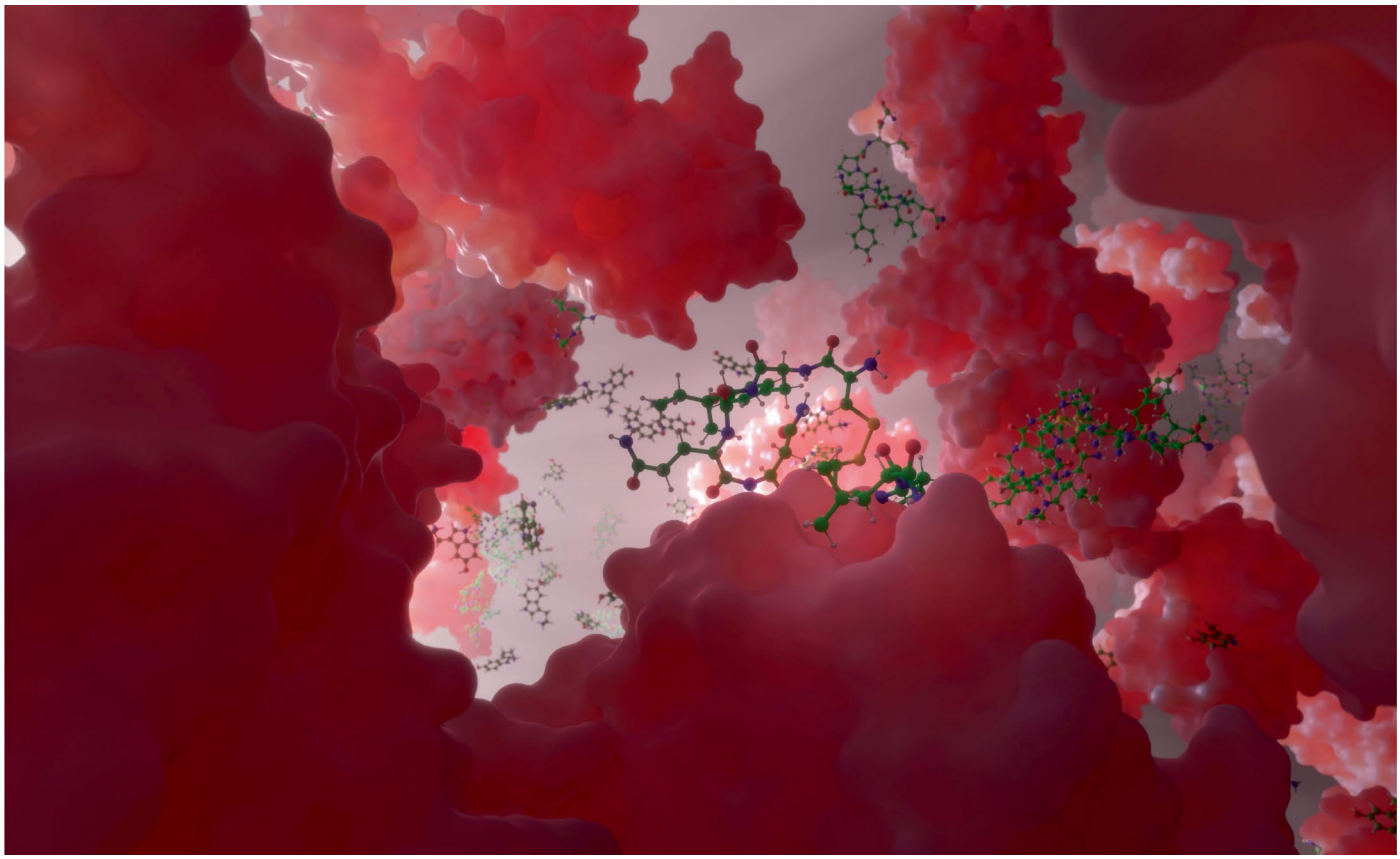
https://doi.org/10.1038/s41587-024-02164-9



