Bispecific antibodies rise again

Amgen's blinatumomab is setting the stage for a bispecific-antibody revival, enabled by new formats that may solve the field's long-standing problems.

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On September 22, Amgen submitted its bispecific antibody blinatumomab for regulatory review by the US Food and Drug Administration (FDA). An approval — for adult acute lymphoblastic leukaemia (ALL) — would be the first in the United States for a bispecific antibody. At least eighteen other bispecifics are in clinical development (TABLE 1; BOX. 1). The field is "blossoming," says Bassil Dahiyat, Chief Executive Officer of Xencor, a company that develops bispecifics.

Bispecifics are not new. The first were described over 30 years ago, and Medarex developed a bispecific that reached Phase III in 2001. But by then, manufacturing problems and clinical failures were sending the field into dormancy. And although Trion Pharma succeeded in seeing its rat–mouse hybrid bispecific catumaxomab to market in Europe in 2009 for malignant ascites, few if any other companies are still designing bispecifics in unwieldy and relatively inefficient quadroma-based formats.

Over the past decade, "people have learned a lot of lessons," says Dahiyat. Newer-format bispecifics are more stable, easier to manufacture and less immunogenic, and persist longer in circulation. Blinatumomab has shown dramatic efficacy in its clinical trials, providing clear proof-of-concept for bispecifics. But the antibody also requires cumbersome administration and faces toxicity issues. It remains to be seen whether bispecifics build upon this qualified success, or slip back into latency.

The blinatumomab case study

Blinatumomab, which was first described in 2000, is a 'bispecific T-cell engager (BiTE)' consisting of two single-chain variable fragments (scFvs) joined by a 5-amino-acid peptide linker (FIG. 1). scFvs are fusion proteins of the variable regions of the heavy and light chains of an antibody, and have the same antigen specificity as does a natural antigen-binding fragment (Fab). BiTE developers have needed to overcome manufacturing challenges, including lack of stability (as scFvs tend to aggregate), low expression titres and poor solubility. But once produced, the T-cell redirectors excel at bringing T cells and tumour cells together, inducing an immune

Table 1 | **Select bispecifics in clinical development**

Candidate	Company	Targets	Phase (indication)
Blinatumomab	Amgen	CD19 and CD3	III (ALL)
MEHD7945A	Genentech	HER3 and EGFR	II (colorectal cancer, head and neck cancer)
ABT-122	AbbVie	TNF and IL-17	II (rheumatoid arthritis)
ABT-981	AbbVie	IL-1α and IL-1β	II (osteoarthritis)
SAR156597	Sanofi	IL-4 and IL-13	II (IPF)
MM-111	Merrimack	HER2 and HER3	II (gastric cancer)
IMCgp100	Immunocore	GP100 and CD3	II (melanoma)
RO5520985	Roche	ANG2 and VEGFA	II (colorectal cancer)
XmAb5871	Xencor	CD19 and CD32B	I/II (rheumatoid arthritis)
COVA322	Covagen/ Johnson & Johnson	TNF and IL-17A	I/IIa (psoriasis)
ALX-0761	Ablynx	IL-17A and IL-17E	I (psoriasis)
AFM13	Affimed	CD30 and CD16A	I (Hodgkin's lymphoma)
AFM11	Affimed	CD19 and CD3	I (non-Hodgkin's lymphoma)
MEDI-565	MedImmune	CEA and CD3	I (GI adenocarcinoma)
Ertumaxomab	Trion	HER2, CD3 and FcR	I (solid tumours)
MGD006	MacroGenics	CD123 and CD3	I (AML)
MGD007	MacroGenics	GPA33 and CD3	I (colorectal cancer)
LY3164530	Eli Lilly	MET and EGFR	I (advanced cancer)

ALL, acute lymphoblastic leukaemia; AML, acute myeloid lymphoma; ANG2, angiopoietin 2; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; FcR, crystallizable-fragment receptor; GI, gastrointestinal; GP100, glycoprotein 100; GPA33, glycoprotein A33; HER, human epidermal growth factor receptor; IL, interleukin; IPF, idiopathic pulmonary fibrosis; MET, hepatocyte growth factor receptor; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A.

