Trispecific antibodies take to the clinic

Sanofi and others are testing whether trispecific antibodies might have applications in cancer and infectious disease indications.

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With around 100 bispecific antibodies already in the clinic, drug developers are working on new ways to add even more functionality to the ever-growing antibody toolbox. For Sanofi and a few others, that means an interest in multispecific candidates that can bind multiple therapeutic targets. And in August, Sanofi advanced its second trispecific candidate into the clinic.

"I would be really surprised, and probably disappointed, if in a few years we didn't see more use of this approach," says Gary Nabel, CSO at Sanofi. "It just makes sense." The company has highlighted infectious diseases, immuno-oncology and autoimmunity as therapeutic areas that could benefit from these types of candidate.

A few other trispecific programmes are also moving through the pipeline (TABLE 1). GT Biopharma advanced its trispecific natural killer (NK) cell engager GTB-3550 into a phase I/II trial earlier this year, for high-risk haematological malignancies. Innate Pharma is working on trispecific NK cell engagers for cancer settings. Companies including Numab Therapeutics have presented preclinical data on multispecific immune cell engagers in recent years. And Molecular Therapeutics is planning on advancing a multispecific antibody-like candidate for COVID-19.

Paul Parren, CEO of the γδ T cell-engaging bispecific company Lava Therapeutics, is watching this space. "I like bispecifics a lot, and I also like trispecifics a lot," he says. But just as with the bispecifics, drug developers need to follow the science, rather than just the technological opportunity, he adds. "I am not a fan of just putting things together just for the purpose of making bispecifics or trispecifics," he says.

In some circumstances, combinations of antibodies may perform as well as or better than multispecific candidates. Even as Sanofi advances its trispecific T cell engager into the clinic, Regeneron is preparing to test bispecific combination strategies that could achieve similar immunotherapeutic effects.

From HIV to oncology

Sanofi's first stab at a trispecific antibody focused on HIV, a long-term focus of Nabel's work. "We explored it in HIV because it was a great proof of concept," he explains.

Already, Nabel and others had identified broadly neutralizing antibodies that could shut down the HIV virus. But HIV mutates quickly, rapidly picking up escape mutations that limit the clinical utility of single-agent approaches. Cocktails of monoclonal antibodies could be used to address the emergence of resistance, says Nabel, but these face practical hurdles. Toxicology and pharmacokinetics need to be assessed for each antibody, increasing the preclinical workload. Regulators typically ask for each antibody to be tested alone and in combination, making for unwieldy and expensive trials. Manufacturing and quality control requirements bring their own challenges. Trispecific antibodies, by contrast, offered a streamlined strategy.

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"There's some very pragmatic reasons to start thinking about trispecifics," says Nabel.

Reporting in *Science* in 2017, his team showed that a trispecific candidate that can independently bind to three different epitopes of the HIV envelope conferred non-human primates with complete immunity to a mixture of simian forms of HIV.

The lead candidate from this programme, SAR441236, entered phase I trials in April.

While discovering and optimizing this antibody, Sanofi's findings bolstered Nabel's enthusiasm for trispecific antibodies in other diseases. For one, the firm's trispecifics had pharmacokinetic properties that were in line with those of canonical antibodies, reassuring the team that these agents would most likely function as expected in humans. For another, the discovery and manufacturing technology was up to task. "The success rate in terms of making the trispecifics was remarkably high," recalls Nabel. "We would come in with any antibody, and plug it into the system, and there was a pretty good chance that that antibody would be functional and have all three of the specificities that we wanted it to have."

Sanofi also found that production yields for trispecifics were better than for bispecific agents. "That, from a manufacturing point of view, is a big deal because it means that we now can make a product at the quantities needed at an effective price," says Nabel.

