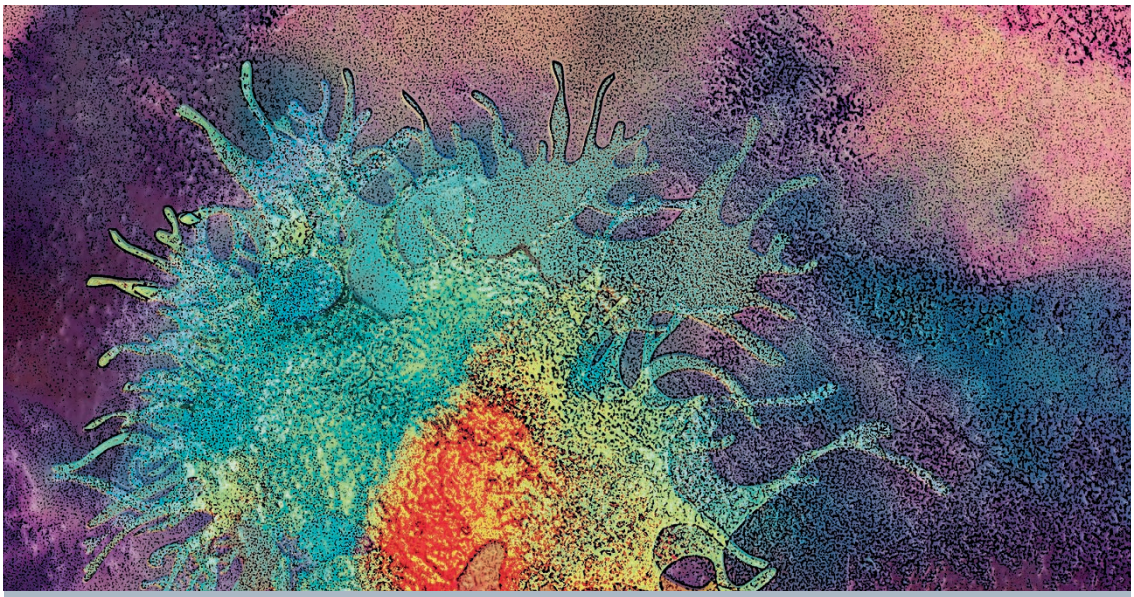
Nature Reviews Drug Discovery | Published online 28 Dec 2017; doi:10.1038/nrd.2017.264



Can innate immune system targets turn up the heat on 'cold' tumours?

STING, RIG-I and NLRP3 agonists might increase the effectiveness of immuno-oncology checkpoint inhibitors, while antagonists of these targets offer an anti-inflammatory bonus.

Asher Mullard

Drug developers have launched more than 1,000 combination trials to try to improve patient outcomes with checkpoint inhibitors that harness the body's T cells to kill cancer cells. Until recently, these trials have mostly focused on up-regulating the adaptive immune system — a slow-starting but long-lasting protective network that has to learn the signatures of specific threats before it can use T cells and other mechanisms to defend itself. Recently, however, a few firms have started to explore whether the broader acting, rapid-onset innate immune system might provide the complementary anticancer targets that everyone is looking for.

Merck & Co., for example, launched a trial early in 2017 of its pro-inflammatory STING agonist MK-1454 alone and in combination with the approved checkpoint PD1 blocker pembrolizumab (see TABLE 1). In September, Novartis and partner Aduro Biotech likewise launched a phase I trial that combines Aduro's STING agonist ADU-S100 with Novartis's experimental PD1 inhibitor PDR001.

> STING seems to be a flavour of the month

In related activity, Bristol-Myers Squibb (BMS) acquired the biotech IFM Therapeutics in August for US$300 million upfront and $2 billion in milestones to get their hands on preclinical agonists of STING and of NLRP3, a component of the innate immune system's inflammasome. Just a month later, Merck bought Rigontec for $135 million upfront and $410 million in milestones to access the phase I drug candidate RGT100, an agonist of another innate immune system target known as RIG-I.