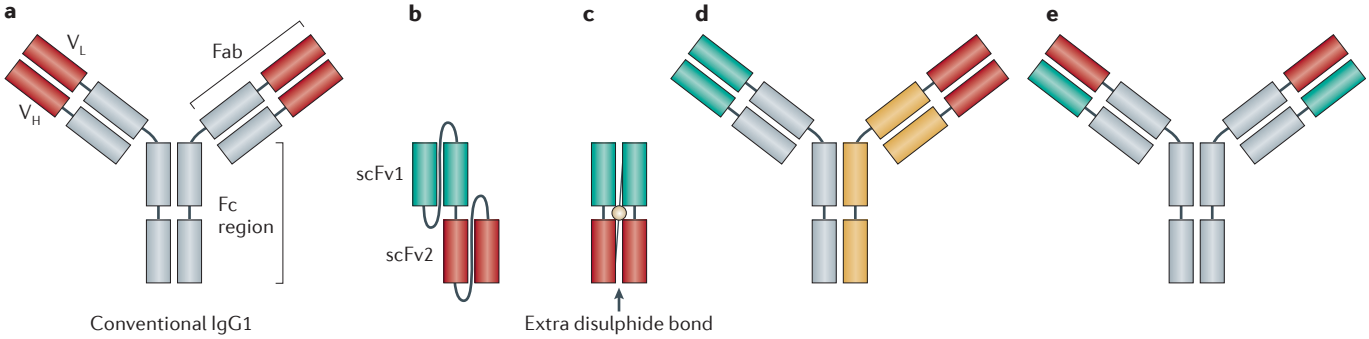

Figure 1 | **Assorted bispecific formats.** **a** | A conventional antibody consists of two antigen-binding Fab arms and one crystallizable Fc fragment region. The two variable domains (V_H and V_L) provide antigen specificity. **b** | In bispecific T-cell engagers (BiTEs), two single-chain variable fragments (scFvs) are joined together by a short linker. **c** | In dual-affinity re-targeting molecules (DARTs), the heavy variable domain from one antibody is linked with the light variable domain of another, and the two chains associate. **d** | A heterodimeric immunoglobulin G-like bispecific. **e** | A 'two-in-one' bispecific.

response. Blinatumomab, specifically, targets CD19 (an antigen expressed on B cells) and CD3 (the signal transduction domain of the T-cell receptor).

In 2011, the drug's original developer, Micromet, reported a remarkable 80% complete remission rate in a small Phase II trial in adult ALL patients who had minimal residual disease following chemotherapy (*J. Clin. Oncol.* **29**, 2493–2498; 2011). At this year's annual meeting of the American Society of Clinical Oncology (ASCO), Amgen — which acquired Micromet in 2012 for US$1.16 billion — reported that in a larger Phase II trial in high-burden relapsed or refractory B-cell ALL (B-ALL), blinatumomab achieved a 43% complete response rate, and a median overall survival of 6.1 months.

"It's super-exciting, but not as exciting as I had hoped," says Wendy Stock, a University of Chicago Medical Center haematologist who worked on the Phase II trial. Other experimental drugs may prove even more effective in adult B-ALL, she notes. Although the patient populations are not directly comparable, Pfizer's inotuzumab ozogamicin antibody–drug conjugate (ADC) achieved a 68% complete remission rate in a Phase I trial, as reported last December at the annual meeting of the American Society of Hematology.

Efficacy aside, blinatumomab has some disadvantages. First, whereas Pfizer's ADC is infused intravenously once weekly, blinatumomab is continuously infused via a portable mini-pump for 28 days and, in most US states, requires visits to the hospital every 48 hours to change infusion bags. "That's going to be one of the big issues with the drug in the future," says Stock. Blinatumomab's relatively small size for a biologic (55 kDa) and lack of a crystallizable fragment (Fc) region means that it is quickly eliminated through the kidneys and can't engage the neonatal Fc receptor that promotes the longevity of an antibody in the serum. The drug's serum half-life is less than 2 hours.

Second, although Pfizer's conjugate carries the risk of liver toxicity, blinatumomab has side effects of its own: neurotoxicity and symptoms of cytokine-release syndrome. These manageable side effects point to a design limitation: the compactness that produces potency allows penetration across the blood–brain barrier and possible nonspecific binding to T cells in the absence of tumour.

To Fc, or not to Fc

Companies and academics have generated a bewildering variety of novel bispecific formats — at least 35 to date — to try to improve on blinatumomab's performance. Bispecifics can be roughly divided into two categories: those that include an Fc region — the 'trunk' of the typical immunoglobulin G (IgG) antibody formed by the pairing of two heavy chains — and those without an Fc region (FIG. 1). Amgen's BiTE, Affimed's Tandab and MacroGenics' dual-affinity retargeting molecules (DARTs) are among the bispecific formats that lack an Fc domain. On the plus side, they are small and easily penetrate tissues and tumours. But there are trade-offs.

In DARTs, for example, the heavy variable domain from one antibody is linked with the light variable domain of another, and the two such chains associate to form a 'diabody' structure, linked tightly by adding a disulphide bond. But basic DARTs, like BiTEs, have a short serum half-life, and MacroGenics' Phase I MGD006 T-cell-redirecting acute

Box 1 | **Bispecifics beyond cancer**

Several bispecifics are being developed for noncancer — and often, autoimmune — indications (TABLE 1). As in cancer, these often seek to co-cluster two targets that would not otherwise come together, rather than simply targeting two independent disease-related proteins. Xencor's Phase II XmAb5871, for example, binds CD19 (an accessory protein to the B-cell receptor (BCR)), and CD32B (an inhibitory receptor on B cells). BCR linkage to CD32B triggers a negative-feedback mechanism that may be disabled in human autoimmune diseases.

