skeptical about whether these would ever be translated into molecules that you could take into the clinic. Some of my colleagues at the time described it as a crazy idea," says Churcher, who is now CSO at Amphista Therapeutics, a degrader-focused biotech.

At issue was the unusual chemistry of these compounds. Heterobifunctional degraders consist of a ligand that binds a protein of interest, a linker and a warhead that recruits an E3 ubiquitin ligase, a key component of the cell's trash disposal machinery. These molecules — epitomized by the PROTACs pioneered by Yale's Craig Crews and colleagues — bring a target into proximity with the E3 ligase, initiating the target's ubiquitination and subsequent degradation. But because of all of the elements involved, these degraders are unlike what medicinal chemists are used to working with.

"When I came to C4, I thought 'Jeez, these molecules look really big and ugly. Are we ever going to get them into cells? Will we ever make them bioavailable? And are we ever going to see a CNS-penetrant bifunctional degrader?'" recalls Stewart Fisher, CSO at C4 Therapeutics. "The reality is those hurdles — all of them — fell over very quickly."

Researchers are also making progress with related molecular glue degraders, compounds that also subvert E3 ligases but without the use of long linkers and bulky target-recruiting ligands.

The degrader toolbox is growing rapidly. Many large pharmaceutical companies are now investing in these small molecules. Dozens of biotechs are embracing the technology. Academic groups, too, are making nearly clinic-ready degraders. One PROTAC-tracking database now lists more than 1,600 publicly disclosed heterobifunctional degraders, acting on more than 100 targets.

"Here's a field that's receiving a lot of hype and a lot of attention. But it feels quite justified," says Jay Bradner, president of the Novartis Institutes for BioMedical Research (NIBR) and co-founder of C4 Therapeutics.

Degraders certainly offer a long list of strengths. They can achieve striking selectivity, because they depend on tertiary interactions between the target, the drug and the E3 ligase. They offer efficient target coverage, because they act via transient binding events rather than occupancy-based ones. As such, a single degrader can take out many copies of a pathogenic protein. Whereas small molecules block just a target's active site, degraders ablate all of its functions, including a protein's ability to help hold a complex together. And because degraders don't have to bind in an active site, they can tackle targets that have been hard to take on.

The key is to find the best use cases, adds John Tallarico, head of Chemical Biology and Therapeutics at NIBR. "We're very thoughtful now about the situations where degradation will be superior to inhibition," he says. For targets with a slow resynthesis rate, the ability to use a low dose to achieve a durable effect can be fantastic, he says. But higher-hanging opportunities still top the list. "It's a great mechanism for drugging the undruggable," says Tallarico.

With the burgeoning clinical pipeline, drug companies are putting the various preclinical promises to the test. For now, all but one of these programmes are in cancer. Many are degrading targets with proven therapeutic utility, where efficacy, safety and commercial risks are understood. But some are taking on otherwise intractable targets too (TABLE 1).

"The next 2 years are going to be fundamental to the validation of this approach," says Fisher.

Minimizing target risk

Arvinas, founded by Crews in 2013, was one of the earliest entrants into the degrader space. As such, risk reduction was key during project prioritization. Drug development is hard enough without venturing into unexplored biology.

The androgen receptor (AR), one of the targets Arvinas focused on, had a few things going for it, including a long history. Anti-androgen therapies that prevent AR signalling include flutamide, first introduced in 1989, and Astellas's enzalutamide, approved in 2012 and now a US$3 billion per year drug. These have demonstrated the clinical efficacy of AR antagonism in prostate cancer. But patients develop resistance to anti-androgen drugs, often through overexpression or amplification of the AR. An agent that can degrade AR, even in patients with resistance mutations, could have broad prostate cancer applications.

