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p53 programmes plough on

Despite recent setbacks with p53-activating small molecules including the nutlins, the cancer target keeps drug hunters coming back for more. Could immuno-oncology combinations, stapled peptides and targeted degraders unleash the therapeutic potential of the 'guardian of the genome'?

Asher Mullard

When Lyubomir Vassilev, then a scientist at Roche, started in 2003 to write up his team's discovery of small-molecule inhibitors of the p53–MDM2 interaction, he knew the paper would be big. p53 is the most frequently mutated gene in human cancer, after all, and his group had found tool compounds that could selectively boost the activity of the tumour suppressor. Disregarding the convention of naming new chemical matter with impenetrable company codes, he instead dubbed these compounds nutlins, after the facility in Nutley, New Jersey, where they were discovered.

The nutlins were set to become a 'household' name in the research community, Vassilev believed, and a complicated moniker would be unfair to the many researchers who would want to use and remember these compounds. "The idea was met with a strong resistance from the management, but I did not surrender," he recalls.

This work — published in *Science* in 2004 — provided a first glimpse into a programme that has now been running for more than 20 years at Roche. It attracted competitors including Sanofi and Merck & Co. to p53. It re-invigorated industry interest in the potential of drugging protein–protein interactions (PPIs). It has been instrumental in the invention and validation of new therapeutic modalities. And with 4,365 citations on Google Scholar, the *Science* paper has been referenced on average nearly five times a week since it was published.

2020 could have been another landmark year for the nutlins. Roche's phase III trial in acute myeloid leukaemia (AML) of idasanutlin — a next-generation nutlin — was set to read out this year, with a regulatory submission planned shortly after. Instead, it provides another example of just how hard drug discovery and development can be. The phase III MIRROS trial failed, Roche disclosed at the European Hematology Association Congress in June.

Other companies have also suffered setbacks with small molecules that target the interaction between p53 and MDM2. Novartis disclosed in January that it was discontinuing development of siremadlin (HDM-201) in AML. Amgen has out licensed its clinical candidate, AMG-232, to Kartos Therapeutics for further development, as KRT-232. And J&J, Sanofi and Merck & Co. have all suspended programmes in this space in the past decade.

Some academic researchers are turning their back on p53. "We've actually closed our MDM2 projects, because we think this might be a dead end," says Lukasz Skalniak, a medicinal chemist in a group at Jagiellonian University that had been working on MDM2-targeted small molecules for a decade. These drugs might still have roles in the right combinations and the right cancer settings, he says, but they are unlikely to offer the broad-based anti-cancer activity the community once hoped for.

Others are persevering, convinced that they can crack this 'undruggable' target.

Small molecules seem to be stalling, but along with stapled peptides, targeted degraders and even mRNA-based drugs, they might still deliver. "These things go in cycles," says Anthony Partridge, a senior principal scientist at Merck & Co. who is working on stapled peptides that could unleash p53. "After putting a lot of effort into something and not seeing the return on investment, people can lose appetite for it. But that can change in a heartbeat with the right data."

The appeal of p53

p53 is a complicated protein that has been misunderstood before. First discovered in 1979, it was named p53 on the basis of its apparent molecular mass of 53 kDa by SDS-PAGE analysis. But its actual mass is 44 kDa. And while researchers initially thought that it was an oncogenic driver of cancer development, only later did they realize that it was actually a powerful tumour suppressor that keeps fledgling cancer cells in check. Upon the detection of DNA damage, p53 signalling leads to either cell cycle delay, so that a cell can repair its damage, or to the induction of apoptosis, if the damage is already too far gone.

It has long been a compelling cancer target. Individuals with certain inherited loss of-function-mutations in p53 have a 50% chance of developing cancer by 30 and a 90% chance of developing cancer by age 70. p53-knockout mice develop tumours quickly. And up to 50% of cancers carry p53 mutations in both alleles of the gene. Drugs that can re-activate p53's tumour-suppressing ability, drug hunters have theorized, might therefore hold powerful anti-cancer activity.

While it is easier to inhibit proteins than to activate them, p53's interaction with MDM2 provided a critical way in (FIG. 1). MDM2 not only binds p53 to physically block its tumour-suppressing transactivation domain, but is also an E3 ligase that tags p53 for degradation by the proteasome. By taking out the interaction between p53 and MDM2, the hope is that patients with wild-type p53 will regain its cancer curtailing activity.

