Antibody–oligonucleotide conjugates enter the clinic

Could antibody targeting solve the long-standing challenge of delivering oligonucleotide drugs to a wider range of tissues?

Asher Mullard

Avidity Biosciences has advanced a first antibody–oligonucleotide conjugate (AOC) into a phase I trial. Its AOC 1001 uses an antibody to carry an siRNA payload to muscle cells, for a rare muscle disease. If successful, this approach stands to broaden the reach of oligonucleotides, a modality that is still struggling to gain traction beyond the liver and a few other tissues.

"The oligonucleotide field is littered with great ideas and great targets that fail because people can't get the drug where it needs to go," says Avidity CSO Arthur Levin. Levin previously held senior drug development roles at Ionis Pharmaceuticals and Santaris Pharma. "Having been beaten by this many times, we took the opposite approach: let's take advantage of where we can get the drugs."

AOCs build on the success of antibody–drug conjugates (ADCs), antibodies that carry small molecules to specific cells. The FDA has approved 11 ADCs, all delivering cell-killing drugs to cancer cells. Over the years, ADC engineers have learned a few tricks about how to design these constructs, contributing to a burst of AOC activity.

"The idea of an antibody as a delivery vehicle is natural," says Hong Wan, CEO of Tallac Therapeutics, another antibody-conjugated oligonucleotide company. "We definitely took some of the learnings from the ADC experience, both in terms of how you design the molecules as well as in terms of manufacturing," says Wan, who previously worked on ADC development at Pfizer.

Advances in oligonucleotide science have also contributed, adds Levin.

Avidity and Tallac — as well as Dyne, Denali and Gennao Bio — are exploring related but different applications of AOC technologies. These span muscle diseases, central nervous system (CNS) diseases and cancer (TABLE 1). Avidity was first into the clinic, but others will join in 2022.

Infectious ideas

Over the decades, researchers have proposed various strategies to smuggle oligonucleotides into cells. Next-generation chemistries, ligand conjugation, cell-penetrating peptides, nanoparticles and exosomes are all on the list. Harvard's Judy Lieberman showcased the potential of antibodies in 2005.

"Delivery was in my opinion always the main problem for RNA therapeutics," says Lieberman. Whereas the field quickly solved issues related to degradation and immunogenicity, delivery remains a challenge.

As an immunologist, she wanted to get these drugs to subsets of immune and cancer cells. Her team attached various siRNA payloads to an antigen-binding fragment (Fab) that engaged the HIV-1 envelope protein. As a linker they used protamine, a protein that binds and packages DNA. These conjugates successfully knocked down gene expression in HIV-infected T cells, she reported in *Nature Biotechnology* over 15 years ago. The same general approach could silence *c-myc*, *MDM2* and *VEGF* in cancer cells.

"When we started out doing this stuff, we didn't think it would take that long to advance. When I'd present, I'd say I think we could be in the clinic in a couple of years," recalls Lieberman.

It took longer, and lost its early advocates along the way.

Lieberman licensed this technology to Alnylam early on, and was on the company's Scientific Advisory Board until 2019. AstraZeneca's MedImmune also explored Lieberman's approach. But these first-generation AOCs were clunky to work with, she says — a pain to purify, susceptible to cleavage and difficult to manufacture.

AOCs "are obviously an approach that works. There's no doubt about that," says departing CEO John Maraganore. But chemistry, manufacturing and controls (CMC) remain complicated, he agrees. The reliance on bulky antibodies also ties AOC developers to relatively high drug dose regimens.

"We think smaller ligands that you can append to siRNA are going to give us better results," says Maraganore. Small molecules and peptides top their delivery list.

