Faster, deeper, smaller—the rise of antibody-like scaffolds

In early May the pharmaceutical giant AstraZeneca completed a deal with Boston-based Pieris Pharmaceuticals worth up to $2.1 billion to bring Pieris' anticalin asthma drug PRS-060, an engineered protein that mimics antibodies, to the clinic. And on June 1, Bicycle Therapeutics in Cambridge, UK, pulled in $52 million in a series B funding round with several high-profile investors to continue developing its bicycle peptides for a variety of cancer types.

Those are just two of the wide variety of protein scaffold drugs currently in development (**Table 1**). "There's a whole zoo of non-antibody scaffolds out there," says Daniel Christ, an immunologist at the Garvan Institute of Medical Research in Sydney, Australia.

These tiny protein scaffolds take advantage of the high binding specificity of monoclonal antibodies—a key therapeutic modality for a range of diseases—while overcoming some of their drawbacks. In principle, the scaffolds should have considerable advantages over antibodies," says Christ, adding that there are three major features driving the field.

The first is size. Protein scaffolds are much smaller than antibodies—with a molecular weight of anywhere from 2 to 20 kDa, compared with 150 kDa on average for antibodies. That allows them to penetrate into tissues much more easily and seek out binding sites that antibodies can't reach. This provides advantages when dealing with solid tumors or creating inhaled drugs.

Second, they tend to be more stable at high temperatures, and are easy to produce in bacteria, yeast or even by chemical synthesis, rather than in eukaryotic cells. This makes the scaffolds much cheaper to produce.

Third, scaffolds are sufficiently different from antibodies that they can be patented. In the mid-1990s when research into scaffolds began in earnest most antibody drugs were still patent-protected. An alternative drug candidate could claim novelty and be considered a patentable invention for the same therapeutic action.

These intellectual property and cost advantages are beginning to erode, however. "The antibody field is not standing still, it has been improving over the years," says Christ. Patents, including for those protecting antibody blockbusters Herceptin (trastuzumab), Humira (adalimumab) and Avastin (bevacizumab), are beginning to expire before the alternative scaffolds make it to market, and new manufacturing techniques are driving down the cost of biologics drug production anyway. Even scaffolds' small size is not always desirable. "Small size is a double-edged sword," says Christ. "It means they also have a short half-life."

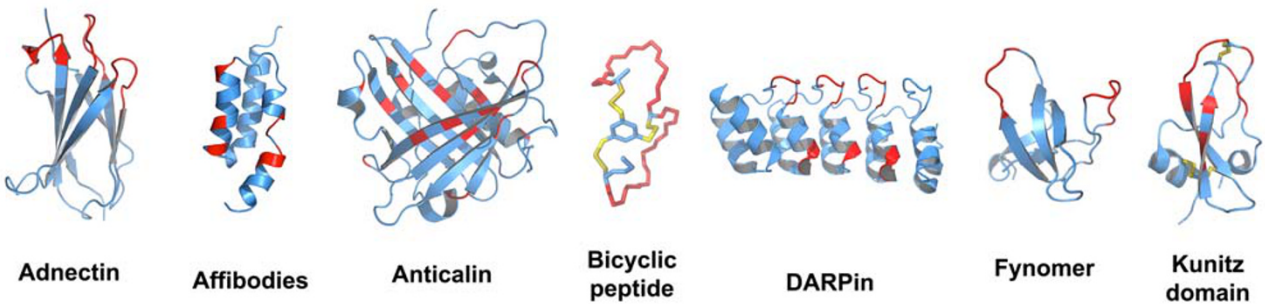
Tiny protein scaffolds are quickly filtered out of the body by the kidneys, so the companies developing them as drugs need to either find ways to make them stick around longer—by adding extensions to them to make them more antibody-like, engineering them to cling on to proteins in the blood or focusing on applications for which a short half-life is desirable.

So far, only one antibody-like scaffold drug has made it to market. In 2009 the US Food and Drug Administration approved Kalbitor (ecallantide), made by Dyax, a Burlington, Massachusetts–based company that was acquired by Dublin-based Shire in 2016 (*Nat. Biotechnol.* **34**, 7, 2016). Kalbitor is a long-acting, 60-amino-acid, 6-kDa peptide based on the first Kunitz domain of human lipoprotein-associated coagulation inhibitor D1, which inactivates the protein kallikrein in blood to prevent attacks of hereditary angioedema.