THE GLUE DEGRADERS

Companies are hoping to discover small molecules that remove undruggable proteins. It won't be easy. **By Ken Garber**

In December 2023, two days after the US Food and Drug Administration approved separate gene editing and gene therapy treatments for sickle cell disease, Novartis biochemist Pamela Ting made a plenary presentation at the American Society of Hematology annual meeting[1]. She described a phenotypic screen that yielded hits causing a surge of fetal hemoglobin, the same protein that the recently approved gene editing therapy is engineered to produce. But unlike that treatment, which is priced at $2.2 million, Novartis's compounds are small-molecule protein degraders, molecular 'glues' that would be much cheaper to produce and administer. Animal studies were positive. "We are currently conducting the experiments necessary to translate these findings to a human clinical trial," Ting said at the meeting. The Novartis work is the latest sign that molecular glue degraders, which hijack the cell's disposal machinery to remove disease-related proteins, have arrived.

Much of pharma is invested, directly or through partnerships. In 2019 Bristol Myers Squibb spent $74 billion to acquire Celgene and its portfolio of molecular glue degraders. More than two dozen biotech companies are now seeking these drugs (Table 1). "We're very active in this space and see tremendous potential in molecular glues," says Ryan Potts, head of the induced proximity platform at Amgen.

Yet the field faces some serious obstacles. Prospective screening for molecular glue degraders is a major undertaking (Fig. 1). It's often done in cells, unlike standard biochemical assays with recombinant proteins, adding time and expense, and involves extensive follow-up work to validate hits and understand mechanism of action. And those hits are rare because it is hard to drug protein–protein interactions. With hit rates low, small-molecule libraries must be sizable. And the field does not yet know what chemical features molecular glues have in common, making it difficult to select these libraries. Biological information on the more than 600 E3 ligases — the enzymes that molecular glues recruit to degrade a drug's target — is scant, except for a handful of these proteins. For all these reasons, molecular glue discovery remains a high-risk enterprise. "The field needs a success story," says Simon Bailey, head of drug discovery at Plexium.

Table 1 | Selected molecular glue degrader companies discussed

Company	Pharma partners	Discovery approach	Deployed E3 ligases	Lead program
Monte Rosa Therapeutics	Roche	Remodel cereblon to recruit neosubstrates; proximity assays, proteomics	Cereblon	MRT-2359, GSPT1 degrader, phase 1 (cancer)
Plexium	Amgen, AbbVie	Miniaturized, cell-based DNA-encoded library screening; target-centric	Cereblon, DCAF11, others undisclosed	IKZF2 degrader, phase 1 (cancer) December 2023
Seed Therapeutics	Eli Lilly	Target centric; detect basal E3–target interactions; proximity assays	Working with 25–30 E3s, including DCAF15	ST-00937, RBM39 degrader (cancer), IND filing, 2H24
Novartis	Dunad Therapeutics	Phenotypic screens, cereblon binders, others undisclosed	Cereblon, others undisclosed	Wiz degrader (sickle cell anemia), IND-enabling studies
Proxygen	Boehringer Ingelheim, Merck KGaA, Merck & Co.	Broad range, from unbiased phenotypic screens to target-centric	Many; undisclosed	Undisclosed
A-Alpha Bio	Amgen, Bristol Myers Squibb, Kymera Therapeutics	Detect basal E3–target interactions using yeast cell surface display, mutagenesis to interrogate interface	Many; undisclosed	Undisclosed

Others in this space include Ambagon Therapeutics, Astellas Pharma, AstraZeneca, Bayer, Biotheryx, Celgene (Bristol Myers Squibb), ChemPartner, Coho Therapeutics, Degron Therapeutics, Gandeeva Therapeutics, GSK, GluBio Therapeutics, Magnet Biomedicine, Neomorph, Orionis Biosciences, PhoreMost, Pin Therapeutics, Progenra, Proximity Therapeutics, Ranok Therapeutics, Revolution Medicines, Salarius Pharmaceuticals, SK Biopharmaceuticals, SyntheX and Triana Biomedicines. IND, Investigational New Drug.