Table 1 | **Selected degraders in and approaching the clinic**

Drug	Sponsor	Properties	Lead indication	Status
Heterobifunctional degraders (PROTACs, BiDACs, etc.)				
ARV-110	Arvinas	Androgen receptor degrader	Prostate cancer	Phase II
ARV-471	Arvinas	Oestrogen receptor degrader	Breast cancer	Phase II
ARV-766	Arvinas	Androgen receptor degrader	Prostate cancer	Phase I in 2021
AR-LDD	Bristol Myers Squibb	Androgen receptor degrader	Prostate cancer	Phase I
DT2216	Dialectic	BCL-XL degrader	Liquid and solid cancers	Phase I
KT-474	Kymera/Sanofi	IRAK4 degrader	Autoimmune including AD, HS and RA	Phase I
KT-413	Kymera	IRAK4 degrader with IMiD activity	MYD88-mutant DLBCL	Phase I in 2H2021
KT-333	Kymera	STAT3 degrader	Liquid and solid tumours	Phase I in 2H2021
NX-2127	Nurix	BTK degrader with IMiD activity	B cell malignancies	Phase I
NX-5948	Nurix	BTK degrader	B cell malignancies and autoimmune	Phase I in 2H2021
CG001419	Cullgen	TRK degrader	Cancer and other diseases	IND in 2021
CFT8634	C4 Therapeutics	BRD9 degrader	Synovial sarcoma	IND in 2H2021
FHD-609	Foghorn	BRD9 degrader	Synovial sarcoma	IND in 1H2021
Molecular glue degrader (CELMoDs, MonoDACs, etc.)				
DKY709	Novartis	Helios (IKZF2) degrader	Solid cancers	Phase I
CC-90009	Bristol Myers Squibb	GSPT1 degrader	Acute myeloid leukaemia	Phase I
CC-92480	Bristol Myers Squibb	Ikaros/Aiolos (IKZF1/3) degrader	Multiple myeloma	Phase I
CC-99282	Bristol Myers Squibb	Ikaros/Aiolos (IKZF1/3) degrader	Lymphoma	Phase I
CFT7455	C4 Therapeutics	Ikaros/Aiolos (IKZF1/3) degrader	Multiple myeloma and lymphoma	Phase I in 1H2021

AD, atopic dermatitis; DLBCL, diffuse large B cell lymphoma; HS, hidradenitis suppurativa; IMiD, immunomodulatory drug; RA, rheumatoid arthritis.

Arvinas's lead AR degrader, ARV-110, leans on the event-driven activity of the drug class, whereby each degrader catalyses the destruction of multiple copies of a protein. One benefit of this pharmacology is that a low drug dose should be able to achieve sustained activity, potentially translating into improved safety profiles over other anti-androgen approaches. Another is that, if ARV-110 can degrade AR as fast as the cells can make it, it could leave few openings for AR-addicted cells to develop resistance.

In 2019, ARV-110 became the first PROTAC to enter the clinic. Data to date show that the orally bioavailable degrader is safe, a win for any first-of-its-kind approach. There are also signs of clinical activity, including biopsy data from one patient that show AR degradation of 70–90%. (Biopsy data are hard to get in this setting, as metastases are often in the bone.) "The initial data look positive," says Churcher.

But there are hurdles ahead. The AR target is so well established and prostate cancer patients have access to so many other treatment options that patient selection is complex. Arvinas's first trial recruited heavily pretreated patients with metastatic disease, who had received a median of five prior therapies. Genetic sequencing revealed that these patients had highly heterogeneous disease, and 84% of patients had non-AR gene mutations that would not be expected to respond to ARV-110.

Arvinas has identified a molecularly defined patient population that appears to respond, and an ongoing phase II trial is enriching for these patients. The company also expects that the drug will have a role in earlier disease settings. And follow-on AR degraders from Arvinas and others could have better profiles still, against a broader suite of AR mutations. But this early clinical experience highlights a downside of tackling a well-validated target in a crowded therapeutic space.

Arvinas is due to disclose the structures of ARV-110 and of its lead oestrogen receptor degrader, ARV-471, at the American Association for Cancer Research (AACR) in April.

"I'm glad Arvinas is out there at the bleeding edge. But because they had to be a pioneer, they picked targets that people were familiar with. And those are challenging targets," says Fisher.

Nurix Therapeutics has taken on a different well-validated target with NX-2127, a degrader of the B cell development kinase BTK. Here, too, target risk reduction is key. "If NX-2127 doesn't work, it's not the target; it's something about the ability of NX-2127 to affect the target," says Robert Brown, Nurix's Senior Vice President of clinical development.

Indeed, Johnson & Johnson secured approval for its first-in-class BTK inhibitor ibrutinib in 2013. This drug, now approved for various blood cancers, earned $7.6 billion in 2020. But a degrader could bring new benefits to the table, says Brown.

For one, prior work with next-generation BTK inhibitors shows that better target selectivity results in better safety. Nurix can therefore explore the effects of the improved selectivity of its degrader over existing BTK inhibitors. Second, whereas patients develop resistance to approved BTK inhibitors through mutations in the target's active site, NX-2127 has preclinical activity against BTK's most common active site resistance mutation. Nurix also believes that its BTK degrader may delay the emergence of resistance compared with competitive inhibitors, because of its event-driven activity. "I think degraders will probably do a better job of disrupting the entire signalling cascade," says Brown.