Targeting PPIs, however, brings its own challenges. The large and mostly flat surfaces of such interfaces offer few footholds for ligands, and large protein partners are generally unaffected by the small molecules that seek to keep them apart. By the late 1990s, when Roche started working on the p53 programme, the company was already downbeat on PPIs, recalls Bradford Graves, who worked on the nutlins for more than a decade at Roche. "Roche was probably no different than many other companies, who were kind of souring on the likelihood of success of targeting PPIs," says Graves, who is now retired.

But MDM2 possessed a deep hydrophobic groove on its PPI interface — a well-defined pocket that Novartis had developed a small synthetic peptide against — and so Roche set aside its doubts. Preliminary work was slow, says Graves. "We tested our entire compound collection, and there wasn't a whole lot there. That was not unexpected, because we knew that there had been a lot of industrial groups and a lot of academic groups that had tried to do this, and nobody was finding anything." But after toiling away on a few weak hits, they started to see activity.

These compounds became the nutlins. "We had to work really hard on our management to get approval to publish the paper," says Graves. "Their feeling was that we were giving away the farm. But our argument was that this really represented a major step forward not only for the field of MDM2 antagonists but also more generally for the field of PPIs."

"It was spectacular stuff," recalls Michael Andreeff, an oncologist at MD Anderson Cancer Center. "I was super impressed, and I jumped up and down when I saw it in *Science*." Andreeff met Vassilev at a conference, and subsequently partnered with Roche on trials of the nutlins.

The nutlins are indeed also often cited along with AbbVie's BCL-2 inhibitor venetoclax as key to a resurgence of interest in PPI targets. While some of the enthusiasm for PPIs has since waned, current excitement around KRAS inhibitors highlights willingness of large and small firms alike to keep working on these once-dismissed targets. "The pendulum has kind of swung back," says Graves. "It's more in the middle right now."

MDM2 inhibitors in the clinic

Despite considerable investment by various pharmaceutical firms into MDM2 inhibitors over the years, these small-molecule PPI blockers have yet to deliver in the clinic.

The first MDM2 inhibitor into clinical trials was Roche's RG7112, an optimized member of the nutlin family. A phase I trial in solid tumours was initiated in 2007, and a phase I trial in haematological cancers was started in 2008. Andreeff, a lead investigator on the haematological cancer trial, was especially optimistic then — and now — about the opportunity in AML. Although p53 is mutated in around 50% of cancers overall, it is only mutated in 5–10% of patients with AML. Because patients need wild-type p53 to benefit from MDM2 inhibition, this setting holds better odds of activity. MDM2, meanwhile, is frequently overexpressed in AML.

But RG7112 had to be dosed at extremely high levels in this trial and caused off-putting levels of gastrointestinal toxicity, neutropenia and thrombocytopenia. Andreeff and his collaborators concluded that a more potent MDM2 inhibitor was needed.

Idasanutlin, formerly RG7388, filled this role. A nutlin by nomenclature, this small molecule is based on a different chemical scaffold than the original nutlins. The first were *cis*-imidazoline analogs, but idasanutlin is a pyrrolidine. Shaomeng Wang, a medicinal chemist at the University of Michigan, first reported activity with a related spiro-oxindole scaffold in 2005, and he has licensed MDM2 inhibitors based on this chemistry to both Sanofi and to Ascentage Pharma, a biotech he founded that is still active in this space.