Sanofi's second trispecific programme showcases the potential of these agents in immuno-oncology applications. Already, drug hunters have embraced bispecific T cell engagers, which bind to a T cell target with one arm and a cancer cell target with the other. These agents help to activate immune cells and bring them into proximity with cancer cells, ideally driving remission. The FDA approved the pioneering T cell engager in 2014, Amgen's CD3xCD19 blinatumomab for acute lymphoblastic leukaemia (ALL). Now, nearly 100 such programmes are in the clinic, showed a recent review of bispecific antibodies by Parren and colleagues. And yet, these T cell engagers are still struggling with toxicity challenges, as well as with limited activity in solid cancers.

For Sanofi, a CD3xCD28xCD38 trispecific provides a possible way forward. Naive T cells need at least two signalling events to become activated, and so Sanofi's approach was to design an antibody that would bind both CD3 and CD28 on the T cell, providing activating and co-stimulatory signalling at the same time. CD38, for its part, is highly expressed on multiple myeloma cells. The approval of Johnson & Johnson's anti-CD38 antibody daratumumab last year highlighted the potential of this target, with some analysts forecasting peak sales of up to US$10 billion for this antibody.

Reporting last year in *Nature Cancer*, Sanofi showed that in preclinical models of disease its trispecific had 3-log to 4-log higher killing potency than daratumumab.

Table 1 | **Selected trispecific antibodies in and approaching the clinic**

Drug	Company	Properties	Indication	Status
SAR441236	Sanofi	HIV-1 Env triparatopic	HIV	Phase I
SAR442257	Sanofi	CD3xCD28xCD38	Cancer	Phase I
GTB-3550	GT Biopharma	CD16xCD33xIL-15	Cancer	Phase I/II
NKp46 NKCE	Innate/AstaZeneca	NKp46xCD16xundisclosed	Cancer	Preclinical
MP0420	Molecular Partners	Triparatopic DARPin	COVID-19	Preclinical

MM, multiple myeloma; NHL, non-Hodgkin lymphoma.

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Although Sanofi included CD28-targeting activity to boost T cell proliferation and survival, the trispecific also bound to CD28 on the surface of cancer cells, adding further specificity. "We got two for one with CD28," says Nabel. "We got the ability to stimulate these T cells and give them a longer half-life, and we got better targeting for the myeloma. This was a happy accident."

A phase I trial of SAR442257 started recruiting patients in August.

"I look forward to seeing what happens with these molecules," says Parren, about SAR441236 and SAR442257. The scientific and pragmatic rationale for both make sense, he adds. But Parren, who worked on the discovery of daratumumab at its originator firm Genmab, has concerns about the safety profile of the immuno-oncology programme.

CD3 binders are associated with cytokine release syndrome, and blinatumomab carries a black box warning to this effect. Another concern traces back to the 2006 trial of TeGenero's CD28-specific agonist antibody TGN1412, where cytokine release syndrome led to the hospitalization of all six volunteers, and caused multiple organ dysfunction in four of them. CD38, meanwhile, is broadly expressed across many cell types, raising the possibility that this trispecific T cell engager might target healthy cells.

"I would be worried," says Parren. "We'll see what happens, but this is definitely a very courageous programme."

Despite these concerns, Nabel believes that clinical trials of the trispecific can be performed safely. Whereas canonical antibodies have two binding sites for each target, Sanofi's trispecific has only one binding site per target. The trispecific's total binding strength, or avidity, consequently, is thousands of fold lower than that of a canonical candidate. "What we're trying to do here is minimize what I would call the off-target effects, and maximize the ability to be specific," explains Nabel. When Sanofi recreated the assay that was used to dissect TGN1412's CD28-induced cytokine release, they found that a monovalent CD28-specific antibody did not produce a toxicity signal. The toxicity profile of SAR442257 in non-human primates was also acceptable.

Sanofi is exploring other ways of keeping toxicity in check as well, including alternative administration routes and schedules. "You want to avoid giving a big bolus of the antibody that will immediately activate T cells to do what they normally do, which is to release cytokines," says Nabel.