Lieberman has also moved on, but to RNA aptamers. These oligonucleotides offer

Table 1 | **Selected antibody–oligonucleotide conjugates in development**

Drug	Sponsor	Antibody properties	Therapeutic payload	Tissue target	Indication	Status
AOC 1001	Avidity	TfR1-targeted mAb	*DMPK* siRNA	Muscle	DM1	Phase I
AOC 1044	Avidity	TfR1-targeted mAb	Exon-44-skipping PMO	Muscle	DMD	Phase I in 2022
AOC FSHD	Avidity	TfR1-targeted mAb	*DUX4* siRNA	Muscle	FSHD	Phase I in 2022
DYNE-251	Dyne	TfR1-targeted Fab	Exon-51-skipping PMO	Muscle	DMD	Phase I in 2022
DYNE-101	Dyne	TfR1-targeted Fab	*DMPK* ASO	Muscle	DM1	Phase I in 2022
DYNE-301	Dyne	TfR1-targeted Fab	*DUX4* ASO	Muscle	FSHD	IND in 2022
TAC-001	Tallac	CD22-targeted mAb	CpG (TLR9 agonist)	B cells	Cancer	Phase I in 2022
ALTA-002	Tallac/ALX Oncology	SIRPα-targeted mAb	CpG (TLR9 agonist)	Dendritic cells	Cancer	IND in 2022
NA	Denali/Secarna	TfR1-targeted mAb, via Fc	Undisclosed ASO	Brain	NA	Preclinical
NA	Gennao Bio	Cell-penetrating antibody	Undisclosed	Cancer and muscle	NA	Preclinical

ASO, antisense oligonucleotide; DM1, myotonic dystrophy type 1; DMD, Duchenne muscular dystrophy; IND, investigational new drug application; Fab, antigen-binding fragment; Fc, constant fragment; FSHD, facioscapulohumeral muscular dystrophy; mAb, monoclonal antibody; NA, not available; PMO, phosphorodiamidate morpholino oligomer.

some of the binding properties of antibodies, without the potential immunogenicity and manufacturing liabilities of an antibody-fusion construct. She reported in *PNAS* this year that EpCAM-directed aptamer–siRNA chimeras can knockdown *CD47* and *PARP1* in cancer cells.

"I'm in discussions with a few venture capitalists. There's interest," says Lieberman.

Muscle power

Avidity's winding scientific path has meanwhile led it towards AOCs. When Avidity launched in 2013, its focus was instead on nanoparticle-based delivery of oligonucleotides. By studding polymeric nanoparticles with antibodies, they hoped to achieve tissue-specific delivery of nanoparticles packed full of siRNAs.

But they too hit technical barriers. The antibodies weren't as useful for delivery as hoped. "It was like putting feathers on a bowling ball and expecting the feathers to direct where the bowling ball would go," says Levin. Worse, the oligonucleotides leaked out of the permeable nanoparticles.

To secure the payload in the nanoparticles, Avidity's researchers conjugated the antibodies to the cargo siRNAs. Subsequent studies showed that the nanoparticles themselves were no longer needed.

"Nanoparticles are a brilliant concept," says Levin. Their ability to carry multiple siRNAs, against different targets, to specific cells remains appealing. "But there are many engineering challenges," he cautions.

AOCs bring their own protein science problems — and lots of design decisions.

Avidity's lead programme, AOC 1001, consists of three components: a full-length monoclonal antibody (mAb) targeting transferrin receptor 1 (TfR1); a linker; and an siRNA payload that targets *DMPK* mRNA.

"Everyone always asks what the secret sauce is, and it turns out that there is no secret sauce. Every ingredient matters, and how these pieces are engineered and put together matters," says Levin.

Avidity opted for a full-length antibody to make the most of the proven properties and safety of this large therapeutic class. Its delivery antibody targets TfR1, a broadly expressed receptor that transports iron into cells. Muscle cells use a lot of iron, making it especially useful for delivery to these cells. To make the candidate less visible to the immune system, and to avoid unwanted toxicity, the team deleted the antibody's effector function.

Avidity uses the same anti-TfR1 antibody for all of its muscle programmes, and is exploring other receptors for other tissues.

"Every ingredient matters, and how these pieces are engineered and put together matters"

Its linker is non-cleavable, ensuring maximal delivery into cells. Upon binding TfR1, the construct is internalized into endolysosomes. Proteases and hydrolases there quickly destroy the antibody, but the siRNA cargo is hardier, says Levin.

"We've gone from the traditional view that the endosome is our enemy, to a less traditional view. Endosomal escape works in our favour as a controlled release depot," says Levin. Novartis and Alnylam's *PCSK9*-targeted siRNA inclisiran — dosed twice a year — makes the most of this slow-release potential, he adds.

On the therapeutic payload front, antisense oligonucleotides (ASOs) and siRNA drugs can both deplete mRNA targets, through different mechanisms. But Levin is an siRNA enthusiast, taken by the potency and safety of the double-stranded oligonucleotides. He plans to use siRNAs for all of Avidity's mRNA-knockdown programmes. Exon-skipping candidates will use phosphorodiamidate morpholino oligomers (PMOs), a form of ASO.