Combining two monospecific antibodies, of course, will not co-cluster two receptors in this way. Bispecifics may have an additional advantage over standard antibodies — avidity. The 'avidity hypothesis' holds that bispecifics are more likely to bind to cells that express both targets than to cells that express only one, whereas monospecifics bind indiscriminately; thus, bispecifics, with all else being equal, should be more potent and safer. The avidity hypothesis remains unproven, but in August this year Johnson & Johnson acquired the bispecific company Covagen and its FynomAb platform, which offers tetravalent binding and which, says Janssen's William Strohl, might be used in biological situations that favour avidity effects. The lead FynomAb, which targets interleukin-17A and tumour necrosis factor, is in Phase Ib for psoriasis.

The challenge in treating autoimmunity, as opposed to cancer, will be establishing appropriate safety and pharmacological profiles for chronic diseases that require long-term therapy, says Strohl.

Bispecifics could also have advantages over drug cocktails in infectious diseases, by targeting multiple pathogens. "In addition, if we want to incorporate a domain that recruits immune cells to destroy these targets, we can do that as well," says MacroGenics' Chief Executive Officer Scott Koenig. MacroGenics is working on such constructs for treating latently-infected HIV patients.

myeloid leukaemia treatment, must — like blinatumomab — be delivered by continuous infusion. To address this problem, MacroGenics now fuses an Fc domain onto its next-generation DARTs, creating heavier 'Fc-bearing DARTs' that can bind the neonatal Fc receptor. "We can dramatically lengthen the time that these molecules remain in circulation," says MacroGenics' Chief Executive Officer Scott Koenig. One Fc-bearing DART is beginning Phase I, and three others are set to follow next year.

"There's absolutely a trend to incorporate Fc regions" into bispecifics, says Dahiyat. Advantages include ease of purification and production, and longer serum half-life.

But Fc-bearing bispecifics have posed their challenges as well. A much-sought-after goal has been Fc heterodimers that can carry different specificities on each arm of the antibody (FIG. 1). Genentech started tackling this problem 18 years ago with its 'knobs-into-holes' approach, substituting a large amino acid for a small one in the heavy chain of one antibody (creating the 'knob') and vice versa in the heavy chain of another antibody (the 'hole'). When the antibodies are co-expressed, in theory the heterodimers come together in a lock-in-key fashion. In practice, however, "it was pretty hard to work with," Dahiyat says. (Genentech is now using a 'two-in-one' format, modifying each antigen-binding Fab fragment so that it binds two antigens (FIG. 1)).

Only in the past few years, Dahiyat says, has the field managed to create truly practical heterodimeric IgG-like bispecifics. Xencor used structure- and sequenced-based approaches to design Fc variants that preferentially heterodimerize, and attached different scFvs on each arm. This technology requires only two amino-acid substitutions in the Fc. "Stay as close as possible to what works in nature for monoclonal antibodies," advises Dahiyat. Roche's CrossMAb platform uses knobs-into-holes to heterodimerize the Fc regions and re-arranges the heavy- and light-chain domains in one Fab to minimize the mispairing that otherwise would occur. And Genmab's DuoBody platform uses matched point mutations at the heavy-chain interface of two separate antibodies to drive recombination of the antigen-binding fragments of the antibody and Fc heterodimer formation, following the controlled reduction of the disulphide bridges at the hinge regions. DuoBodies "can be manufactured essentially as a normal IgG, but with a few key steps added to the process," e-mails William Strohl, Head of the Biotechnology Center of Excellence at Janssen Biotech, one of seven companies that have licensed the DuoBody platform from Genmab.

Immunocore's ImmTAC, meanwhile, is a design outlier — a hybrid between a BiTE antibody and a soluble receptor. An affinity-enhanced T-cell receptor is fused to a CD3-specific scFv, creating a bispecific that targets peptide antigens (derived mainly from intracellular proteins) expressed on major histocompatibility complex class I molecules on tumour cells, rather than targeting the surface proteins typically recognized by antibodies. This opens up new targeting possibilities, says Immunocore's Chief Scientific Officer Bent Jakobsen, including the potential to target a handful of known truly tumour-specific antigens — intracellular proteins not expressed in normal adult human tissues. A Phase I dose-escalation trial of Immunocore's lead ImmTAC, reported at ASCO, elicited 4 confirmed partial responses in 31 late-stage melanoma patients.

Besides manufacture, the main limitation for ImmTACs is the same as for other T-cell-retargeting bispecifics: can target antigens be found that are tumour-specific enough to avoid toxicity, but not so specific that some tumour cells escape detection?

Of course, standard antibodies and ADCs made huge strides despite this same challenge, even when bispecifics stalled. "Timing is the critical difference," writes Janssen's Strohl. "The generation of manufacturable bispecific antibodies only occurred in the past 6–8 years, and the leading molecules from these efforts are now being validated in the clinic … In the end, we believe that bi- and multispecific antibodies will have just as much impact as ADCs, if not more."

But that remains to be proven. "Let's see if we can overcome all the challenges that come when you try to do it [according to good manufacturing practice] and scale up," says Dahiyat. "In 2 or 3 years we'll see if those hopes were well founded or if we were all wrong. The clinical data — that's the only real answer."