Bispecific Antibodies: Rising Stars in Antibody Therapeutics

Introduction

Monoclonal antibodies (mAbs) are important therapeutic agents for the treatment of many human diseases, such as cancer, autoimmune diseases, cardiovascular diseases, asthma, and viral infections. Unlike monospecific mAbs, bispecific antibodies (bsAbs) are antibodies containing two antigen-binding sites and therefore can simultaneously target two different epitopes. In January 2022, Faricimab (Vabysmo, Roche) has been approved by FDA to treat wet age-related macular degeneration (AMD) and diabetic macular edema (DME). Besides the first bsAb Catumaxomab being withdrawn in 2017, to this date, this is the 4th bispecific antibody drug on the market now (table 1). With the increased attention in the antibody field, bispecific antibodies have accounted for nearly 20% of the clinical antibody pipeline, with about 160 bsAbs currently in clinical trials.

Table 1. List of Bispecific Antibody Drugs

Generic Name	Targets	Technology	First Approval	Company	Indication
Catumaxomab	EPCAM/CD3	Quadroma	2009 (withdrawn in 2017)	Trion	Malignant ascites
Blinatumomab	CD19/CD3	BiTE	2014	Amgen	Acute lymphoblastic leukemia
Emicizumab	FIX/FX	Common LC	2017	Roche	Hemophilia A
Amivantamab	EGFR/c-Met	DuoBody	2021	Genmab	Non-small-cell lung cancer
Faricimab	VEGF-A/Ang-2	CrossMab	2022	Roche	Diabetic macular edema, wet or neovascular, age-related macular degeneration

Design and Engineering of Bispecific Antibodies

Initially, bispecific antibodies were produced using the quadroma method (hybrid hybridoma). Due to the random assembly of two different heavy and two different light chains, only one functional bispecific antibody exists and other nine variants are either non-functional or monospecific. This leads to a low yield of target bsAbs which poses a big challenge to the downstream purification process.

To overcome the heavy and light chain association issue, scientists have focused on recombinant DNA technology to engineer bispecific antibodies. Based on their different properties, bsAbs are classified into two distinct types: IgG-like bsAbs and non-IgG-like bsAbs. IgG-like bsAbs have a conserved immunoglobulin constant domain, thus retaining Fc-mediated effector functions, such as CDC and ADCC. Owing to their large molecular weight, they also have a long serum half-life, improved solubility, and stability. On the other hand, because of the lack of Fc fragments, non-IgG like bsAbs have a smaller size, less immunogenicity and better tissue penetration but shorter half-life in serum.

IgG-Like Bispecific Antibodies

The homodimerization of the two heavy chains in IgGs is achieved by the interaction between the CH3 domains. To tackle the heavy chain mispairing problem, different technologies can be applied to engineer the CH3 domain for Fc heterodimerization. The "knobs-into-holes" (KiH) approach was first proposed in the 1990s and has been extensively used for Fc engineering. It involves substituting a large amino acid for a small one in the CH3 domain (the "knob") of one antibody and vice versa (the "hole") of the other antibody. Alternative approaches such as SEEDbody, are also used to generate heterodimeric bispecific antibodies. Alternatively, it's also essential to solve the light chain mispairing problem and the CrossMab approach is one of these technologies. As shown in Figure 1, three major CrossMAb formats are CrossMab^{Fab}, CrossMab^{VH-VL} and CrossMab^{CHI-CL}. By swapping the regions of one side heavy chain and light chain, the BsAb light chain can be assembled correctly. To minimize mispairings, it is usually combined with other strategies such as KiH, DEEK and ART-Ig. By combining KiH and CrossMab, Roche developed a blockbuster Faricimab with dual specificities for Ang-2 and VEGFA.