The enthusiasm for these candidates stems from the hope that the innate immune system

Table 1 | **Selected precision innate immune system agonists**

Drug candidate	Companies	Target (drug modality)	Status
MK-1454	Merck & Co.	STING (cyclic dinucleotide)	Phase I monotherapy and combination
ADU-S100	Aduro Biotech/Novartis	STING (cyclic dinucleotide)	Phase I monotherapy and combination
STING agonist	IFM Therapeutics/BMS	STING (cyclic dinucleotide)	Preclinical
STING agonist	Nimbus Therapeutic	STING (small molecule)	Preclinical
NLRP3 agonist	IFM Therapeutics/BMS	NLRP3	Phase I to start in Q1 2018
RGT100	Rigontec/Merck & Co.	RIG-I (oligonucleotide)	Phase I/II
IMO-2125	Idera Pharmaceuticals	TLR9 (oligonucleotide)	Phase I/II

BMS, Bristol-Myers Squibb; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; RIG-I, retinoic acid inducible gene 1; STING, stimulator of interferon genes; TLR9, Toll-like receptor 9.

can convert uninflamed 'cold' tumours — so-called immune deserts that don't respond to checkpoint inhibitors — into responsive 'hot' cancers. "That's where the value for these kinds of things comes in," says Carl Decicco, head of Discovery at BMS. "The first-in-human trials can't come fast enough for me. I'm just very excited to see that data."

"STING seems to be a flavour of the month in terms of non-antibody immuno-oncology targets," says Rosana Kapeller, CSO at Nimbus Therapeutics, which is working on STING agonists for immuno-oncology, and on STING antagonists for autoimmune indications.

STING makes a buzz

Researchers have long suspected that the innate immune system, which provides a fast-acting but generic barrier to a suite of bacterial and viral pathogens, can keep cancers in check. As far back as the 1890s, the oncologist William Coley observed that cancer patients with bacterial infections sometimes experienced spontaneous disease regression. By the 1970s, researchers had found that the bacterium-based bacille Calmette–Guérin (BCG) vaccine — developed for prevention of tuberculosis — activates the innate immune system's Toll-like receptors (TLRs) and induces the elimination of localized bladder cancer in up to 70% of patients.

The TLRs are a family of innate immune system proteins that respond to various pathogenic warning signs, including DNA and RNA from bacteria and viruses. With the realization that downstream pro-inflammatory signalling can also lead to the death of cancer cells, drug developers piled in, hunting for small molecules and oligonucleotides that activate this system with precision for therapeutic effect.

Most of this work failed, and a slew of clinical trials disappointed (*Nat. Rev. Drug Discov.* **11**, 503–505; 2012). Concerns that innate immune system drugs might induce cytokine storms and autoimmune responses put a further damper on these projects. But STING, NLRP3 and RIG-I now represent a new set of innate immunity targets with different triggers, different inflammatory profiles and different distribution patterns in the body, rekindling interest. As immuno-oncologists have become more comfortable with the toxicity liabilities of immune-activating drugs, the excitement around these targets has surged.

STING, which has attracted the most attention to date, is a transmembrane protein that is expressed in various endothelial, epithelial and haematopoietic cells. Glen Barber, a cell biologist at the University of Miami, first reported in 2008 that bacterial and viral DNA activate STING and the subsequent production of pro-inflammatory type I interferon and cytokines. At that time, recalls Barber, the therapeutic applications seemed to extend only to infectious diseases and vaccine development. But in 2012, he reported that STING can also respond to self-DNA, suggesting that it could drive anticancer responses from sick cells that leak DNA into their cytosol.

> "A combination of innate and adaptive triggers could be highly synergistic"

Barber has since shown that STING-deficient mice are especially vulnerable to cancer, and that STING can be deregulated in colorectal cancer and melanoma. Another group reported in 2014 that radiation therapy kills cancers in part by releasing DNA into the cytosol and activating STING.

Although the exact mechanism remains to be resolved, STING's upregulation of inflammatory signalling seems to attract immune cells to tumours and plays a key role in stimulating T cell priming, says Barber. "And if you've got more T cells, through STING activity, then the checkpoint inhibitors can do their thing a little better," he adds. Based on these findings, Barber co-founded STINGINN to discover STING-modulating drugs. "There was a lag period where nobody was really interested in this work," says Barber. "But once there was one party that was interested, that triggered the interest of the others."

Aduro were one of the first movers, spurred on in part by their findings with bacteria-based drugs that have been designed to boost anticancer immune response. "The genetics and all the preclinical data provided strong rationale for STING," says Aduro CSO Andrea van Elsas. "When you look at the difference between hot and cold tumours, STING-induced signalling is what stands out," he adds.

The company's approach was to engineer STING's natural ligands, cyclic dinucleotides, to make them more stable, more potent and more active against variant forms of STING. Since teaming up with Novartis, they have advanced a lead compound into monotherapy and combination phase I trials.

