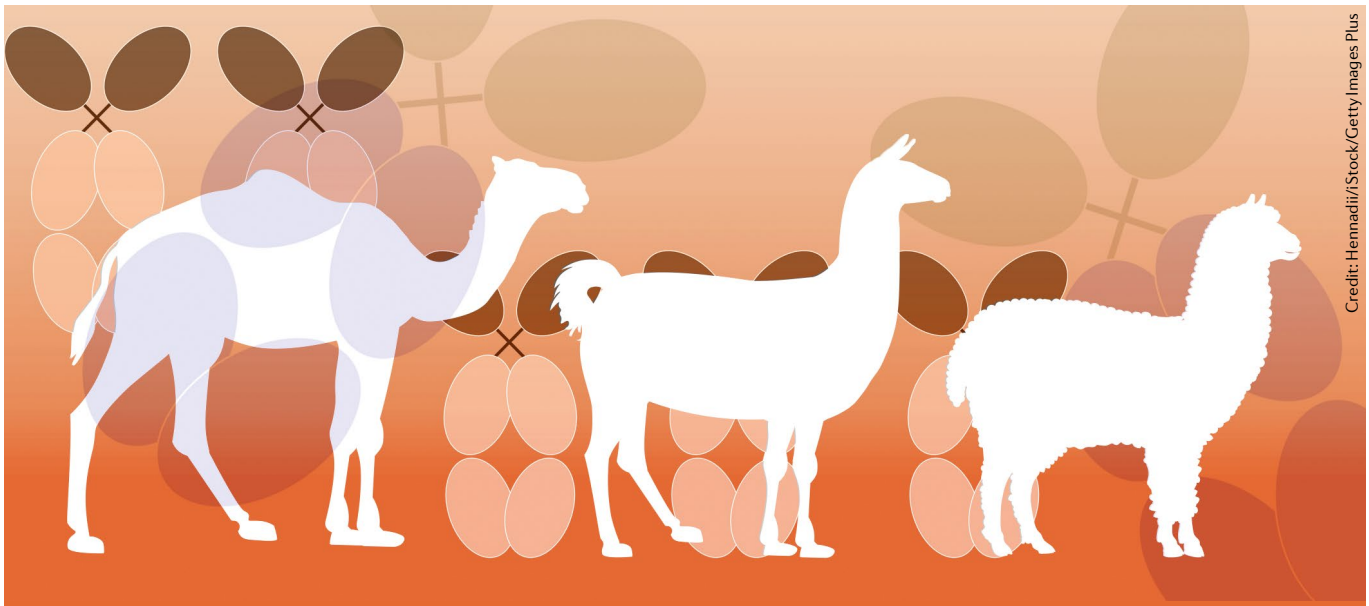

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Nanobody approval gives domain antibodies a boost

The FDA has approved a first nanobody, lifting hopes for companies that are exploring innovative uses for domain antibodies.

Chris Morrison

In February 2019 the FDA approved Sanofi's caplacizumab for acquired thrombotic thrombocytopenic purpura (aTTP), a rare disease characterized by excessive blood clotting in small blood vessels. Caplacizumab is the first drug approved for this disease and the first to target von Willebrand factor (vWF), a key protein in the blood coagulation cascade. It is also the first domain antibody to be approved by the FDA, a belated but potentially lasting landmark event for this modality of small biologics.

Caplacizumab was originally discovered and developed by Ablynx, a biotech that was founded in 2001 to develop and commercialize 'nanobodies' that comprise modified single-variable domains (V_{HH}) of the heavy-chain-only antibodies found in llamas and other camelids. Sanofi acquired Ablynx in January 2018 for €3.8 billion, successfully outbidding rival Novo Nordisk, which had made several offers for the company.

Shortly after the turn of the century, several other firms were also touting novel antibody domain platforms that they hoped could deliver a range of benefits over traditional monoclonal antibodies. These 12–30-kDa domain formats promised new routes of administration, better stability and the ability to bind to targets that were out of reach to full-length ≥150-kDa monoclonal antibodies, as well as the possibility of lower immunogenicity, Lego-like modularity and cheap and fast manufacturing.