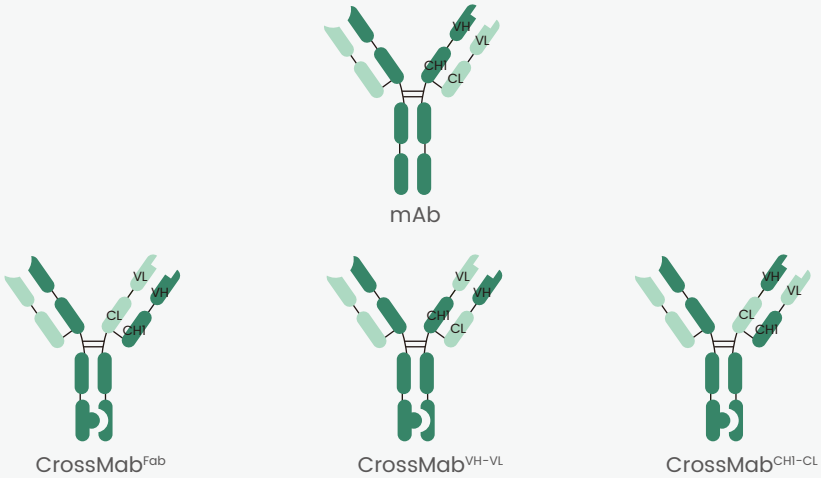

Figure 1. Different CrossMab Crossovers

Non-IgG-Like Bispecific Antibodies

The design of non-IgG-like bispecific antibodies is relatively simple. scFv fragments are widely used as fundamental building blocks in generating bsAbs. Utilizing properly engineered peptide linkers, scFvs can form dimers, trimers, tetramers, pentamers and even higher order oligomers. The bispecific T-cell engager (BiTE) is one type of tandem scFvs that consists of two scFvs, one binds to CD3 on T cells and the other one binds to a surface antigen on tumor cells to redirect T cells to kill tumor cells. Using this approach, Blinatumomab (Blincyto) has been approved by FDA for the treatment of acute lymphoblastic leukemia (ALL). Other common formats include dual-affinity re-targeting proteins (DARTs), tandem diabodies (TandAbs), single-chain diabodies, dock-and-lock (DNL) and nanobodies.

Bispecific Antibody Expression and Production

Choosing proper expression systems is vital to the efficient expression and production of bispecific antibodies. For some non-IgG bsAbs, such as BiTE and tandem bispecific scFv, they can be expressed in *E. coli*, yeast, or in mammalian cells, including CHO and HEK293 cells. *E. coli* is a common choice for scFv expression because bacteria can grow rapidly on cheap media and produce heterologous proteins in large amounts. However, expressed scFv molecules lack the formation of intra-domain disulfide bonds, which is essential to the "immunoglobulin fold" structure. Additional protein refolding and recovery steps are usually required to generate soluble scFv molecules. To overcome these problems, mammalian cells can be used to express bispecific scFvs due to their ability to perform complex post-translational modifications. Since they lack the Fc region, they are usually purified by using a His-tag or protein L chromatography.

Like conventional mAbs, IgG-like bsAbs are expressed predominantly in mammalian cells, especially in CHO cells. However, the production of bispecific antibodies is more challenging due to the double number of heavy and light chain genes. Typically, it requires at least two expression plasmids to be co-transfected into the CHO cells, and the ratio of the two plasmids may also influence both the quality and quantity of the expressed bsAbs. During the early stages of bsAb development, HEK293 cells are often used for transient expression. Sometimes it's difficult to scale up transient expression of IgG and the titers are relatively low compared to stable CHO cells. Due to various structural similarities between monoclonal and bispecific antibodies, many established purification processes for conventional mAbs are compatible with bispecifics. A variety of purification methods such as affinity, charge, size, hydrophobicity and mixed-mode-based separation techniques, are employed for the purification of bsAbs.

Understanding these manufacturing challenges, Sino Biological provides fast and efficient bispecific antibody expression services based on our expertise and experience in mammalian cell expression. Starting from the antibody sequence, we can deliver multiple bsAb formats such as BiTE, Diabody, CrossMab and DVD-IgG (some formats are listed in the figure 2). We have completed many bsAb production projects with >90% of overall success rates, and the highest yield has even reached ~250mg/L.