The rest of the pipeline is moving more slowly. Zurich-based Molecular Partners has one drug in phase 3 clinical trials to treat macular degeneration. The company works with designed ankyrin repeat proteins (DARPins), small 12–18 kDa molecules with highly specific binding domains. "We wanted to look for something very different, so we looked at what nature had evolved for protein binding and found these repeat proteins," says Patrick Amstutz, the company's CEO. DARPins are very flexible, says Amstutz, and can be designed to bind to just about any target protein. Their size is almost like a single-chain antibody fragment, they are cheap to manufacture as they can be made in *Escherichia coli* rather than mammalian cells, and can also be linked to bind multiple targets. So far the company has linked as many as six without changing their activity. "It's one of the key benefits of this tech," says Amstutz.

The anti-angiogenic DARPin drug abicipar, in phase 3 trials for wet age-related macular degeneration, is an antagonist of VEGF used to reduce leakage from the blood vessels behind the retina. By adding polyethylene glycol to the DARPin, it stays in the eye for longer—up to two weeks—and could allow patients to go three months between injections rather than to have one injection per month, as they do currently. Molecular Partners licensed the drug to Allergan in 2011 (*Nat. Biotechnol.* **30**, 1014, 2012), which is taking it forward into the clinic, while Molecular Partners focuses on oncology. The company has one DARPin drug for multiple myeloma in phase 2 trials now, which blocks both the VEGF and HGF tumor-escape mechanisms. This should help re-sensitize tumors that have become resistant to front-line chemotherapy drugs.

Affibodies were among the first protein scaffolds. They were invented by researchers at the Royal Institute of Technology, Stockholm, and Fredrik Frejd, now chief scientist at the Swedish biotech company Affibody in Solna. "We thought antibodies were really lousy molecules, they were difficult to make, tended to clump together and could be highly immunogenic," he says. "So why not take a small, soluble and easy-to-engineer molecule and optimize it for binding?" Affibodies—6 kDa-scaffolds, based on the Z domain of protein A, made up of a bundle of three alpha helices—can be produced by chemical synthesis and can be engineered to bind multiple targets. Their small size makes them ideal for cancer imaging, as they rapidly penetrate the tumor and bind it, while



Daniel Christ

Companies are engineering a wide variety of protein scaffolds to have the binding affinity of an antibody, at a fraction of the size.

Table 1 Selected antibody-like scaffolds in development

Scaffold	Company	Target protein	Drug name	Indications	Status
Adnectins	Bristol-Myers Squibb	PCSK9	BMS-962476	Hypercholesterolemia	Phase 1
				Atherosclerosis	
		VEGFR2	Angiocept	Cancer	Phase 2
		Myostatin	BMS-986089 (licensed to Roche)	Muscular dystrophy	Phase 2/3
Affibodies	Affibody	HER2	ABY-025	Cancer (PET imaging)	Phase 2/3
		IL-17A	ABY-035	Psoriasis	Phase 1/2
Anticalins	Pieris	VEGF-A	Angiocal	Cancer	Phase 1
		Hepcidin	PRS-080	Anaemia	Phase 1/2
		IL-4Rα	PRS-060 (with AstraZeneca)	Asthma	Trial to commence in 2017
Bicyclic peptides	Bicycle Therapeutics	MT1-MMP	BT1718	Cancer	Trial to commence in 2017
DARPins	Molecular Partners	VEGF-A	Abicipar pegol (with Allergan)	Macular degeneration	Phase 3
		VEGF/HGF	MP0250	Cancer	Phase 1/2
Fynomers	Covagen	TNF/IL-17A	COVA322	Psoriasis	Terminated
Kunitz domains	Shire	Kallikrein	Kalbitor	Hereditary angioedema	Approved

Sources: clinicaltrials.gov, clinicaltrialsregister.eu, pieris.com, bicycletherapeutics.com. DARPins: designed ankyrin repeat proteins, HER2: human epidermal growth factor receptor type 2, HGF: hepatocyte growth factor, IL-17A: interleukin-17A, IL-4Rα: interleukin-4 receptor alpha, MT1-MMP: membrane type 1 matrix metalloproteinase, PCSK9: proprotein convertase subtilisin kexin 9, PET: positron emission tomography, VEGF: vascular endothelial growth factor, VEGFR2: vascular endothelial growth factor receptor 2.
Table by Rodrigo Vazquez-Lombardi, Carsten Zimmermann and Daniel Christ

the unbound proteins are quickly flushed from the body, giving high-contrast images within hours. The company is currently testing two drugs designed for imaging: one for breast cancer, targeting the HER2 protein, will enter phase 3 trials this autumn; the other targets EGFR in brain tumors, to help delineate the borders of the tumor for surgeons. The company is now shifting its focus towards other therapeutic applications, says Frejd. They are developing affibody drugs for psoriasis, Alzheimer's and autoimmune diseases. The psoriasis treatment will begin phase 2 trials in the autumn.