But reluctance by investors and pharmaceutical partners to really rev the engines on these platforms, combined with a struggle to select programmes that played to the biological strengths of these small biologics, means that few of these benefits have materialized. "The idea that domain antibodies will open up new realms hasn't lived up to the early billing," says Eric Krauland, CSO of antibody discovery biotech Adimab. When full-length formats work, he adds, they tend to outclass other formats. Large pharmaceutical groups including GlaxoSmithKline and Bristol-Myers Squibb have consequently largely wound down their domain antibody programmes.

But a funny thing is happening on the way to full-length antibody hegemony. "With the [caplacizumab] approval there's a lot more interest in domain antibodies," says Paul Parren, head of R&D at Lava Therapeutics, a biotech that is developing bispecific camelid-based products. Even if the recently approved nanobody only just starts to push the boundaries of the potential of antibodies, it proves that camelid-based V_{HH} domains can make it over the approval hump. Other projects could advance this modality further (TABLE 1).

> "The idea that domain antibodies will open up new realms hasn't lived up to the early billing"

A handful of other camelid candidates, mainly discovered with Ablynx's platform, are also in the clinic. Although Sanofi recently discontinued development of ALX-0171, an inhaler-administered nanobody against respiratory syncytial virus that it acquired from Ablynx, the big pharma says it plans to maintain Ablynx's R&D partnerships and will exploit the technology platform on its own. And preclinical shark-based variable new antigen receptor (VNAR) domain formats are starting to make waves. Together, these domains could yet offer orally available biologics, shuttle other therapies across the blood–brain barrier (BBB), and even increase the efficacy of chimeric antigen receptor (CAR)-T cells. They might also open up new targets, either by binding to them directly or by stabilizing them for small-molecule drug discovery work.

Seeking validation

Alternatives to full-length antibodies had the odds stacked against them from the start. In the absence of evidence of efficacy and proven safety track records, their

Table 1 | **Select list of domain antibodies in development**

Drug	Sponsor	Domain properties	Target	Indication	Status
Caplacizumab	Sanofi (Ablynx)	V_{HH}	vWF	aTTP	Approved
Ozoralizumab	Taisho (Ablynx)	V_{HH}	TNF	Rheumatoid arthritis	Phase III
M1095	Merck KGaA (Ablynx)	V_{HH}, bispecific	IL-17A, IL-17F	Psoriasis	Phase IIb
Vobarilizumab	Sanofi (Ablynx)	V_{HH}	IL-6R	Rheumatoid arthritis	Phase II
LCAR-B38M	Legend/Janssen	V_{HH}, incorporated into a CAR-T	BCMA	Multiple myeloma	Phase II
V565	VHsquared	V_{HH}	TNF	Inflammatory bowel disease	Phase II
M6495	Merck KGaA (Ablynx)	V_{HH}	ADAMTS5	Osteoarthritis	Phase I
BI 836880	Boehringer Ingelheim (Ablynx)	V_{HH}, bispecific	VEGF, Ang2	Solid tumours	Phase I
BI 655088	Boehringer Ingelheim (Ablynx)	V_{HH}	CX_3CR1	Renal disease	Phase I
AD-214	AdAlta	i-body	CXCR4	Idiopathic pulmonary fibrosis	Preclinical
TXB4	Ossianix	VNAR, with mAb payload	TfR1	Primary CNS lymphoma	Preclinical

aTTP, acquired thrombotic thrombocytopenic purpura; CAR-T, chimeric antigen receptor-T cell; CNS, central nervous system; VNAR, variable new antigen receptor; vWF, von Willebrand factor. Source: BioMedTracker.

disadvantages — particularly with regard to shorter half-lives and poorer potency than full-length antibodies — were hard to overlook. For Tillman Gerngross, who founded Adimab in 2007 when new antibody formats were still all the rage, much of the early interest in the alternative formats "was driven by IP [intellectual property] considerations" rather than biological ones. New formats attracted attention if only because they might enable companies to avoid the cost of licensing monoclonal antibody IP. That cost wasn't nominal: Roche and Genentech's Cabilly patents, covering a critical step in the production of monoclonal antibodies, earned US$840 million in licensing fees in 2017, the year before they expired.