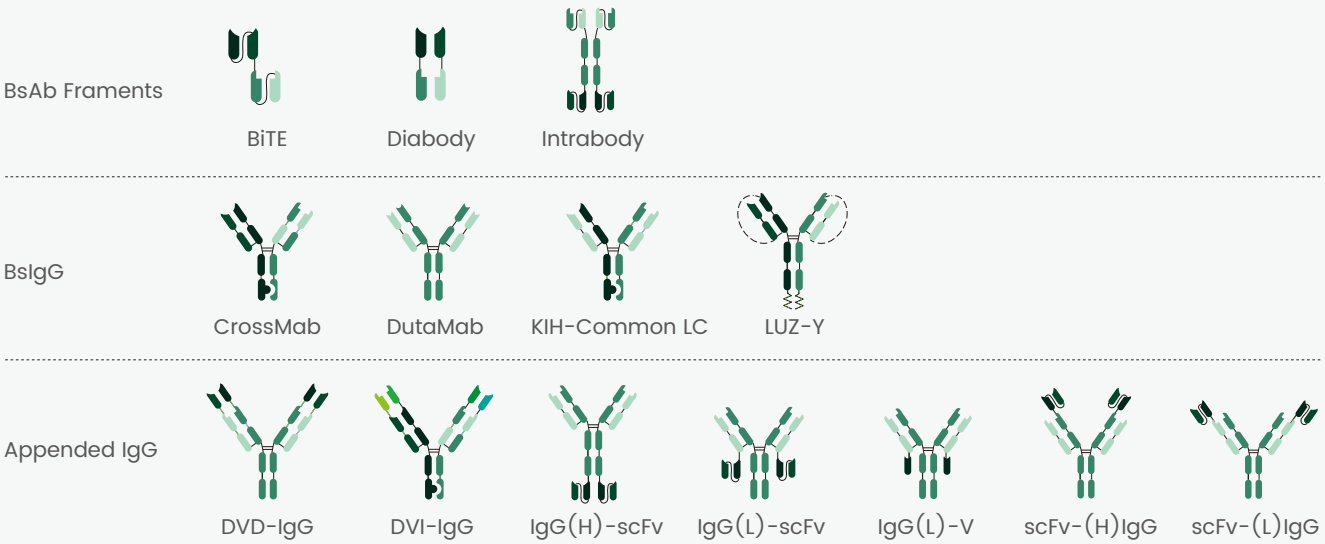

Figure 2. Our Experience with Diverse BsAb Formats
(The bsAb nomenclature - DOI: 10.1016/j.molimm.2015.01.003)

Here, we'd like to share a featured case of bispecific antibody production. The bsAb construct has a knobs-in-holes (KiH) design in its heavy chain. After the three-step purification process (figure 3), monomer purity was changed from 68.5% to 96.6%, meeting the final QC requirements.

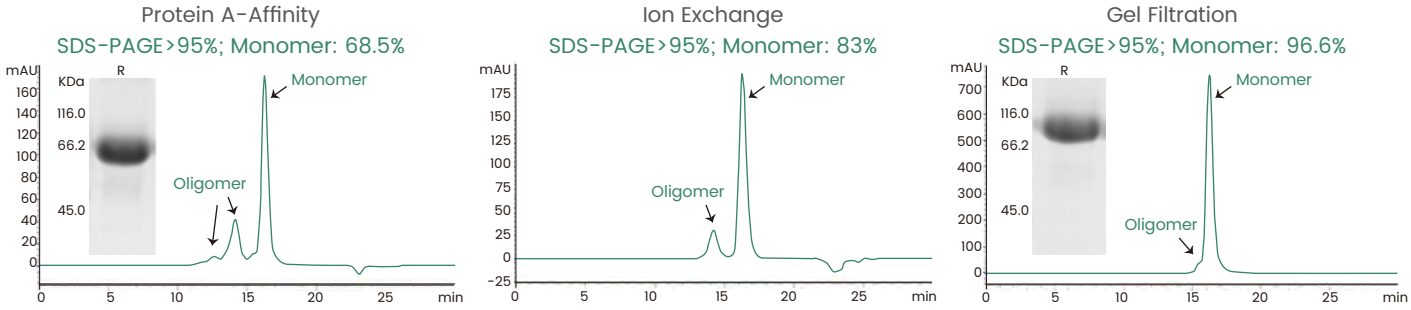

Figure 3. The Three-Step Process to Purify BsAbs

Conclusion Remarks

With the rapid development of recombinant DNA technology and a deep understanding of antibody engineering, diverse bispecific antibody formats are emerging to pursue optimal biological activity and clinical purposes. Bispecific antibodies have already shown great therapeutic potential in cancer and other diseases, such as diabetes, Alzheimer's disease and ophthalmological diseases. Currently, numerous bsAbs are entering clinical development and it can be estimated that there will be more bsAbs getting marketing approval in the future.

Sino Biological US Inc. (U.S.A)
Tel: +1-215-583-7898
Email: order_us@sinobiologicalus.com

Sino Biological Europe GmbH (Europe)
Tel: +49(0)6196 9678656
Email: order_eu@sinobiologicaleu.com

Sino Biological, Inc. (Global)
Tel: +86-400-890-9989
Email: order@sinobiological.com

www.sinobiological.com