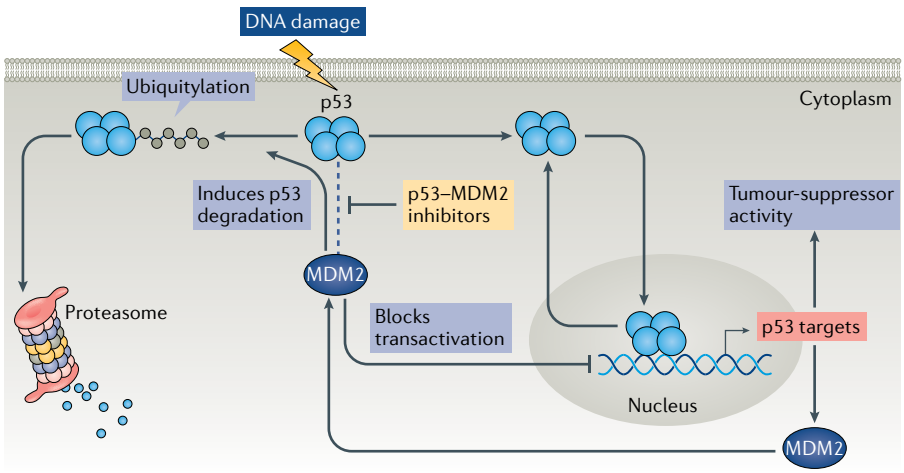

Fig. 1 | **p53 and MDM2.** p53 and MDM2 interact in an auto-regulatory feedback loop. p53 activity increases the expression of MDM2; MDM2 decreases p53 activity by blocking the protein's transcriptional activity and stimulating its degradation. Different cellular signals, such as DNA damage and oncogene activation, induce p53 activation. Inhibitors of the p53–MDM2 interaction can activate p53's tumour-suppressing activity in tumour cells with wild-type p53. Image adapted from *Nature Reviews Cancer*, Springer Nature Limited.

The clinical efficacy with idasanutlin, too, has proven underwhelming. Since the first-in-man study in 2011, Roche has trialled several combinations of the drug in different disease settings in the hunt for activity. The only programme to make it to phase III is the study of the drug in combination with the chemotherapeutic cytarabine in relapsed or refractory AML. But this faltered at a planned interim analysis, the company reported in June at the European Hematology Association Congress. Despite an overall response rate that was nearly double that of the cytarabine-only arm, addition of idasanutlin did not confer a benefit on median overall survival or complete response rates. Roche halted the trial for futility.

Roche had also been developing idasanutlin for first-line AML, hoping that the drug might offer more durable remissions in treatment-naive patients with fewer accumulated mutations and resistance mechanisms. They have now stopped this trial as well. "This decision was made based on the current advances in the AML treatment landscape within our haematology portfolio," says Nancy Valente, senior vice president of oncology product development at Roche's Genentech. "We continue to analyse the overall idasanutlin programme in haematology and solid tumours to help identify areas where idasanutlin has the potential to improve the standard of care," she added.

Table 1 | **Select list of ongoing trials of MDM2 inhibitors**

MDM2 inhibitor	Combination agent (target)	Sponsor	Cancer setting	Status
Idasanutlin	Venetoclax (BCL-2)	Roche	AML	Phase I
Idasanutlin	Atezolizumab (PDL1)	Vanderbilt-Ingram Cancer Center	Breast cancer	Phase I/II
Siremadlin	MBG453 (TIM3) or venetoclax (BCL-2)	Novartis	AML and MDS	Phase I
BI 907282	BI 754091 (PD1) and BI 754111 (LAG3)	Boehringer Ingelheim	Solid tumours	Phase I
Milademetan	Quizartinib (FLT3)	Daiichi Sankyo	FLT3-ITD AML	Phase I
Milademetan	Venetoclax (BCL-2)	M.D. Anderson Cancer Center	AML	Phase I/II
KRT-232		Kartos Therapeutics	Myelofibrosis	Phase III to start
APG-115	Pembrolizumab (PD1)	Ascentage Pharma	Melanomas and solid tumours	Phase I/II
ALRN-6924[a]		Aileron Therapeutics	Chemotherapy-induced toxicity	Phase I/II

[a]Stapled peptide, MDM2 and MDMX inhibitor. AML, acute myeloid leukaemia; MDS, myelodysplastic syndromes.

Trial by combo

Few are surprised by Roche's latest setback with idasanutlin, especially in light of the failures faced by other MDM2 inhibitors in the clinic. Since as early as 2006, researchers were realizing that these agents do not always drive apoptosis in p53 wild-type cancer cells, potentially because of disabled downstream signalling. Cells that are initially sensitive can also develop resistance over time.

Better cancer genotyping might yet point to cancers that will respond, but these look increasingly likely to be niche cancer applications rather than broad ones. And so other strategies are needed for researchers who want to keep working on p53-activating agents.

A possible solution is combination therapies. "I am optimistic that MDM2 antagonists will make their way to the patient's bed with the help of appropriate combination partners," says Vassilev, who is now at EMD Serono.

"It's been clear to me for a long time that we need combination therapies," agrees Andreeff.

There are lots of options to choose from, providing both an opportunity and dilemma. "If you look at the signalling and interaction pathways of this protein, it seems to be connected to everything," says Partridge.

"The biology is incredibly complicated, so really finding the nuances of how to tackle this therapeutically will be a fundamental challenge going forward."

Dozens of combination strategies have been proposed, points out Skalniak, using everything from traditional DNA-damaging chemotherapies to antibiotics, kinase inhibitors, proteasome inhibitors and therapeutic antibodies. Several have already failed, and others remain ongoing (TABLE 1).

