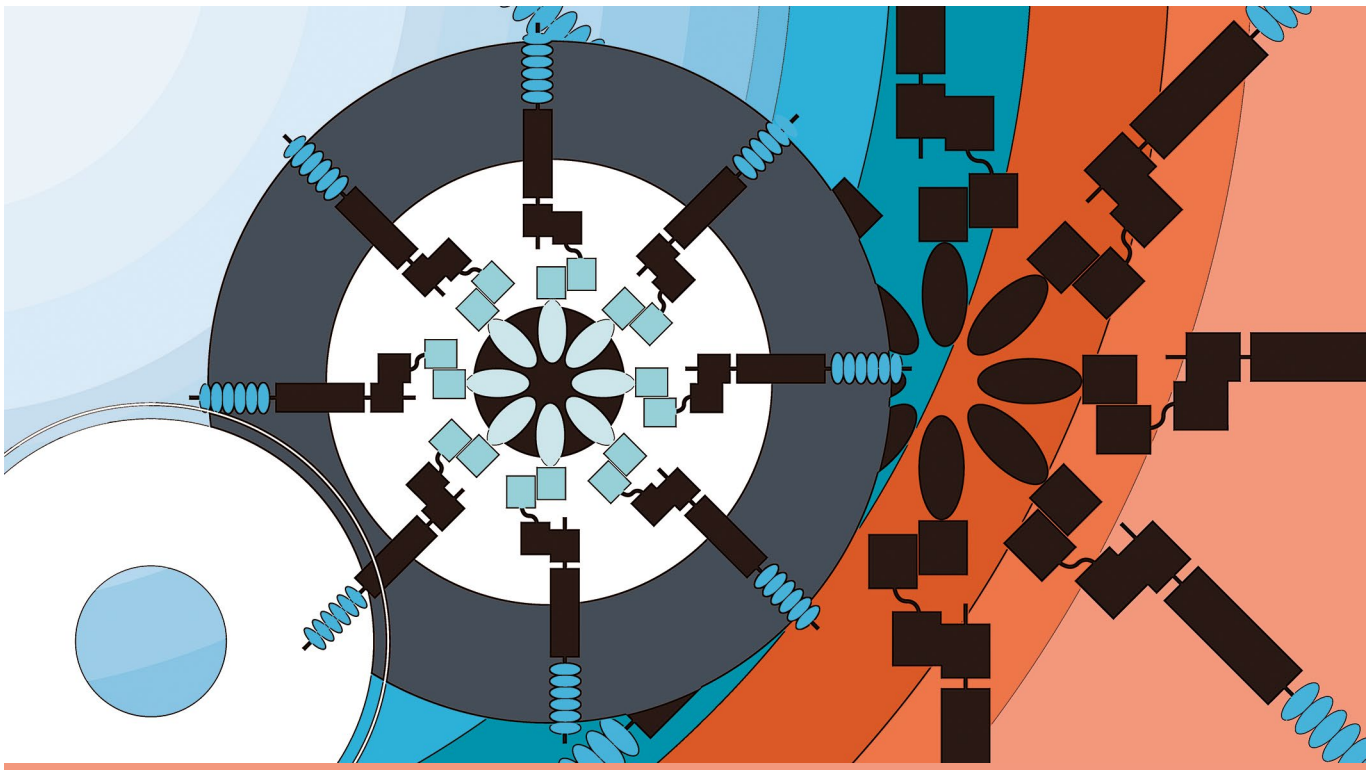

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NLRP3 inhibitors stoke anti-inflammatory ambitions

Inhibitors of the innate immune system's NLRP3 inflammasome promise potential in Parkinson disease, Alzheimer disease, non-alcoholic steatohepatitis, gout and much more, catching the eye of Novartis, Genentech and others.

Asher Mullard

With Novartis's April acquisition of IFM Tre for US$310 million upfront plus future milestone payments, the innate immune system's NLRP3 target continues to excite industry interest.

Novartis acquired a portfolio of three NLRP3 inhibitors, the most advanced of which went into the clinic just days before the deal was disclosed. Just 4 months earlier, Genentech acquired Jecure Therapeutics, for an undisclosed amount, to secure rights to its preclinical NLRP3 inhibitors. At least two other biotechs have NLRP3 candidates that are approaching the clinic. And more than two dozen inflammatory indications — including both rare and common conditions — could be in play.

"I've worked in inflammation for over 30 years and I've never seen a target like NLRP3. It's fantastic," says Luke O'Neill, CSO of Inflazome, one of the biotechs that are working in this space. O'Neill, who is also an immunologist at Trinity College Dublin, was a lead author on a 2015 *Nature Medicine* paper showing that NLRP3 is druggable.