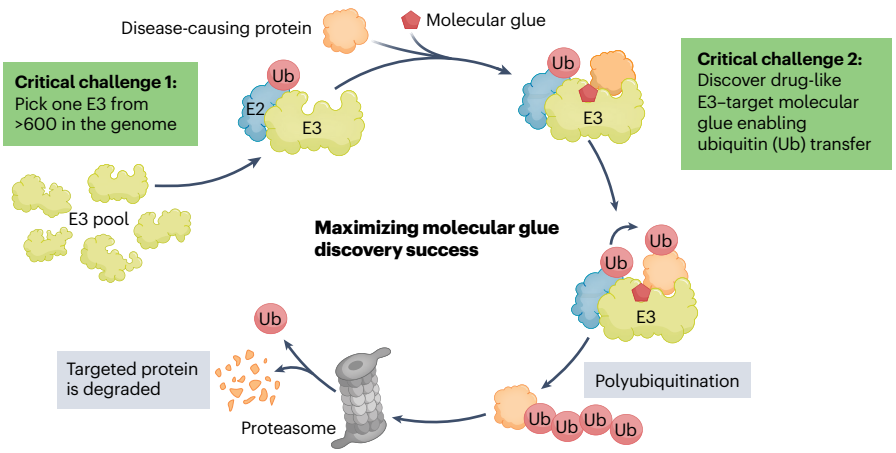


Fig. 1 | Molecular glue degraders. Molecular glue degraders recruit target proteins to ubiquitin ligase enzymes, which attach ubiquitins that deliver the target for disposal.

Some natural product molecular glues, identified after the fact, are successful drugs. Harvard chemical biologist Stuart Schreiber first coined the term 'molecular glue'[2] after his lab showed, using affinity chromatography, that the immunosuppressants cyclosporine and FK506 bind and remodel proteins that then recruit the ultimate target, calcineurin[3]. The term faded from use. Then University of Washington structural biologist Ning Zheng recoined it for the plant hormone auxin, the first molecular glue degrader to be described[4], in 2007. Auxin sits in the gap between two proteins to boost an existing protein–protein interaction. All molecular glue degraders do one or the other – either enable a new protein–protein interaction or enhance an existing one. Either way, this three-way cooperative interaction forms a ternary complex that triggers ubiquitin transfer and substrate degradation[5].

Though high risk, the rational development of molecular glues promises high reward. Less than a quarter of cellular proteins are thought to be druggable, with the rest lacking pockets for small molecules to bind. For example, transcription factors cannot be targeted by standard methods. Yet the IMiD (immunomodulatory imid drug) class of molecular glue degraders, epitomized by Revlimid (lenalidomide), degrade transcription factors. "That by itself is super-exciting," says Ben Ebert, chair of medical oncology at the Dana-Farber Cancer Institute.

Thalidomide's redemption

The molecular glue degrader story is intimately tied to thalidomide's. Originally a nausea treatment for pregnant women, thalidomide was shelved in the early 1960s because of horrific birth defects. It was approved for leprosy in 1998 and for multiple myeloma in 2006, but its mechanism remained unknown. In 2010 Hiroshi Handa's group at the Tokyo Institute of Technology discovered that thalidomide bound the E3 ubiquitin ligase cereblon[6]. (Handa first assumed the drug inhibited ubiquitination.) The 2014 revelation by Ebert's group and two others[7–9] that Revlimid, a thalidomide derivative, is a molecular glue, enabling the ubiquitination and degradation of two transcription factors, would soon open the field.