ADC developers tend to load their cancer-cell killing candidates with as much drug as possible. AOC pioneers, by contrast, are taking a more nuanced approach, optimizing for product homogeneity and safety. AOC 1001 has a drug to antibody ratio (DAR) of 1.

AOC 1001 is designed for myotonic dystrophy type 1 (DM1), a rare genetic muscle disorder caused by CUG repeats in the *DMPK* gene. In these patients, aberrant *DMPK* mRNA accumulates in the nucleus of muscle cells, trapping key RNA-processing proteins there and preventing these from carrying out their usual function. AOC 1001 aims to deplete *DMPK* mRNA, releasing the trapped RNA-processing proteins.

Biogen and Ionis previously took a stab at *DMPK* mRNA with the ASO ISIS-DMPK$_{Rx}$. A phase I/IIa trial hinted at efficacy, the partners reported in 2017. They have since shelved the programme, and noted the need for muscle-targeting chemistries that might provide better potency.

AOC 1001 reduces *DMPK* mRNA levels by over 80% in preclinical models of DM1, without any observed toxicities, says Levin.

A phase I trial is recruiting patients.

Avidity will launch trials of other candidates — for Duchenne muscular dystrophy (DMD) and facioscapulohumeral muscular dystrophy (FSHD) — in 2022.

Pharmacokinetics and safety data from these trials will be key. Many AOC applications will require repeat dosing, contributing to potential immunogenicity concerns. "If there's a place that I've lost sleep in the past, it's on that biologic component," says Levin.

Design differences

Dyne Therapeutics is focused on the same areas. But it made different design choices.

Its lead candidates also target TfR1, but via a Fab rather than a full-length antibody. Fabs are a less proven entity, but Dyne CSO Oxana Beskrovnaya sees four reasons for optimism regarding AOCs. Full-length mAbs can induce the degradation of TfR1, resulting in decreased receptor availability for drug and iron uptake. Fabs do not have any effector function. Their smaller size translates into a lower protein load per dose, with potential tolerability benefits. And, their shorter half-life reduces the risks of long-term drug exposure, a benefit for programmes optimized for rapid drug uptake.

Dyne then uses a cleavable linker, releasing the cargo in the endolysosome. "We did our homework, compared it to multiple other linkers, and this combination allows us the highest modification of the target," says Beskrovnaya.

The company plans to use siRNAs, ASOs and PMOs as therapeutic payloads, with DARs ranging from 1–4.

Dyne's lead programmes also take on DM1, DMD and FSHD. DYNE-251, for DMD, will be the first into the clinic.

Exon-skipping PMOs are already approved for DMD, albeit setting a controversial precedent. DMD is caused by mutations in the dystrophin gene, ablating protein production. PMOs can induce the protein translational machinery to jump over faulty parts of the gene, producing shorter but functional forms of the protein. The FDA granted accelerated approval to Sarepta's exon-51-skipping eteplirsen in 2016. It has since used the same accelerated approval pathway to greenlight Sarepta's exon-53-skipping golodirsen and exon-45-skipping casimersen, as well as NS Pharma's exon-53-skipping viltolarsen.

These drugs leave room for improvement. The FDA approved all four based on their impact on dystrophin levels, a predicted biomarker of clinical benefit. But the dystrophin boost is modest, at 0.5–1% with eteplirsen. Confirmatory clinical trials have yet to prove that these drugs help patients.

AOCs could fare better. In a mouse model of disease, a single high-dose injection of Dyne's lead candidate restored 90% of dystrophin in the diaphragm and nearly 80% in the heart, the company recently reported. Both of these tissues are hard to reach with naked exon-skipping PMOs.

Toshifumi Yokota, a medical geneticist at the University of Alberta, calls this data "very encouraging". But quantification of dystrophin levels is notoriously difficult, adds Yokota, whose worked paved the way for viltolarsen. And animal data only tells part of the story.

The initial efficacy focus for both Dyne and Avidity in DMD will be on dystrophin production. Any evidence of functional improvement will be a bonus.

"These are exciting times in muscle diseases, and it's great there are so many programmes that are now entering the clinic," says Wildon Farwell, Dyne's CMO.

For Yokota, the liver and kidney safety profile of these AOCs will be key. Sarepta recently ran into unexpected kidney toxicity with SRP-5051, a peptide–PMO conjugate for DMD, he points out. Phase II data suggested that SRP-5051 outperforms eteplirsen on dystrophin production, but three patients had serious side effects, including hypomagnesemia. The trial is ongoing.