Innate immunity provides a rapid-onset defence against both infectious and non-infectious insults by triggering the release of inflammatory cytokines. But while the innate immune system is evolutionarily hard-wired to fend off threats early in life, long-term and persistent activation of this system can contribute to a suite of inflammation-related conditions.

NLRP3, in particular, is triggered by various non-infectious molecules, including molecular byproducts of ageing, physical inactivity and overnutrition. Once activated, it boosts the downstream production of the inflammatory cytokines interleukin-1β (IL-1β) and IL-18. And because it is primarily tuned to sterile host tissue damage signals, rather than opportunistic infectious ones, NLRP3 blockade shouldn't carry the increased risk of infection that limits the use of many approved anti-inflammatory drugs.

NLRP3 also offers a clear initial drug development path forward, followed by lots of potential room to grow. Gain-of-function mutations in NLRP3 cause cryopyrin-associated periodic syndromes (CAPS), a rare set of autoinflammatory conditions that provide a first testing ground to ensure that a small-molecule drug is working as intended. NLRP3 has also been linked via preclinical data with nearly two dozen other conditions — including atherosclerosis,

Box 1 | **Canakinumab development continues**

Novartis's 10,000 patient CANTOS trial of its anti-interleukin-1 (IL-1) canakinumab revitalized interest in anti-inflammatory drugs in both cardiovascular disease and cancer settings in 2017.

Past studies had struggled to show that anti-inflammatory agents can provide cardiovascular benefit, despite considerable scientific rationale and preclinical evidence that this should be the case. In CANTOS, however, patients on a mid-dose arm had a 15% lower risk of non-fatal myocardial infarction, non-fatal stroke or cardiovascular death than did those on placebo. The treatment was also associated with a higher incidence of fatal infection than was placebo, highlighting a major limitation of IL-1 blockade.

The CANTOS study also provided an intriguing oncology signal. High-dose canakinumab was associated with a more than 50% reduction in total cancer mortality compared with placebo. Most of that benefit came from a reduced incidence of lung cancer. These findings were in line with previous experimental work that suggested that IL-1 can promote angiogenesis and tumour growth, and that the cytokine is essential to tumour invasiveness in malignant cells, the authors noted.

In November 2018, the FDA rejected canakinumab for cardiovascular risk reduction. Novartis has not disclosed the full reasons for the rejection, but insiders say it was due at least in part to the use of a post-hoc stratification tool to see benefit. The company discontinued further development of canakinumab for secondary prevention of cardiovascular events later in the year. "With what we now know about this trial, I think that the future game play might be slightly different," says Jörg Eder, executive director leading inflammasome drug discovery at Novartis.

Phase III trials of canakinumab in non-small-cell lung cancer are ongoing, including a combination trial with PD1 blocker pembrolizumab and chemotherapy. The company hopes to file for approval of the antibody in oncology in 2021.

Canakinumab is already approved for multiple other indications, including CAPS and systemic juvenile idiopathic arthritis.

Alzheimer disease, gout, Parkinson disease, rheumatoid arthritis and non-alcoholic steatohepatitis (NASH).

"All the big pharma companies want to block this target. It ticks all the boxes," says O'Neill.

With so much to play for, development teams can choose from multiple paths to market. But interplay between NLRP3 and related immunity targets — including indirect upstream actors such as IRAK4 and NF-κB as well as downstream effector cytokines such as IL-1 and IL-18 — means that they will have to think carefully about choosing the right patients for the right drugs.

"We are fully aware that this is a highly competitive field," says Jörg Eder, executive director leading inflammasome drug discovery at Novartis, a company that has been exploring this space for more than 20 years through its work on its IL-1 blocker canakinumab. In 2017, its 10,000-patient CANTOS trial of canakinumab highlighted the potential that IL-1 blockade could have in atherosclerosis and cancer (BOX 1). "I think the company who figures out most efficiently and fastest where each inhibitor has the best efficacy will win the race."

From pill to probe to pill

The origins of the coming wave of NLRP3 inhibitors can be traced back to Pfizer's work in the late 1990s, when the company was hunting for compounds that could block the secretion of IL-1. Through a phenotypic screening strategy, it identified a series of anti-inflammatory compounds with this effect, which it figured at the time were acting on glutathione *S*-transferase omega 1-1.

Pfizer advanced a lead compound from this series called CRID3 or CP-456,773 into clinical trials for rheumatoid arthritis. But it was not developed further, and anecdotal but unpublished accounts suggest that it increased liver enzyme levels in the clinic.