Revlimid's sales exploded (to $9.2 billion in 2018), but few saw business opportunity in molecular glues. "The predominant view was that this was the very limit of what you could do with such things," said Philip Chamberlain, co-founder of molecular glue company Neomorph, at a 2022 symposium sponsored by Guggenheim Securities[10]. "At meetings, this was the consensus opinion: 'This is great, nice trick, but you'll never be able to do anything new.'" But then more glue degraders, identified retrospectively, appeared[11,12], as well as

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novel degradation substrates (targets) for cereblon, the E3 ligase that is bound by the IMiD drugs. The conviction grew that molecular glues could, in theory, be prospectively discovered or designed to target otherwise undruggable proteins. Companies soon formed around this concept. Unlike bulky PROTACs[13], which connect target and ligase binders with a linker, molecular glues are low-molecular-weight compounds – a delivery advantage – and they do not need to bind to the target protein, just sit in the interface of a protein–protein interaction.

Gluing to cereblon

All marketed glue degraders were identified retrospectively. Making new ones on purpose is not simple. "That's not turnkey, in general," says Ebert. "The central challenge is, you're not trying to drug just one protein, you're trying to manipulate a broad protein–protein interface."

Companies employ a range of strategies. These go from the very narrow – screening for binders to a single E3 ligase against specific targets – to unbiased phenotypic screens looking for degradation events, then working backwards to identify E3, target and mechanism. Each has its advantages and drawbacks.

Boston-based targeted protein degradation company Monte Rosa Therapeutics works at one end, using the cereblon E3 ligase to degrade specific targets. Co-founder Raj Chopra, while at Celgene, led the team that identified the first cereblon degradation substrates[9], and saw potential for new ones, rationally discovered. "From an early stage, the goal was to take the guesswork or serendipity out of identifying these molecular glue degraders," says Sharon Townson, Monte Rosa's chief technology officer.

Monte Rosa's hypothesis is that remodeling the surface of cereblon with different molecular glues can recruit a variety of proteins for degradation. Most reported cereblon substrates are zinc finger transcription factors that feature a degron (the degradation-enabling structure) called the G loop. Monte Rosa uses artificial intelligence tools to predict how different small molecules will reshape cereblon's surface to recruit and degrade G loop-containing proteins and others. The company synthesizes such molecules, often based on known cereblon–molecular glue co-crystal structures. It then screens them against proteins and in cells, identifying the degraded proteins using biochemical and cellular proximity assays to confirm protein–protein interactions and using proteomics to verify degradation events and to ensure specificity.

This work flow, says Townson, is not exceptionally long. "We're applying a lot of the rules that have been defined for how to effectively develop selective small molecules, but now just applying it to glues," she says. "Which just carry slightly different types of assays and requirements. But I wouldn't say it was that much different in terms of heavy lifting."

But the proteomics part, says Ebert, can be limiting. "If we could do proteomics ten times better, or ten times cheaper, that would be fantastic for the field," he says.

The Monte Rosa hypothesis that chemical diversity could remodel cereblon's surface to identify novel targets so far appears justified. "We've now identified new targets through that screening effort," says company CSO Owen Wallace. "And those targets actually bind in many cases quite uniquely and differently to cereblon than would have been predicted." Two of the company's disclosed pipeline programs target proteins – cdk2 and Vav1 – that do not contain the classic G loop structure. (Two other pipeline projects do display G loops.) "This is why we're still very much excited about cereblon," says Townson. "We're basically teaching it new tricks, and we don't really know the limit of the target space at this point, because we keep finding novel discoveries."

Expanding the ligase universe

Cereblon's degradation versatility is a welcome surprise to the field. "New chemical matter is leading to the recruitment of more neosubstrates than we ever thought possible," says Ebert. This glue discovery strategy works, he adds: "There are drugs that have come out of that and have moved forward." But none are close to approval. And the cereblon molecular glue target space is still limited. "It's absolutely critical to identify new ligases," says Ebert. "Only a small percentage of the proteome will have a sufficiently complementary protein–protein interface with cereblon." Degrading more targets will require other E3 ligases.