Oligos on the brain

For Denali Therapeutics, TfR1 is the gateway to the brain. The cells that line the blood–brain barrier express the receptor, making it an appealing means of shuttling oligonucleotides, proteins and antibodies into the CNS.

The company's phase I/II candidate DNL310 is a first test of this potential. It consists of a TfR1-binding constant fragment (Fc) antibody domain fused to an enzyme replacement therapy, for Hunter syndrome. And a similar delivery tactic can be used for oligonucleotides. Systemic delivery of an oligonucleotide transport vehicle (OTV) candidate slashed the expression of a proof-of-concept target by 50% in the cortex and the spinal cord in mice, the company reported at an R&D day in 2020.

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Denali is partnered with Secarna to advance ASO-loaded OTVs. They have yet to disclose lead candidates, but neurodegenerative disease opportunities could include Alzheimer disease, Parkinson disease and Huntington disease.

"Stay tuned, as we look forward to sharing more about our OTV, especially in 2022," says CEO Ryan Watts.

Notably, Denali relies on an engineered Fc domain — rather than antigen-binding arms — to engage TfR1. This results in candidates with low affinity for the receptor, says Watts. These can interact strongly enough with TfR1 to catch a ride across the blood–brain barrier, but they are then released into the brain rather than processed right away. "Low affinity gives us broad distribution in brain tissue," explains Watts.

The antigen-binding arms of their antibody carrier are non-targeting, for now. "With version two, we could consider cell-type-specific targeting," he says.

But the promise of deep brain penetration is compelling enough. Antisense drugs can be injected directly into the CNS, but the physics of diffusion still limit distribution, he says. Systematically administered agents that cross over to the brain via more than 400 miles of blood vessels may offer better distribution.

"There's been a real challenge with deep brain penetration of ASOs," says Watts. "The idea here is that getting across these capillaries allows for knockdown across all regions of the brain."

Innate activity

At Tallac Therapeutics, the emphasis instead is on priming the innate immune system to recognize and destroy cancer cells.

Its therapeutic payload of choice is therefore synthetic oligonucleotides containing CpG motifs, mimicking bacterial fingerprints that activate the innate immunity target TLR9. These have long attracted industry interest. But the best signs of clinical efficacy as yet have come from candidates that are injected directly into tumours, limiting their utility to patients with accessible, injectable cancers.

Tallac expects that antibodies can deliver these immunotherapeutic payloads to exactly where they need to go.

With TAC-001 they target the oligonucleotides to B cells, via the CD22 receptor.

"It's actually pretty novel biology. We're trying to nudge the memory B cells to be more active," says Wan. This should result in improved antigen presentation to T cells

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as well as production of pro-inflammatory and anti-tumour cytokines, amplifying anti-tumour immunity, she explains.

ALTA-002, partnered with ALX Oncology, delivers the immuno-stimulatory payload to SIRPα-expressing cells. Other companies are targeting the CD47–SIRPα axis to override a 'don't eat me' signal that cancer cells hijack to evade the immune system. Tallac's goal is instead to preferentially prime SIRPα-expressing dendritic cells. Because renal cell carcinoma and melanoma can overexpress SIRPα, this candidate might also directly boost innate immune activity in the tumour microenvironment.

Tallac plans to advance TAC-001 into the clinic in 2022. ALTA-002 is close behind.

Future programmes might more fully embrace tumour-directed delivery, adds Wan. "It's a little more akin to an ADC, where you'd use a tumour-specific antigen as a target to enrich your drug in the tumour microenvironment, amplifying the local immune response. That's an approach that we think could be very productive," says Wan.

Here too, the components of each AOC are optimized carefully. Tallac's test candidates incorporate antibodies aimed at different targets, with various Fc engagement levels, linkers and CpG payloads.

"As you can imagine, you need to test a lot of different hypotheses," says Wan. "Having antibody, conjugation and oligonucleotide expertise in the same team is key to driving the technology's development."

Gennao Bio is meanwhile exploring a linkerless possibility. Yale University's Peter Glazer co-founded the company based on his work with the lupus autoantibody 3E10. 3E10 can bind directly to oligonucleotides and crosses the cell membrane via the ENT2 receptor. A preclinical 3E10-derived candidate carried the oligonucleotide payload 3p-hpRNA into cells, triggering the innate immune target RIG-I, the firm has reported.

"I didn't start out my career thinking about drug delivery," says Glazer. But for oligonucleotide-based drugs, he adds, tissue issues keep bringing people back here.