NX-2127 also provides an opportunity to test the ability to pack two drugs into one with a degrader. Bristol Myers Squibb (BMS)/Celgene's immunomodulatory drug (IMiD) lenalidomide — an inadvertently discovered degrader that uses the E3 ligase cereblon to degrade the transcription factors Ikaros and Aiolos — is approved for some of the same B cell malignancies as ibrutinib. And in 2019, clinical researchers reported in *Blood* that ibrutinib plus lenalidomide and CD20-targeting rituximab showed impressive phase I/II promise in diffuse large B-cell lymphoma. By using a cereblon-recruiting IMiD-like warhead in NX-2127, Nurix expects to tap the benefits of combined degradation.

With NX-5948, another BTK degrader, Nurix instead engineered out the IMiD-like activity. This candidate might have a cleaner safety profile as a result, making it better suited for autoimmune diseases. NX-5948 is also brain penetrant, so it might prove useful for blood cancers that have made it into the brain, such as CNS lymphoma. NX-5948 is headed to the clinic later this year.

First-in-class openings

Other heterobifunctional programmes are taking on targets that industry has had a harder time with in the past.

A key selling point of degraders is their ability to drive the destruction of proteins that have multiple functions. Proteins with catalytic active sites, including kinases, can also serve as scaffolding proteins, holding protein complexes together, for example. This kind of function has thwarted many drug development programmes over the years.

Take IRAK4, for example, a kinase that enables both IL-1 family receptor and Toll-like receptor (TLR) inflammatory signalling. Despite links to arthritis, atherosclerosis, Alzheimer disease, gout, systemic lupus erythematosus, psoriasis and more, drug developers have only recently made headway with small molecules targeting IRAK4. One possible explanation for why IRAK4 inhibitors have lagged is that the protein provides a scaffolding function, holding the myddosome complex together and facilitating downstream inflammatory signalling even when its kinase function is blocked.

Not everyone agrees that this is the case: Pfizer and a few others are now testing IRAK4 inhibitors in the clinic in autoimmune indications. But Kymera believes degraders offer a better way forward. Its KT-474 "will allow us not only to showcase the power of the technology, but also to bring transformative therapies to patients," Kymera's CEO Nello Mainolfi told *Nature Biotechnology* last year, after Sanofi committed $150 million in up-front payments and up to $2 billion in milestones to partner on this programme.

KT-474 is the only non-oncology degrader in the clinic to date. Lead indications will include atopic dermatitis and hidradenitis suppurativa. The ability to use a degrader safely in these chronic settings could speed up the acceptance for other indications too. "We don't have any data as of today that show that these molecules, and this technology, should not be compatible with chronic dosing. The proof will be in the pudding," Mainolfi said.

Kymera's KT-413, an IRAK4 degrader with conserved IMiD activity, is headed for cancer clinical trials later this year.

C4 Therapeutics, meanwhile, has a programme focused on BRD9, a member of the bromodomain family of proteins. Bromodomain-containing proteins recognize acetylated lysines on histones and other proteins. They have attracted considerable industry attention over the past decade because they have druggable pockets and a range of biological functions, including activity as epigenetic readers. And, emerging data suggest that BRD9 has a critical role in a rare form of sarcoma, potentially driven by a scaffolding function.

The exact biology is still being worked out. Although small molecules can bind and inhibit BRD9's bromodomain with high selectivity, efforts to use these to kill cancer cells have yielded contradictory results. But when academic researchers used a CRISPR screen to look for cancer dependencies in synovial sarcomas, a rare soft-tissue sarcoma, BRD9 lit up. Further analysis, reported in *eLife* in 2018, showed that BRD9 is a component of an aberrant chromatin-remodelling complex that is thought to be the primary driver of this disease. Critically, degradation of BRD9 stopped tumour progression in mouse models of disease.

C4 Therapeutics has compared the effects of a degrader with an inhibitor in this cancer. "From our preclinical data, it was night and day," says Fisher. "An inhibitor has no effect on the cells, and a degrader ablates the activity and stops cell growth."

With its BRD9-degrader CFT8634, C4 Therapeutics sees a clear development path as well. Synovial sarcoma is a deadly cancer that primarily affects adolescent and young adults, with limited treatment options. It is driven by a chromosomal translocation, making for a relatively genetically homogeneous and identifiable patient population. And the translocation's ability to cause cancer appears to be dependent on BRD9.

"This is the cleanest play that we see," says Fisher. "There's a validation of the platform here."

C4 Therapeutics plans to submit an investigational new drug application to start phase I trials of this drug later this year. No one has publicly advanced a BRD9 inhibitor into the clinic.