Companies are working on this. At the other extreme from Monte Rosa's tight focus on cereblon is Proxygen, a Vienna biotech that spun out of the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences in 2020. CeMM investigator and Proxygen cofounder Georg Winter's lab developed an unbiased phenotypic screen for molecular glue degraders that immediately became the gold standard[14]. For that project, the group tested 2,000 cytotoxic compounds against individual cells lines mutated to eliminate neddylation, a post-translational modification required for E3 ligase activity. Winter's lab was seeking compounds that killed only cells with active E3 ligases present, indicating protein degradation. It then used a variety of methods to identify the target, the ligase and the mechanism of action, which in the case of a cyclin K degrader was completely unanticipated.

"That was the idea behind Proxygen, to really pick this up and develop it into a glue degrader platform," says Proxygen CEO Bernd Boidol. It has expanded and evolved since then. Boidol declines to elaborate, except to say that molecular glue assays range from the very broad – like the Winter cytotoxicity screen – to target- and ligase-specific assays. "There are companies with one single approach or one target pair that they throw everything against the wall that they have," says Boidol, citing the Monte Rosa example. Proxygen, in contrast, is more academically oriented (it has avoided venture capital). "Some of these mechanisms we would never have been able to uncover if we were going for a purely structural approach," Boidol says. "We really try to find out about glue degraders, E3 biology, as much as we can, in order to … bring it to the next level."

At the same time, screening purely for an outcome, because it requires target identification and mechanism validation, adds time, expense and technical risk to an already cumbersome and unproven discovery enterprise. Proxygen, Boidol says, tries to offset that risk with "a very nice mix of targets that are … more validated clinically, to strike a nice balance between technical and biological risk."

Sticking without glue

Molecular glue company Seed Therapeutics, like Proxygen, is looking beyond cereblon. It's a majority-owned subsidiary of Beyond-Spring Pharmaceuticals, a drug company co-founded by Lan Huang, who published the first E3–E2 crystal structure[15], and Ning Zheng, who solved the structure of auxin bound to its transport inhibitor response 1 (TIR1) receptor[4].

Seed emphasizes proper E3 selection. The discovery process is lengthy: pick a candidate E3 on the basis of complementarity with the target protein (as predicted by AlphaFold and other computational methods) and cell location of the E3; detect a basal E3–target interaction in a cell system; confirm ability of the E3 to ubiquitinate the target; and perform high-throughput screening for degraders, followed by validation assays and then medicinal

chemistry to improve drug potency, specificity and longevity. "The goal is to select an E3 that has the best chance of making it through all the tests, especially HTS [high throughput screening] and cell-based activity, because we don't want to go through all that, and in a year or more later have to turn around and pick another E3," says Seed president and CSO James Tonra.

It's critically important, says Tonra, to show that the E3 and the target already interact weakly in cells, without the glue. "It's definitely a prerequisite; it's been shown in every molecular glue system that's known," he says. For example, in 2022 Zheng reported that cereblon and two of its substrates interact without the glue[16]. These interactions take place at higher-than-normal protein concentrations — dissociation constants are in the micromolar range — so they are "biologically inconsequential," Zheng said in a recent talk at the Dana-Farber Cancer Institute[17]. "But once they're pushed into the nanomolar range by a small molecule, they will become productive. And in the case of E3 target interaction, it will lead to target protein degradation."

Ebert shares this view. "These are interactions that don't happen natively in a cell, but there's a high degree of complementarity of the proteins, the surfaces," he says. "They are almost interacting, but not quite, and the glue provides just a little extra binding energy to help to stabilize that protein–protein interaction."

