WHITE PAPER

Exploring the strengths, challenges, and therapeutic applications of multispecific antibodies

Introduction

Antibody-based therapeutics have provided huge clinical benefits in a variety of diseases—and particularly in cancer.[1,2] Traditionally, antibody-based therapeutics have used monospecific formats (antibodies that recognize one antigen), but research into multispecific formats has gathered momentum in recent decades.[3,4] Multispecific antibodies can bind two or more epitopes at the same time—with antibodies that bind to two different epitopes more specifically referred to as 'bispecific antibodies'.[2] To date, 11 bispecific antibodies have been approved by the U.S. Food and Drug Administration (FDA) to treat various hematological and solid cancers, hemophilia A, neovascular age-related macular degeneration, retinal vein occlusion, and diabetic macular edema.[5-8] With the field expanding and many multispecific antibodies undergoing clinical trials (Figure 1), this captivating area of research offers a variety of clinical applications.[3] Here, we explore multispecific antibody formats and therapeutic applications, along with their advantages and challenges.

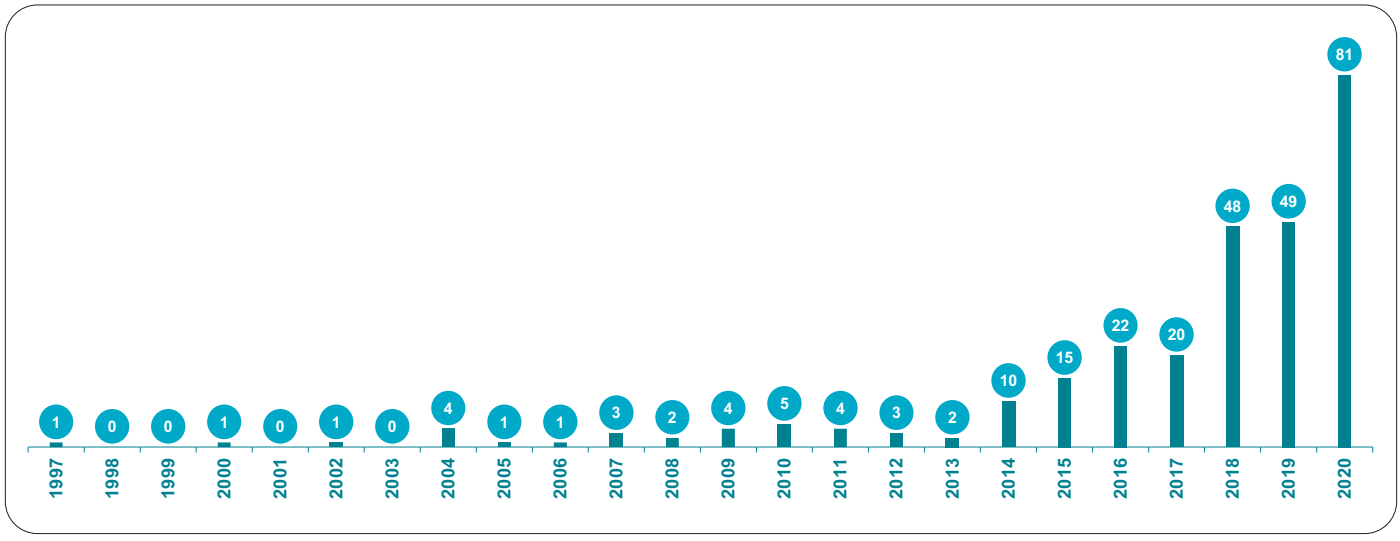

Figure 1. Number of clinical trials involving multispecific antibodies for cancer per year from 1997 to 2020. Data was collected from, and graph adapted from, Elshiaty M, Schindler H, Christopoulos P. Principles and current clinical landscape of multispecific antibodies against cancer. Int J Mol Sci. 2021;22(11):5632. doi:10.3390/IJMS22115632/S1. Elshiaty et al. used the terms "bispecific antibody" "trispecific", and "oncology" in ClinicalTrials.gov to obtain the data.

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Common multispecific antibody formats

Multispecific antibodies are typically classed into immunoglobulin G (IgG)-based antibodies and fragment-based antibodies—where fragment-based antibodies lack a fragment crystallizable (Fc) region (Figure 2).[9,10] The first report of bispecific antibodies was in 1961, where bispecific antibodies were generated by mixing and recombining univalent antibody fragments together.[11] Breakthroughs in antibody engineering techniques – such as hybrid hybridomas (quadromas), crosslinking methods, and genetic engineering methods – have propelled multispecific antibody research, enabling a wide array of multispecific formats and techniques to be generated, such as:[4]