On cell selectivity

Another major benefit promised by degraders is the ability to improve cell selectivity, offsetting on-target but off-tissue toxicity that can sink a drug. After all, there are an estimated 600 E3 ligases in the human proteome, each with a different cell expression profile. If drug developers can properly harness more of these, they might be able take on tantalizing targets that need a surgical strike. Here, Dialectic Therapeutics is trailblazing with its BCL-XL degrader DT2216.

BCL-XL is an anti-apoptotic protein that drug hunters have circled for decades. Abbott and Roche made it to phase II trials with navitoclax, which frees up apoptotic activity by inhibiting both BCL-XL and the related BCL-2. But on-target toxicity killed platelet cells, resulting in dose-limiting thrombocytopenic toxicity. Could a degrader be used to achieve platelet-sparing activity, wondered Guangrong Zheng and Daohong Zhou, both at the University of Florida?

Industry insiders suspect that many of the front-running heterobifunctional degraders rely on cereblon as their E3 ligase of choice. But cereblon is expressed nearly everywhere. Only a handful of other ligases have been successfully co-opted for preclinical degraders as yet. Zheng and Zhou realized, however, that the VHL ligase is poorly expressed in platelets. Reporting in *Nature Medicine* in 2019, they showed that DT2216 — navitoclax fused to a VHL-recruiting warhead — is better at killing cancer cells than is navitoclax, while also being less toxic to platelets.

Zhou and Zheng co-founded Dialectic to advance this academically originated candidate into the clinic. "We really had translation in mind from the beginning," says Zheng. The only changes that Dialectic made were with regard to formulation, for intravenous delivery, adds David Genecov, another co-founder of the company, and a co-founder of the gene therapy firm AveXis.

DT2216 is now in a phase I trial. "This is a first test of whether we can use a degrader to overcome on-target drug toxicity," notes Zhou. Because of the focus on platelet-sparing capabilities, a key readout will be the effect on platelet count. The lead indications for DT2216 are T cell lymphoma and small cell lung cancer.

The ability to use degraders to tune for selectivity in other cell types will ultimately depend on access to an arsenal of E3 ligases with different expression profiles. Researchers in both academia and industry are working on identifying contenders, but it remains slow going. "We're getting better and better at it as a community. But it's really, really hard," says Mark Rolfe, Senior Vice President of Oncogenesis at BMS.

Sticking with it

Low-molecular-weight molecular glue degraders are also gaining steam.

These candidates are exemplified by BMS/Celgene's IMiD lenalidomide, a blood cancer drug that earned $12 billion in 2020. The FDA approved lenalidomide in 2005, but it was only in 2013 that researchers traced its activity back

to its ability to make use of the E3 ligase cereblon to degrade Ikaros and Aiolos. This realization fuelled interest in heterobifunctional degraders, showing that IMiD-like molecules could be turned into cereblon-recruiting warheads. But it also showed that degraders need not rely on ligands and linkers to deliver targeted activity. Instead, molecular glues reshape or create new surfaces on E3 ligases to facilitate degradation.

BMS, which acquired Celgene in 2019, is capitalizing on its accumulated experience with lenalidomide and the rest of the IMiDs. Before the degradative capabilities of these agents had been unravelled, the company used phenotypic screening to discover its next-generation IMiDs. Afterwards, it realized that phenotypically discovered agents such as iberdomide "still left something on the table with respect to depth and efficiency of degradation of Ikaros and Aiolos," says Rolfe.

With CC-92480 and CC-99282, the company has put degrading activity front and centre. These candidates are tuned to maximize both the speed and depth of Ikaros and Aiolos degradation, says Rolfe, while curtailing the degradation of neosubstrates that contribute off-target toxicity. CC-92480 is optimized for bone marrow distribution, where myeloma largely grows. CC-99282 is designed for activity in the lymph nodes and spleen, where lymphoma grows.

Preliminary phase I data for CC-92480 in multiple myeloma are promising, says Rolfe, and combination trials of the drug are ongoing. The company has yet to report phase I data on CC-99282 in lymphoma, "but we're excited about that one as well," he adds.

BMS will soon face competition. C4 Therapeutics has been benchmarking its cereblon recruiters against BMS/Celgene's published structures. "They've done really good work, so we have to do better to compete here," says Fisher. C4 Therapeutics' lead compound, CFT7455, is 100-fold more potent than CC-92480, he adds.

C4 Therapeutics will present preclinical data on CFT7455 at the upcoming AACR. The company plans to advance the candidate into the clinic within months.