Enlisting NK cells

At Innate Pharma, Eric Vivier and colleagues plan to use trispecifics to try to unlock the anticancer activity of NK cells, a class of innate immune cells that are marked by their ability to quickly recognize and shut down threats. "I think that more and more people are switching out from a T cell-only-centred view of the world into a more integrated view of the immune system," says Vivier, CSO of the company. And because NK cells are about one-tenth as prevalent as T cells, NK engagers may have better safety profiles than T cell engagers, he speculates.

Vivier and his colleagues have focused on NKp46 and CD16 as the keys to the NK cell-engaging approach. NKp46 is a glycoprotein that, while expressed in a subset of immune cells, is particularly important for NK cell function. "NKp46 is by far the most specific activating NK cell receptor known today," says Vivier. CD16 is expressed on a broader set of immune cells, but it has a key role in NK-mediated antibody-dependent cell-mediated cytotoxicity (ADCC). Vivier and colleagues consequently reasoned that these two targets would synergize well.

The preclinical data has borne this out. In a paper in *Cell* last year, his team tested the activity of various permutations of NK engagers. These recruited immune cells either via only NKp46 or via both NKp46 and CD16, and bound cancer cells through CD19, CD20 or EGFR. The bispecifics were active, and the trispecifics fared even better. "We can basically increase the potency by a thousand fold when we combine the engagement of NKp46 and CD16," says Vivier.

Innate is now working with AstraZeneca on the development of NKp46-based trispecific and multispecific candidates. Candidates that can bind more than one cancer antigen might benefit from even more specificity, speculates Vivier. Innate is also working with Sanofi on bispecific NKp46-based products. No timelines have been disclosed as yet for the advancement of these NK engagers.

But Vivier is happy with the progress to date. "At this stage, we do not see any disadvantages to the development of trifunctional engagers," he says.

Cocktails instead

Despite growing interest in trispecific immune cell engagers, it might be possible to achieve similar or better effects with cocktail approaches, says Dimitris Skokos, senior director of cancer immunology at Regeneron.

Earlier this year, his team showed how CD3- and CD28-binding bispecifics can be combined to achieve a co-stimulatory T cell-engaging effect. Combinations of these agents outperformed monotherapeutic approaches, they reported in *Science Translational Medicine*, while also avoiding the risks of cytokine release syndrome associated with CD28. In another paper in the same journal later in the year, his team showed that a CD28-binding bispecific could be combined with an anti-PD1 antibody to similarly boost the activity of the bispecific candidate.

For Skokos, this combination approach provides more flexibility than a trispecific approach. With Sanofi's SAR442257, for example, the company has had to commit to a predetermined ratio of CD3 to CD28 to CD38 activity. But what if it turns out that this ratio needs to be tweaked in the clinic? With a cocktail strategy, by contrast, agents can be titrated in trials to optimize activity. Similarly, if the sequence of co-stimulatory signalling matters, researchers can vary the order of administration of bispecifics to figure out which timing works best.

Regeneron plans to test this approach with bispecifics that are nearing or that have been already been advanced into the clinic. The company plans to start testing a CD28xMUC16 bispecific in combination with either a CD3xMUC16 bispecific or with its PD1 inhibitor cemiplimab in ovarian cancer later this year, for example. It is also already testing a CD28xPSMA bispecific in combination with cemiplimab for prostate cancer. And it plans to start testing a CD28xEGFR bispecific in combination with cemiplimab later this year.

"We are investing a lot in moving multiple combination strategies that use our CD28 co-stimulatory bispecific platform," says Skokos. But that's not to say that Regeneron has turned its back on trispecifics, either. "We are very interested in and we do explore what I call sophisticated, multi-targeted approaches," he adds. Ultimately, he says, each programme needs to be assessed on its own merits, the preclinical data and the clinical opportunity.

At Sanofi, Nabel is also taking the empirical approach. "I don't want to leave anyone with the impression that there's a preference for one versus two versus three. We want to use, for whatever medical application, the very best molecule that we can use," he says.