"A combination of innate and adaptive triggers could be highly synergistic," says van Elsas.

The decision to take a cyclic dinucleotide-based approach has pros and cons, says Barber. On the one hand, because cyclic dinucleotides have a relatively short half-life and have to be injected directly into tumours, they have a lower risk of overstimulating the innate immune system and causing cytokine storms or autoimmune adverse events.

But these safety safeguards also limit the activity profile and delivery options for these drugs. And although preclinical data suggest that local STING activation at one tumour site can lead to the inflammation of distal tumours, this has yet to be shown in humans. Small molecules, by contrast, can be delivered intratumourally and orally, offering systemic exposure as well as higher

potency and longer half-lives. STINGINN and Nimbus are both focusing on this approach.

"Everyone in the field is dealing with this question of cyclic dinucleotide versus small molecules," says Decicco. The STING agonist that BMS acquired from IFM is a cyclic dinucleotide, but the company is also exploring small-molecule options for oral delivery if need be. "This will really play out in the clinic."

In the first instance, trials are likely to combine STING agonists with PD1 checkpoint inhibitors in patients who do not respond to PD1 alone, searching for activity across multiple tumour histologies and genetic landscapes.

"The home run would be that these agents can be used routinely to treat people who are either becoming unresponsive to checkpoint inhibitors or who are unresponsive at the beginning of the treatment," says Decicco. "That is what pretty well everyone in the industry is looking for right now."

If this doesn't work out, says Barber, there may also be merit in focusing on cancers in which STING has not been mutationally deregulated, as it is in 30–55% of patients with colon cancer or melanoma.

RIG-I and NLRP3 offer alternative means of activating the innate immune response and warming up cold tumours. The RIG-I pathway is physiologically activated by viral RNA and also triggers type I inteferon signalling. By contrast, NLRP3 triggers include pathogen-associated molecules, such as bacterial lipopolysaccharides, and stress signals, such as reactive oxygen species. These lead to upregulation of proinflammatory interleukin (IL)-1β and IL-18.

> "There's a huge anti-inflammatory market out there for these drugs"

"We actually think that NLRP3 could in fact be the most effective way to activate the innate system," says Decicco. "That's one of the reasons we're advancing it so quickly." BMS plans to start phase I trials of this candidate early in 2018.

Autoimmune opportunity

Nimbus, like its competitors, first started thinking about STING for its immuno-oncology potential. But as the team boned up on this science, they realized that STING antagonists that can dampen the innate immune system also offer compelling opportunities in autoimmune conditions. "It was just such a perfect fit for us," says CEO Don Nicholson.

In part, STING agonist discovery programmes were likely to turn up STING antagonists anyway, so the firm figured they might as well make the most of this chemical matter. But, more than that, human genetic data provide an efficient means of validating STING antagonists in autoimmune diseases.

The rare disease STING-associated vasculopathy with onset in infancy (SAVI), a severe and often fatal genetic autoinflammatory condition, is caused by constitutive activation of the STING protein — offering a clear path to proof-of-concept clinical trials in a genetically defined patient population. A number of other rare conditions that are defined by overactivation of type 1 interferon signalling might also be responsive to STING inhibitors (*Nat. Rev. Immunol.* **15**, 429–440; 2015). And systemic lupus erythematosus, a more common condition with high unmet medical need and little therapeutic progress in recent years, has phenotypic and pathological overlap with these conditions.

These opportunities resonated with Celgene, which partnered with Nimbus in October to explore the use of STING and TYK2 inhibitors in autoimmune diseases. TYK2 acts downstream of STING to control inteferon-stimulated genes.

"The ultimate clinical programme will be some hybrid between looking at lupus and at some of the genetically definable interferonopathies," says Nicholson.

Aduro, too, is advancing STING antagonists for interferonopathies towards the clinic.

And beyond autoimmune conditions, says Barber, the innate immune system plays a role in an even broader subset of 'inflammatory' diseases — which might include everything from atherosclerosis and inflammatory bowel disease to depression and Parkinson disease. One recent paper explored the role of STING in sepsis, for example, highlighting another possible way forwards for these drugs. BMS spun IFM's non-oncology assets out into a new company, again named IFM, which is now developing NLRP3 antagonists for inflammatory conditions that include liver fibrosis, inflammatory bowel disease and gout.

"There is a huge anti-inflammatory market out there for these drugs," says Barber. "Some people have started to cotton on to this."