All three of these agents could one day be case studies for the benefits of understanding the mechanism of action of a phenotypically discovered drug. But lenalidomide has derisked the way.

Novel neosubstrates too

Glue degraders can take on novel targets as well, including ones that are inaccessible to heterobifunctional degraders. Whereas most small molecules rely on ligand–protein interactions for activity, glue degraders seem to capture their targets by modulating protein–protein interfaces. Ikaros and Aiolos are both zinc finger transcription factors, for example, targets that are hard to drug with small molecules because they lack a catalytic active site.

Novartis has already discovered and advanced a glue degrader that binds another zinc finger transcription factor. "Helios is an attractive cancer target, both because of its role in immuno-oncology signalling and the provocative suggestion that it is an intrinsic cancer cell dependency," says Bradner.

A phase I trial of this degrader, DKY709, is ongoing in advanced solid tumours, as monotherapy and in combination with Novartis's anti-PD1 PDR001.

For Bradner, this programme exemplifies how molecular glues can disrupt novel targets. Although chemists are overcoming the lack of drug-likeness of heterobifunctional degraders, the pharmacological advantages of low-molecular-weight glues are not to be underestimated either. "We opportunistically create heterobifunctional degraders where appropriate, but our concerted emphasis has been around intractable targets and molecular glues," says Bradner.

BMS's GSPT1-degrading CC-90009 shows that glue degraders can take on other classes of proteins too.

GTPases are difficult to drug both because GTP concentrations in the cell are high and because the GTP-binding pocket binds GTP strongly. As a result, says Rolfe, it is hard to make compounds that can compete. In 2016, researchers from Celgene reported in *Nature* that they could degrade the GTPase GSPT1 with a cereblon glue, and that patient-derived acute myeloid leukaemia cells were highly sensitive to this agent. They then optimized a follow-on compound, CC-90009, to maximize GSPT1 degradation and minimize degradation of Ikaros, Aiolos and other neosubstrates associated with toxicity.

CC-90009 also kills acute myeloid leukaemia blasts and leukaemia stem cells, they recently reported in *Blood*. It entered phase I in 2016, and is now being combined with various agents.

"It's an undruggable target that we can degrade to get clinical benefit for patients. It's super exciting," says Rolfe.

But drug developers may have to adopt a different target-selection mindset when working with molecular glue degraders. With bifunctional degraders, they can choose a target up front and then look for relevant ligands that they can use to trap it. With molecular glues, by contrast, they are constrained by the activity profiles of the small set of E3-recruiting molecules that have been identified to date. "It's about having diversity of E3 ligase binders, and asking 'do any of these have activity against targets I want to pursue'", says Fisher.

BMS has had the same experience. But there are still plenty of targets to choose from. Only around "24 to 30" IMiD neosubstrates have been reported to date, but "the final tally is going to be much, much, much higher than that," says Rolfe. Rupert Vessey, BMS's head of R&D, has pegged the accessible neosubstrate number in the hundreds already, many of them "literature deserts".

Resistance bands

As heterobifunctional and glue degraders move through the clinic, drug developers will scrutinize trial results to identify both the challenges and opportunities ahead.

As always, safety needs to be watched. The regulatory perspective here is encouraging, however. "Our experience with the FDA has been very straightforward. There hasn't been anything that you wouldn't expect for any small-molecule drug discovery programme," says Fisher. "That's the beauty in some ways of this approach. We're still small molecules."

Beyond efficacy, there are other readouts researchers are also keeping a close eye on. Pharmacodynamic and pharmacokinetic data, and the ability of these degraders to deliver on their event-driven promise by providing durable target coverage with low doses, will be key. So too is depth of target degradation, and how well preclinical and animal data can be used to predict degradation levels and target protein resynthesis rates in humans.

Then there is resistance to these agents, which will arise through mutations in the degradation pathway. "This is not a hypothetical consideration. We should expect emergent resistance," says Bradner. This view is backed by lessons learned from lenalidomide in the clinic as well as preclinical CRISPR screens. The speed with which these mutations accumulate and the ability to use combination strategies to slow resistance down will be important in the short term. Access to a broader set of E3 ligases will also be important here, if the community hopes to keep focusing on cancer applications.

Other limitations might arise as well. Despite hopes that oligonucleotide drugs would unlock a world of undruggable targets, that field is still grappling with delivery to tissues other than the liver.

But as more degraders enter the clinic, the community will at last get to evaluate which of the preclinical benefits are hype, and which are helpful. "You cannot judge an approach on a single molecule," says Churcher. "It's encouraging that we now have a broad range of molecules in the clinic."