A-Alpha Bio, a Seattle-based company that spun out of the University of Washington in 2017, is premised on molecular glues working this way. The company has collaborations with Bristol Myers Squibb, Amgen and Kymera Therapeutics. "Our view is that it is far more likely that a weak interaction is enhanceable, rather than creating an interaction out of nowhere," says CEO David Younger. The company uses a high-throughput yeast cell surface display system to test for these interactions. It engineers yeast cells to express different intracellular proteins on their surfaces by fusing a given protein to a yeast anchor protein, typically a cell wall protein, and to a signal peptide sequence involved in protein secretion. These yeast cells are mixed and shaken in liquid culture, with protein–protein interactions leading to cell fusion. The diploid cells that result are sequenced to identify the interacting proteins. Biophysical validation assays follow. Then the company mutates the protein–protein interface to see whether the interaction can be strengthened — for example, by forming a pocket for a small molecule. This in turn informs structural models for the rational design of molecular glues. The main goal is to reduce the high risk of glue discovery by first identifying and optimizing existing protein–protein interactions. "They're out there, they're prevalent, but if one were to randomly select two proteins and try to embark on a small-molecule discovery campaign, it would be fraught with a lot of risk," says Younger.

Potts says that A-Alpha Bio helped Amgen discover several weakly interacting ligase–target pairs for new molecular glues to enhance. "This combination of experimental evidence combined with in silico modeling will hopefully allow us to do things more rapidly and effectively," he says. But until drugs emerge, that remains unproven.

And finding basal interactions is only one way to shorten the discovery process. "It's a first step," says Boidol. "It's like … using a flashlight to shine on a huge field in the dark. I think it's one of these flashlights, but there must be more flashlights, otherwise it's going to be very, very tedious."

What makes a good glue?

Another flashlight is a glue-like small molecule library. "Rationally designed proprietary libraries are absolutely critical to success in this space," said Neomorph's Chamberlain in his 2022 Guggenheim talk. Such libraries exist for cereblon glues because cereblon structure and IMiD pharmacology are known. "Our increased understanding has really given rise to the design of more and more chemical structures that we anticipate will open up more and more targets," says Monte Rosa's Wallace. But other such libraries are scarce. "How would you build something like that?" Proxygen's Boidol asks. "Likely by looking at glue degraders that are already around." Few of these recruit non-cereblon ligases.

"The molecular features that make a good molecular glue have not been determined," agrees Seed's Tonra. "I don't know that they will be, though. I think with a new E3 and a new target it all might change." Even structural predictions for compound–pocket binding might not work, because a glue could have an allosteric effect, binding anywhere on the surface. To cover all possibilities, Seed incorporates scaffold diversity and three-dimensional structures into its screening libraries, along with features that might be important, such as brain permeability for CNS drugs. But, for finding glues, which are rare, unfocused conventional libraries must be very large — up to a million compounds — and are thus expensive to acquire or synthesize and to screen, since each well must be separately analyzed and each target requires a new functional assay.

As the field learns more about ligases, Potts says, screening efficiency will improve. "We may not be able to design molecules rationally, but we can certainly go from finding a rare needle in a haystack to … building haystacks of needles," he says. One solution that Amgen uses is DNA-encoded libraries (DELs), where compounds are ligated to DNA sequences, which serve as barcodes[18]. These tagged compounds are pooled with an affinity-tagged target on a bead, and those that bind are isolated, eluted, PCR amplified and sequenced. Such libraries can include a billion or more compounds, since they are not analyzed individually, while PCR amplification means only picomolar quantities are needed. All this saves time, reagents and money while boosting library numbers. Potts says DELs have allowed Amgen to screen against many ligases. "You can then start to build molecular glue libraries in a little bit more rational, directed fashion, off of DNA … DELs for the first step, then you can go for more traditional high-throughput screening approaches afterwards." Schreiber's group recently reported new molecular glues using DELs[19].

DELs have limitations. They are hard to screen against membrane proteins, which are critical drug targets, because these proteins are not easily kept in solution and captured on beads. Compound synthesis conditions must be compatible with DNA synthesis, which limits the chemistries that can be employed and thus the library diversity. Also, DEL screening can identify binders, not degraders, requiring follow-up assays.