This early work grabbed the attention of O'Neill and Inflazome CEO Matthew Cooper, who renamed the compound MCC950 and used it as a probe to further unravel the biology of IL-1 secretion. In 2015 they reported in *Nature Medicine* that the target for MCC950 was not glutathione *S*-transferase omega 1-1, as researchers at Pfizer had originally thought, but NLRP3.

Prior to this and related work — including a 2009 paper in the *Journal of Cell Biology* by Genentech's Vishva Dixit and colleagues, showing that glyburide inhibits NLRP3 — people had struggled to block NLRP3 activity effectively, despite widespread appreciation for its importance as a key mediator of innate immunity. "It's a funny target, pharmacologically. People didn't know whether they could do it," says O'Neill.

The biology of NLRP3 is complicated, as an auto-inhibited form of the protein has to be primed and post-translationally modified before it can assemble into an inflammasome complex — with ASC and caspase 1— to trigger downstream signalling. It's not an enzyme, and has no known natural ligands. It's a big protein that has been hard to make via recombinant approaches. And cell-free biochemical assays long remained elusive.

"Those are things that are oftentimes good pieces to have when starting on a drug discovery programme, because they take one layer of subjectivity out of some of the early parts of a screening funnel," says Gary Glick, CEO of IFM Tre. "It's only really been in the last 10 years where a lot of work on this has come out."

Novartis's Eder points to O'Neill's 2015 work as "a game changer", and adds that Novartis's efforts to solve the crystal structure of NLRP3 bound to MCC950 also opened things up internally. "Once we could understand that binding at the molecular level, I think it was normal drug discovery and it's actually not such a difficult target."

Jecure, Inflazome and NodThera were all founded in 2016, suggesting they felt the same. IFM Tre was spun out of IFM Therapeutics in 2018, following Bristol-Myers Squibb's acquisition of the company in 2017 for access to NLRP3 and STING activators that might be able to turn cold tumours into hot ones for immuno-oncology applications.

These companies have disclosed few details about their NLRP3 inhibitors. But there is plenty of room for improvement over MCC950, be it from structurally related compounds or entirely new chemical matter. Most notably, Pfizer reportedly dosed its drug at high levels in its rheumatoid arthritis trial, for example, and some researchers speculate that this caused the rumoured elevation of liver enzyme levels in the clinic.

"Our compounds have a different chemistry, and they are much more potent," says Glick.

Novartis acquired three compounds in its deal with IFM Tre, which were separately optimized for systemic administration, for gut-directed activity and for the ability to cross the blood–brain barrier. Novartis has also developed its own NLRP3 inhibitors in house, potentially expanding the scope of its NLRP3 portfolio further still.

"Currently we see the main differentiation of these in terms of tissue access, and that might be very important for the different indications that we can go after," says Eder.

Spoiled for indications

IFM Tre advanced its systemic NLRP3 inhibitor IFM-2427 into a phase I trial in healthy volunteers, just days before Novartis announced that it was acquiring this portfolio.

Planning is already underway for phase II trials in various patient populations. First up is likely to be diseases with a clear-cut link to NLRP3, says Guido Junge, Novartis's head of translational medicine for autoimmunity, renal and transplantation. "We have two indications in mind, which are CAPS and a crystal disease like gout."

CAPS is an appealing first indication for a few reasons. Because it is caused by gain-of-function mutations in NLRP3, it is backed by strong human genetic rationale. And biologic IL-1 blockers — including Novartis's own canakinumab and Regeneron's IL-1 decoy receptor rilonacept — are already approved for CAPS, showing that therapeutics that modulate the downstream effects of NLRP3 provide benefit. CAPS's status as an orphan indication with approved agents means, moreover, that it provides a well-trodden clinical development pathway, with trial designs that should offer little room for surprise.

"Unless everyone has got this wrong, inhibiting NLRP3 will have a therapeutic role in CAPS," says Glick.

The existing biologic IL-1 drugs also have limitations in this setting that leave room for small-molecule competitors to shine. The biologics don't have good penetrance into the brain, for example, and so can't address central nervous system inflammation, for example, in patients suffering from a particularly severe and fatal form called neonatal onset multisystem inflammatory disease (NOMID). The IL-1 blockers also increase the risk of infection, because the cytokine is needed to protect against pathogenic viruses and bacteria.

"The beauty of NLRP3 is that you shouldn't have the same risk of infection because there are other ways to make IL-1 during infection," says O'Neill. "There's redundancy because there's more than one inflammasome, basically."