But for new platforms to sidestep IP considerations, entrepreneurs still needed to show that they could deliver safe and effective biologics. And to prove this capability as quickly as possible, companies typically first went after known targets. For Abylnx that meant targeting TNF, a target that was also accessible to full-length antibodies. "We chose TNF because the target was validated and everything was available — the animal models, the assays, etc. It was the quickest way," says the company's founding CEO Mark Vaeck, who held that position until 2006 and is currently CEO of the cell-penetrating peptides company Complix. "We made great inhibitors," he adds.

Other companies with alternative antibody technologies pursued similar strategies to avoid stacking target risk on top of technology risk. "You could say that's a mistake, but we all had investors and boards to take into account and we had to work within certain financial boundaries that made it difficult," says Vaeck.

Ablynx licensed its anti-TNF ozoralizumab, a trivalent molecule comprising two anti-TNF domains linked to albumin to extend half-life, to Wyeth in 2006. Pfizer later tested it in phase II rheumatoid arthritis trials before returning it to Ablynx in 2011. Ablynx then out-licensed regional rights to Eddingpharm, in China, and Taisho, in Japan, in 2014 and 2015, respectively. It is now in phase III trials in Japan.

Luckily, Ablynx also chose to work on a new target in parallel, where it believed its nanobody platform offered distinct advantages. vWF, for the treatment of aTTP, had been passed over by full-length antibody developers because there was a risk that bivalent IgGs could drive vWF aggregation and disease worsening, says Vaeck. It also turned out to be a smart application for a domain antibody because the biologic has to be used in a setting where patients are undergoing plasmapheresis every day. The now-approved caplacizumab has a much shorter half-life than a full-length antibody, but with daily plasma exchange half-life matters less.

Even as this approval at last demonstrates the efficacy and safety of antibody domain formats, a new business case for these technologies is emerging. The foundational IP in the domain antibody field, held by GlaxoSmithKline via its $454 million acquisition of Domantis in 2006 and by Ablynx, expired in recent years. And this room to manoeuvre provides a further boost to the field.

The right tool for the right job

The challenge now is to ensure that innovators emphasize the advantages of their domain antibody platforms over what can be achieved with regular antibodies.

Janice Reichert, executive director of The Antibody Society and a drug development consultant, points out that there are 675 active antibody programmes of all stripes in clinical development, covering lots of target and biology space. But only 11 of these are domain antibodies. Even expanding beyond domain antibodies into antibody fragment formats, the tally is still only about 10% of the total. "[Antibodies] have just exploded," she says. "And yet because of their limited functionality, the domain antibodies haven't expanded at the same rate. It's about finding a use for that tool."

Parren, too, points out the importance of thinking carefully about where these formats will have the edge on other types of biologic. "Where's the niche? If you keep that in mind there are many possibilities," he says.

At Lava, for example, Parren is taking advantage of domain antibody modularity, and using bispecific V_{HH} domains as therapeutics to bridge the gap between a subset of γδ T cells and antigens of interest on cancer cells, inducing tumour cell killing and secretion of pro-inflammatory cytokines. The presence of this subset of T cells is correlated with better prognoses, in both solid tumours and haematological cancers, says Parren.

One advantage of domain antibodies in these kinds of application is their ability to bind with high affinity to epitopes that are inaccessible to traditional antibodies, says Parren. And Lava's bispecific V_{HH}-based products may also have significant manufacturing and cost advantages over existing bispecific antibody formats, including Amgen's bispecific T cell engager (BiTE) platform. 55-kDa BiTEs comprise two single-chain variable

fragments (scFvs), which are each synthesized with heavy and light chain variable domains, unlike domain antibodies that comprise a single domain.