Screening DELs in live cells solves some of these problems. Plexium, another Amgen collaborator, uses a miniaturized DEL screening platform to interrogate live cells. This approach, says Plexium's Bailey, gets around some of the chemistry limitations of synthesizing compounds together with DNA. (They're arrayed separately on beads, which are individually deposited in wells containing the target protein.) After cells are added to the miniature wells (88,000 per plate), UV light releases compound from bead, the cells are lysed, a fluorescent antibody to the protein of interest is added, and an optical scanner measures relative fluorescence. For hits, Plexium repeats the assay in standard 96-well plates using non-bead versions of the compounds, confirming degradation by western blot and in the presence of a proteasome inhibitor and then a neddylation inhibitor to verify that the proteasome is involved. CRISPR knockout of

individual E3 ligases in cells then identifies the ligase. "In principle, any of the ligases in a cell can be co-opted into a degradation process," says Bailey.

Advancing in the dark

A major bottleneck for the field is the paucity of biological knowledge around the hundreds of E3 ligases. "According to our founders, 20 to 30 E3 ligases are characterized reasonably well," says Seed's Tonra. "That leaves a lot that aren't." Lacking reagents and turnkey assays, including ubiquitination assays, many companies avoid these ligases. Tissue expression may not be known. Also, only E3s that ubiquitinate lysine 48 on ubiquitin proteins (to enable ubiquitin chain formation) are likely to lead to degradation in the proteasome, the desired outcome, and for most E3s the ubiquitin target residue is unknown.

Cereblon may have special qualities. "Cereblon is very, very unique," says Monte Rosa's Townson. "The surface has been shown to be highly adaptable, it's ubiquitously expressed, it's very robust in terms of its degradation activity." It is so far unclear whether other E3s can be reprogrammed by molecular glues this readily. Monte Rosa is looking. "Having a broadly expressed E3 ligase and having another version of [cereblon] would be foundationally a big breakthrough for the field," Townson says.

"The biggest bottleneck is, how do we expand the E3 toolbox?" agrees Plexium's Bailey. "The number of E3 ligases that have been shown to be redirectable to degrade therapeutically useful proteins is very small. And over the last five years there's been intense effort to increase that number. And now much of the discussion in the field seems to be recognizing that that's very difficult." Companies have pulled back from novel E3 ligases, he says. "Now there's more talk about exploiting things like cereblon further, whereas two or three years ago it was all about getting away from cereblon."

Companies are being cautious, mostly advancing cereblon glues to the clinic. Plexium's clinical lead is a cereblon molecular glue that degrades the IKZF2 transcription factor. A clinical trial began in December. Monte Rosa has a cereblon molecular glue in phase 1 that degrades GSPT1, a translation termination factor, for myc-driven cancers. True, Seed's lead molecular glue employs the DCAF15 E3 ligase to degrade RBM39, a protein involved in pre-mRNA splicing. But it was preceded in the clinic by two other RBM39 degraders (whose mechanism was discovered after the fact.) Celgene, meanwhile, is advancing new cereblon degraders.

Clinical trials for intentional molecular glues have disappointed. GSPT1 degraders from Celgene and Biotheryx are no longer in development. Novartis discontinued its IKZF2 degrader after phase 1. The field still awaits clinical proof of concept. "We'll find out soon enough, over the next couple of years," says Potts. To their credit, molecular glue companies are mostly going after undruggable targets, which is important to validate the field. "There are a lot of targets where people want to play kind of safe," Boidol says. "Which is absolutely legit. But I think in order to really bring the message across that TPD [targeted protein degradation] is a modality that has advantages far beyond attacking proteins that are already treatable with inhibitors, that proof has to be made."

There should be time for that, although some companies will drop out. For all the activity across pharma and biotech, the molecular glue field is still young. "We're constantly reminded that we're at the beginning of this story, not the end," says Ebert. "We're still discovering new mechanisms, and that is why we think the field has so much long-term potential — that as we learn more mechanisms, and we're able to degrade or manipulate more proteins, there's going to be a whole world of new targets."

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Published online: 06 March 2024

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