By contrast, gout, an inflammatory form of arthritis, is caused by the accumulation of monosodium urate crystals. These were the first crystals to be shown to activate the NLRP3 inflammasome. Canakinumab is approved for gout in Europe, but was rejected in the USA after an independent advisory panel raised concerns about the increased risk of infection with the antibody.

Other crystals that are associated with NLRP3 activity include cholesterol crystals that drive atherosclerosis and calcium oxalate crystals that cause oxalate nephropathy.

"It's not that there is a genetic link to disease, but those crystals are canonical NLRP3 activator and drivers," says Glick.

> The beauty of NLRP3 is that you shouldn't have the same risk of infection because there's other ways to make IL-1 during infection

NLRP3 has also been implicated in an even broader set of conditions, likely driving Novartis's willingness to pay $310 million upfront for a drug that had only just entered the clinic and two preclinical candidates.

Last year, for example, O'Neill and colleagues reported in *Science Translational Medicine* that α-synuclein, a hallmark of Parkinson disease, drives NLRP3 activation in the brain. They also showed that patients with Parkinson disease have extensive inflammasome activation in the brain. And use of MCC950 in multiple rodent models of Parkinson disease blocks NLRP3 activation, prevents α-synuclein pathology and mitigates the degenerative and motor deficits of disease.

Earlier this year Inflazome secured $1 million in funding from the Michael J. Fox Foundation for the development of a brain imaging probe for patients with Parkinson disease, indicating that it is pursuing further work in this neurodegenerative space.

NLRP3 is similarly implicated in Alzheimer disease, with amyloid-β plaques providing the inflammatory trigger. Researchers reported in *Nature* in 2013 that NLRP3-deficient mice were largely protected from loss of spatial memory and other consequences associated with Alzheimer disease.

"The big money would be behind things like Parkinson's and Alzheimer's," says O'Neill.

Genentech has not yet disclosed when it will advance its NLRP3 inhibitors into the clinic, or publicly prioritized the indications it will work on. But a company spokesperson indicated that they see promise in indications including NASH, liver fibrosis, gout, inflammatory bowel disease, cardiovascular disease and more.

NASH is attracting a lot of industry interest these days because it offers a huge patient population, with massive unmet need and enormous market potential. Researchers reported in *Nature* in 2012 that the NLRP3 and NLRP6 inflammasomes regulate NASH progression through the effector protein IL-18. In 2017, another team reported in the *Journal of Hepatology* that NLRP3 blockade reduces liver inflammation and fibrosis in mouse models of NASH.

"When you have a molecule that works in so many diseases, you've got an interesting challenge in terms of which one you go after first," says O'Neill.

Derisking development

Drug developers will also have to think carefully about how to choose an optimal patient population within each indication for future development plans. "We are looking at this pathway in a holistic way, looking at the different nodes and different points where we can interfere," says Eder.

The data from the 10,000 patient CANTOS trial of canakinumab will be invaluable for unravelling this anti-inflammatory science, he adds. The patients in this large cohort had a lot of comorbidities, he points out, and so biomarker and outcomes data can be mined to understand the different ways forward. Such analyses might show where IL-1 activity alone drives disease, where IL-18 plays a more important role and where an NLRP3 inhibitor that can turn off both these cytokines has the best chance of success.

"This data set is a unique treasure that provides insights that nobody else has," says Christian Bruns, global head of auto-immunity, transplantation and inflammation research at the Novartis Institutes for Biomedical Research.

> This is the next frontier in inflammasome drug discovery

Even so, drug development remains a risky business. It may turn out, for example, that as yet underappreciated and redundant inflammasomes can also drive IL-1 and IL-18 secretion in response to sterile danger signals. "There may be other inflammasomes playing a role under certain conditions, and we just don't know about them yet," says Eder. "For me personally that's the biggest risk."

Glick is already working on launching IFM Quatro, to discover inhibitors of other inflammasomes, including NLRP1, in recognition of industry interest in these targets.

And with so many upstream and downstream nodes in the inflammatory network to choose from, drug companies may have to suffer through some trial and error in the clinic to identify where exactly to act for the best clinical efficacy in any given indication. "This is the next frontier in inflammasome drug discovery," says Eder.

On the safety side, there are other risks — even beyond the unpredictable rare toxicity signals that can sink any drug development programme. The clinical data already show that the main safety limitation of IL-1 inhibition is the increased risk of infection, and on paper at least NLRP3 inhibition shouldn't carry this risk because redundant inflammasomes can still fight off pathogenic organisms. But NLRP3 inhibitors will also block IL-18 secretion and an inflammatory type of cell death called pyroptosis.

"Nobody's blocked all three before, so that's a risk obviously," says O'Neill. "But it's a risk well worth taking."