To get around the constraints of the molecule's short half-life, Affibody has come up with what Frejd describes as "a more elegant solution" than tacking on a large polyethylene glycol extension. A smaller addition binds to albumin in blood, allowing them to hitch a ride and hang around longer, extending the half-life to around 12 days. Delivery is another benefit of their small size.

New Jersey–based Janssen Research & Development is pursuing two different kinds of protein scaffold drugs: centryns and fynomers. Centyrins, developed in-house, are 10-kDa molecules based on the FN3 domain of human fibronectin plasma protein. "We set out to have something well-behaved biophysically," says Karyn O'Neil, venture leader for Centyrex, the Janssen unit developing the technology. "So it's chemically very simple."

Since the centyrins don't have any disulfide bonds and are not glycosylated, it allows O'Neil's team to introduce cysteine residues at various locations, and attach a therapeutic payload. The centyrin can be designed to bind any number of different targets, delivering the payload—whether a toxin to kill tumor cells, or oligonucleotides to regulate gene expression—exactly where it is needed.

The technology is primarily focused on oncology, particularly solid tumors in lung and colorectal cancer where the centyrins' small size allows them to penetrate the tumor deeper than some other drugs. The molecule's short half-life can either be embraced—delivering a large dose to a specific tissue of a drug that is quickly cleared from the body—or mitigated using a strategy similar to Affibody's, that is, binding to proteins in the blood.

Centyrex hopes to begin clinical trials on its first centyrin within the next two years.

Janssen's other protein scaffold is the fynomer, originally developed by Zurich-based Covagen and acquired by Janssen in 2014. Like all scaffold drugs, fynomers are small molecules, but rather than making them work as a drug, Covagen went in the opposite direction. The fynomers, derived from the human Fyn SH3 domain, are fused to full-size antibodies, creating antibodies that can bind two targets at once.

"We founded the company in 2007, so we were late to the field and didn't feel there was room for another small scaffold," says Julian Bertschinger, former CEO of Covagen and now managing director of the unit within Janssen. "We wanted to focus where we could have a competitive edge."

The bi-specific FynomAbs can be optimized for many different applications—they can bring together two cells, such as tumor cells and killer T-cells, or cross-link two sites on the same cell, changing how the cell reacts to an immune response.

Janssen had one fynomer drug, for psoriasis, that was discontinued after phase 1 because of safety concerns after it induced transient rashes and muscle pain. But Bertschinger is hopeful that the other fynomers under development will be successful in the future. "We don't fully understand the safety issue, but we believe it was related to our target, rather than the technology itself," he says. The smallest of the protein scaffolds are the bicyclic peptides, or bicycles. These 2-kDa proteins, consisting of two peptide loops just 9–15 amino acids in total, are designed to deliver quickly and efficiently a tumor-killing toxin to exactly the right place. Rather than try to extend the drug's short half-life, Greg Winter, co-founder of Bicycle Therapeutics, says they have embraced it, aiming to deliver massive doses that are quickly cleared from the system. "We hope to achieve high doses in a tissue for a short period. With a good toxin we can get good tumor killing," he says.

The short half-life can be an advantage, he says, particularly in cases where you want to get rid of the drug quickly. He cites the example of the disastrous trial of Tegenero's immune-stimulating antibody drug TGN1412 in London in 2006, which caused massive swelling and organ failure within hours of being given to patients. Part of the problem was that the drug was eliminated from the patients' system slowly, worsening the adverse reactions. "Every problem can be a solution to someone else's problem," says Winter.

All Bicycle's potential drugs are pre-clinical, but one is expected to begin phase 1 trials later this year, in partnership with Cancer Research UK, in solid tumors including triple-negative breast cancer and non-small-cell lung cancer.

Though few drugs are nearing approval, the rapidly expanding field shows a lot of promise, says Christ. "But there's a lot of technical development still to be done," he adds.

Brian Owens *New Brunswick, Canada*

Corrected after print 9 August 2017 and 13 April 2018.

Erratum: Faster, deeper, smaller—the rise of antibody-like scaffolds

Brian Owens

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In the July 2017 issue, Janssen's fynomer drug for psoriasis was said to be discontinued "when it induced rheumatoid arthritis." In fact, it was discontinued "after it induced transient rashes and muscle pain." The error was corrected in the html and pdf versions of the article on 9 August 2017.

Corrigendum: Faster, deeper, smaller—the rise of antibody-like scaffolds

Brian Owens

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In the version of this article initially published, it was stated that affibodies were invented by "a group led by Fredrik Frejd," instead of "researchers at the Royal Institute of Technology, Stockholm, and Fredrik Frejd." The error has been corrected in the HTML and PDF versions of the article.