Andreeff's favourites include the combination of MDM2 inhibitors with BCL-2-blocking venetoclax. He showed in 2017 in *Cancer Cell* that these agents can act synergistically. p53 drives MCL-1 degradation, thereby overcoming a major mechanism of resistance to venetoclax, and BCL-2 inhibition facilitates p53-driven apoptosis, rather than cell-cycle stalling.

Roche and AbbVie are working together to test this combination, with Andreeff as an investigator. Preliminary phase I/II results at ASH in 2017, 2018 and 2019 showed remission rates of 40–50%. This trial is ongoing.

Daiichi Sankyo is also now testing its MDM2 inhibitor milademetan (DS-3032) with the next-generation FLT3 inhibitor quizartinib in AML, with Andreeff as a collaborator. Novartis previously tested siremadlin in combination with the first-generation FLT3 inhibitor midostaurin in AML, but Daiichi's trial uses a more selective FLT3 inhibitor and is only recruiting patients with a FLT3 mutation that could be particularly sensitive to the combination.

As the field's understanding of p53 evolves, other strategies are emerging. The potential of MDM2 inhibitors as immuno-modulatory agents, especially, is attracting attention because of the role of p53 in tumour immunology. Cancer cells upregulate the expression of PD1 and PDL1 — immune checkpoint molecules that induce and maintain T cell tolerance — via p53. p53 activity also increases the expression of DD1α, a molecule that suppresses T cell activity. "It's becoming increasingly appreciated that nature has selected this protein to be mutated in cancers because in doing so these cancers gain advantages in terms of immune evasion," explains Partridge.

"p53 might be able to be called the master regulator of the immune system, although I'm sure immunologists don't like that," adds Andreeff.

Several companies are exploring this relationship. Boehringer Ingelheim is testing its BI 907282 in combination with both the PD1 blocker BI 754091 and the LAG3 blocker BI 754111. Novartis is running a trial of siremadlin in combination with the TIM3-targeted antibody MBG453. An academic group is trialling idasanutlin in combination with the PDL1 blocker atezolizumab. And Ascentage Pharma has a phase I trial of APG-115 in combination with pembrolizumab ongoing.

"I think the whole field is going to be watching very carefully to see how these immuno-oncology trials bear out. If they are successful, I would predict that people will jump on it," says Partridge.

Sticking with stapled peptides

Another possible explanation for the failures of MDM2-inhibiting small molecules may be that these compounds are too specific to sufficiently reactivate p53. Despite the role of MDM2 in silencing p53, the structurally related MDMX also binds and blocks p53

activity. For Ulrich Steidl, a cell biologist at Albert Einstein College of Medicine, a dual-targeted approach is key to success here. "MDM2-targeting approaches never made too much sense to me, because they just ignore the second p53 inhibitor," says Steidl. "In my mind there's no doubt that you have to go for a dual targeting approach."

Peptide-based strategies currently lead the way here. p53 has a helical region that binds both MDM2 and MDMX, and this helix provides a template for a peptide mimetic that can bind both of these targets. While peptides don't penetrate well into cells, researchers have been trying to show for decades that stapled peptides that are locked into a helical conformation have the stability, cell permeability and efficacy to make it as a new therapeutic modality.

Aileron Therapeutics has been working on this premise since 2005, and its ALRN-6924 is the most advanced stapled peptide in the clinic. The peptide, a dual inhibitor of MDM2 and MDMX, was initially developed in collaboration with Roche researchers including Vassilev. But Roche walked away from this deal by the end of 2013, around the same time that it shuttered its Nutley site.

Researchers elsewhere have questioned the activity of stapled peptides. A team of industry and academic researchers from Genentech and in Australia, for example, called into the question the purported utility of a stabilized BimBH3 peptide in 2013. Stapled peptides have been trying to re-establish their reputation ever since.

Steidl believes that stapled peptides deserve more attention. After finding that MDMX was highly overexpressed in AML, he started looking for dual MDM2 and MDMX inhibitors that he could use to pick apart the biology of their interactions with p53. Aileron provided him with ALRN-6924 to work with, and he reported in *Science Translational Medicine* in 2018 that this stapled peptide had marked antileukaemic effects.

"There is not a shred of doubt in my mind that ALRN-6924 works exactly as advertised," says Steidl, who is a scientific advisor to Aileron. "I think this entire field has just enormous potential, because it can be leveraged to go after things that just haven't been targetable before."