The size and simplicity of the domain formats can come into play in other ways as well. In late 2018, for example, researchers from the Scripps Research Institute and Janssen reported in *Science* on the preclinical effects of a universal flu vaccine candidate. Their candidate comprised four conjugated V_{HH} antibodies, each targeted against a different epitope across influenza A and B, to provide coverage that would be impossible with a single full-length antibody. Because the V_{HH}s are so small, the researchers were able to deliver this multidomain multivalent biologic intranasally using an adeno-associated virus with limited base-pair capacity.

> Where's the niche? If you keep that in mind there are many possibilities

VHsquared is meanwhile leveraging domain formats to control the tissue distribution profiles of their products. The company's lead programme in inflammatory bowel disease, V565, is an orally delivered anti-TNF camelid-derived domain antibody that has been engineered for gut protease resistance.

"A conventional antibody would be cleaved immediately, it's just too difficult to engineer out the protease sites," says VHsquared CSO Scott Crowe. Delivering an anti-TNF orally avoids systemic immunosuppression, he adds. And the short half-lives of these products, considered a shortcoming in many settings, mean that any product that does reach the bloodstream is cleared quickly. V565 in the gut can heal the disruption of the epithelial lining caused by chronic inflammation, says Crowe.

A phase II study in Crohn's disease is fully enrolled, and the company expects results in the late summer.

Shark-based products are circling the clinic too, offering other benefits.

AdAlta's i-bodies consist of a single-domain human antibody scaffold with binding regions engineered to mimic those found in variable domains of shark antibodies (VNARs). These 12-kDa i-bodies are characterized by long complementarity-determining region loops found in VNARs, which can run up to 20 amino acids, compared with 8–10 amino acids for a traditional human antibody, says AdAlta CEO Samantha Cobb. That means that i-bodies can bind targets like G protein-coupled receptors (GPCRs) and ion channels that have proved difficult to block with traditional antibodies. The biotech's lead candidate AD-214 should enter clinical trials for idiopathic pulmonary fibrosis next year. It comprises two anti-CXCR4 i-bodies attached to an Fc fragment to extend the molecule's half-life.

Ossianix is also working with VNARs, but with a focus on using these domains as BBB shuttles. Its lead candidate is TXB4, a VNAR that binds the BBB transporter protein TfR1 to carry a conjugated therapeutic payload into the brain. "Our VNARs interact with high affinity with those buried epitopes on the surfaces of a number of receptors and give a log better transfer into the brain than we've seen with other strategies," says Ossianix CEO Frank Walsh, a former head of research at Wyeth.

The company is building up a portfolio of TXB4-based products that will carry full-length monoclonal antibodies into the brain to treat primary central nervous system lymphoma and glioblastoma. The company is about 2 years from clinical trials, says Walsh.

Since September 2018, Ossianix has also been working with Novo Nordisk to deliver metabolic disease products to the brain. With Ablynx's technology now in-house at Sanofi, Walsh anticipates an uptick in dealmaking between other domain antibody players.

The utility of domain antibodies, in all their various flavours, also extends beyond pure therapeutics. Confo Therapeutics, helmed by an Ablynx alumnus, uses camelid domains as a structure-based drug discovery tool. Its V_{HH}s can stabilize targets such as GPCRs in particular conformations, promising to reveal new nooks for small-molecule binders. The company raised €30 million in May 2019 in a series A round and has struck discovery deals with Lundbeck and Roche.

And even full-length IgG proselytizers Adimab are dipping a toe into domain antibody engineering. "We have at least one domain programme in the clinic," says Gerngross. Working with Kite Pharma, which is now owned by Gilead Sciences, Adimab reformatted an IgG into an easier-to-use single-domain construct for expression on CAR-T cells. The heavy and light chains of an IgG introduce complexity to CAR-T design that "you don't want to deal with," he says. The firm has also carried out projects for others to improve the properties of existing domain antibodies.

"Many of our pharma partners are now asking us if we have a single-domain library," he says. "There is interest in these constructs."