But Aileron is a small biotech with a limited budget, derisking a new modality against a biologically complex target. On the basis of Steidl's research, Aileron initiated a phase I trial of ALRN-6924 in patients with AML and myelodysplastic syndrome in 2016. The company has since scaled back its clinical trial plans. Now, the company's primary goal is the development of ALRN-6924 for the reduction of chemotherapy-related toxicities — a potentially easier path to market, with a myelopreservation end point rather than a survival one.

Aileron's rationale is that the stapled peptide can be used at a low dose to pause the cycling of non-cancer cells that are otherwise affected by chemotherapies that mess with DNA replication. Faster-cycling cancer cells — especially those with mutant p53 that would be unresponsive to dual MDM2 and MDMX blockade — should still be sensitive to these chemotherapies.

Full data from an ongoing phase I/II trial of ALRN-6924 for the prevention of topotecan-induced toxicities in small-cell lung cancer are expected later this year.

Others are meanwhile investing anew in stapled peptides. Merck & Co. and collaborators at the A*STAR, including p53 co-discoverer David Lane, are optimizing stapled peptides against MDM2 and MDMX in the hopes of eventually moving these forward. Partridge, who is heading up this work, is encouraged by what he has seen so far. "These molecules are real, and they have authentic cellular activity," he says.

Merck & Co. is now working on understanding how to best design these peptides to maximize properties such as efficacy, stability, cell permeability and solubility. "I think we're just about to turn a corner as a field really, and once those things become clear then we can go after targets in earnest with stapled peptides," says Partridge.

Modality maker?

Targeted degraders could provide another means of re-activating wild-type p53.

Targeted degraders are bifunctional molecules that bind a protein of interest with one arm and an E3 ligase with the other to co-opt the proteasome to degrade their target. And nutlins played a role in the early history of this emerging modality. The first all-small-molecule targeted degrader, a PROTAC discovered by Yale University's Craig Crews and colleagues in 2008, used a nutlin to recruit MDM2 to kick-start the degradation cascade.

MDM2 has since fallen out of favour as an E3 ligase for targeted degraders. But Crews and colleagues at Arvinas — the PROTAC company he founded — demonstrated in *Cancer Research* in 2018 that there could be reason to revisit it. His team built a PROTAC using idasanutlin as the E3 recruiter, and found that it offered a dual anti-cancer mechanism of action. Not only did this PROTAC drive the breakdown of BRD4, but it also re-activated p53 by blocking the tumour suppressor's interaction with MDM2.

"Not only can nutlin-based PROTACs mediate degradation far more potently and effectively than previously realized, but that by virtue of the p53-stabilizing activity particular to nutlins, the PROTACs derived from them can have biological activity surpassing that of equipotent degraders that harness other E3 ligases," Crews and colleagues wrote.

Wang is also now using targeted degraders to re-activate p53, but in a different way. MD-224 — made by tethering a spiro-oxindole to the Cereblon E3 ligase ligand lenalidomide — induces rapid degradation of MDM2 and re-activation of p53, he reported last year in the *Journal of Medicinal Chemistry*.

Part of the appeal of this approach is the presumed efficiency of targeted degraders. These are event-driven compounds rather than occupancy-driven ones, and so each degrader catalyses the destruction of multiple targets before it is cleared by the body. This is a particularly appealing characteristic for MDM2 blockade, because one of the consequences of p53 reactivation is increased MDM2 expression. Efficient degradation might therefore translate into longer lasting MDM2 suppression with lower and less frequent dosing, potentially alleviating some safety considerations.

"I think MDM2 degraders may be much more effective than MDM2 inhibitors," says Wang, who is exploring opportunities to advance his MDM2 targeted degrader programme into the clinic through a partnership. "I'm pretty excited about this."

At least one other group has discovered an MDM2 degrader, using a nutlin-based ligand and a lenalidomide-based E3 recruiter.

p53 could also push the boundaries with other modalities. "There's nothing like p53," says Andreeff. "There's a never ending stream of discoveries around this protein."

In a recent *Science Translational Medicine* paper, for example, researchers showed how mRNA-based therapeutics might have potential for the many cancer patients with mutant p53. Jinjun Shi, of the Harvard Medical School, and colleagues used a nanoparticle-based approach to deliver p53 mRNA to p53-null cancer cells. This led to expression of functional p53 in these cells, and delayed cancer cell growth. When they combined this approach with the mTOR inhibitor everolimus in animal models of disease, they saw marked anti-tumour effects.

"I think this is sort of emblematic of where the field is headed," says Partridge. "This is arguably the most important target in human cancer, and so I would expect scientists to continue to be interested in it and to keep applying new approaches to tackle it."