- Bispecific T-cell engager (BiTE) – a bispecific antibody that binds to a tumor antigen and a T-cell antigen, such as CD3, recruiting and activating T cell cytotoxicity.[9,12] .
- Immune mobilizing monoclonal T-cell receptors against cancer (ImmTAC) – a fragment-based bispecific antibody that is a fusion protein, involving a T-cell receptor linked to a single chain antibody fragment that recognizes an HLA-displayed antigen.[13]
- Dual variable domain immunoglobulin (DVD-Ig) – a bispecific antibody that has two antigen-binding domains from two monoclonal antibodies, generating an antibody-based structure with four antigen-binding sites.[14]
- Diabody – a bivalent antibody that involves two single-chain variable fragments linked together through a short peptide chain.[15]
- Tandem diabody – a tetravalent antibody that is generated by linking two diabodies.[16]
- DuoBody – an IgG-based bispecific antibody that is generated through the DuoBody® platform (Genmab) that results in controlled Fab-arm exchange between two different monoclonal antibodies.[3]
- CrossMab antibody – a multispecific antibody generated through CrossMab technology that ensures correct light-chain association in bispecific antibody assembly.[17]
- Knobs-into-holes antibody – a multispecific antibody generated through knobs-into-holes technology that ensures correct heavy-chain association in bispecific antibody assembly.[18]

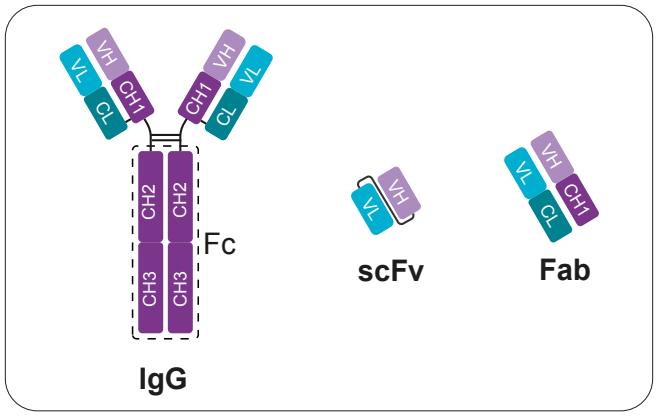

Figure 2: Overview of IgG antibody structure and key antibody-based building blocks in fragment-based multispecific antibodies (scFv and Fab).

Advantages of using multispecific antibodies over monospecific antibodies

Multispecific antibodies can offer a variety of strengths over monospecific antibodies (also referred to as monoclonal antibodies), such as reduced risk of drug resistance and improved specificity and efficacy (Figure 3). As monospecific antibodies target one antigen, multifactorial diseases such as cancer can develop resistance to monospecific antibodies. Cancer drug resistance can occur by cancer cells triggering or upregulating alternative pathways, which can help cancer cells to avoid the effects of treatment or the immune system; or by mutations, such as in the target antigen structure which can restrict antibody binding.[1,19,20] In contrast, multispecific antibodies target two or more antigens and can act through multiple mechanisms, making it more difficult for cancer to develop resistance to this therapeutic strategy. A variety of multispecific formats also recruit immune cells, reducing the ability of cancer cells to avoid detection and clearance by the immune system.[1]

Resistance can also emerge in other applications such as viral therapeutics, like antibody-based therapeutics for SARS-CoV-2. Through mutations – typically in its receptor binding domain – SARS-CoV-2 can develop resistance to neutralizing monoclonal (monospecific) antibody therapies.[21,22] However, multispecific antibodies can be designed to target several sites on viral targets, such as antibodies that bind different epitopes in the receptor binding domain[22] or antibodies that bind epitopes in the spike protein and the receptor binding domain.[21] This multispecific format can also increase binding affinity, which may reduce the dose needed for a clinical response.[22]

Multispecific antibodies can also improve efficacy over monoclonal antibodies. For example, multispecific antibodies can recruit cytotoxic immune cells to enhance killing of target cells.[23] In addition, as mentioned above, multiple pathways can be targeted – creating synergistic effects – improving efficacy compared to monospecific antibodies by acting on multiple pathways involved in disease.[24] Multispecific antibodies can also target pathways or antigens that can increase its uptake or access to a target site, improving efficacy over monoclonal antibodies that may have limited access and action to the target cell/ sites. For example, antibody-based therapies that target the central nervous system, such as neurodegenerative disorders, can struggle to cross the blood-brain barrier. One strategy to improve access to the central nervous system – and consequently efficacy of treatment – is to design a multispecific antibody that can bind to a receptor for blood-brain barrier transportation (such as receptor-mediated transcytosis receptors) as well as bind the disease molecular target/s.[25]

In addition, multispecific antibodies can also improve specificity compared to monospecific antibodies, reducing off-target effects (such as killing healthy cells)—particularly for antibodies that target tumor-associated antigens that are expressed at low levels on healthy cells and can consequently be bound and acted on by monospecific antibodies. Targeting multiple antigens, through multispecific antibodies, is one way to improve selectivity and consequently reduce off-target effects.[26] Targeting multiple antigens on a pathogen surface (such as HIV) can also improve affinity and can allow these therapeutics to act on multiple viral variants.[27]

Therapeutic applications of multispecific antibodies

Many different multispecific antibodies are in clinical development, with 11 bispecific antibodies approved for clinical use by the FDA (Figure 4).[3,5] The majority of these are approved to treat cancer:

- Blinatumomab (Blincyto) was approved in 2014 to treat relapsed or refractory acute lymphoblastic leukemia. Blinatumomab is a BiTE that binds CD19 on cancer cells and recruits T cells by binding to CD3.
- Amivantamab (Rybrevant) was approved in 2021 to treat metastatic non-small cell lung cancer. Amivantamab is a duobody that binds epidermal growth factor receptor (EGFR) and c-Met on cancer cells, blocking downstream growth and survival signaling, as well as binding to CD16 on immune cells through its Fc domain to induce immune cell-mediated cytotoxicity.[28]
- Tebentafusp (Kimmtrak) was approved in 2022 to treat metastatic uveal melanoma. Tebentafusp is a soluble TCR/ scFv fusion protein or ImmTAC that binds gp100 peptides displayed by HLA (HLA-A*02:01) on cancer cells and recruits T cells by binding CD3.[29]

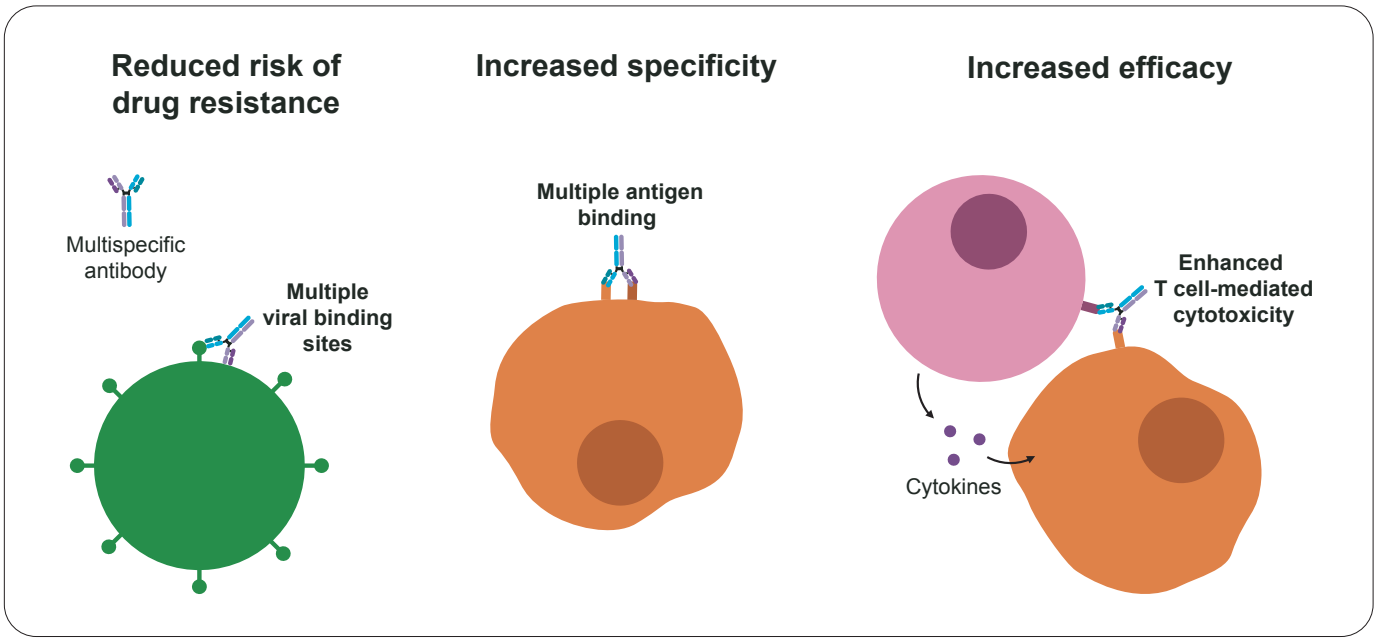

Figure 3. Overview of advantages of multispecific antibodies compared to monospecific antibodies.

- Mosunetuzumab (Lunsumio) was approved in 2022 to treat relapsed or refractory follicular lymphoma. Mosunetuzumab is a knobs-into-holes bispecific antibody that binds CD20 on cancer cells and recruits T cells by binding CD3.[31,32]
- Elranatamab (Elrexfio) was approved in 2023 to treat relapsed or refractory multiple myeloma. Elranatamab is a duobody that binds BCMA on cancer cells and recruits T cells by binding CD3.[33]
- Talquetamab (Talvey) was approved in 2023 to treat relapsed or refractory multiple myeloma. Talquetamab is a duobody that binds GPRC5D (G-protein-coupled receptor class 5 member D) on cancer cells and recruits T cells by binding CD3.[34]
- Glofitamab (Columvi) was approved in 2023 to treat relapsed or refractory diffuse large B-cell lymphoma. Glofitamab is a 2: 1 CrossMab that binds CD20 on cancer cells and recruits T cells by binding CD3.[35]
- Epcoritamab (Epkinly) was approved in 2023 to treat relapsed or refractory diffuse large B-cell lymphoma and high-grade B-cell lymphoma. Epcoritamab is a duobody that binds CD20 on cancer cells and recruits T cells by binding CD3.[5,36]

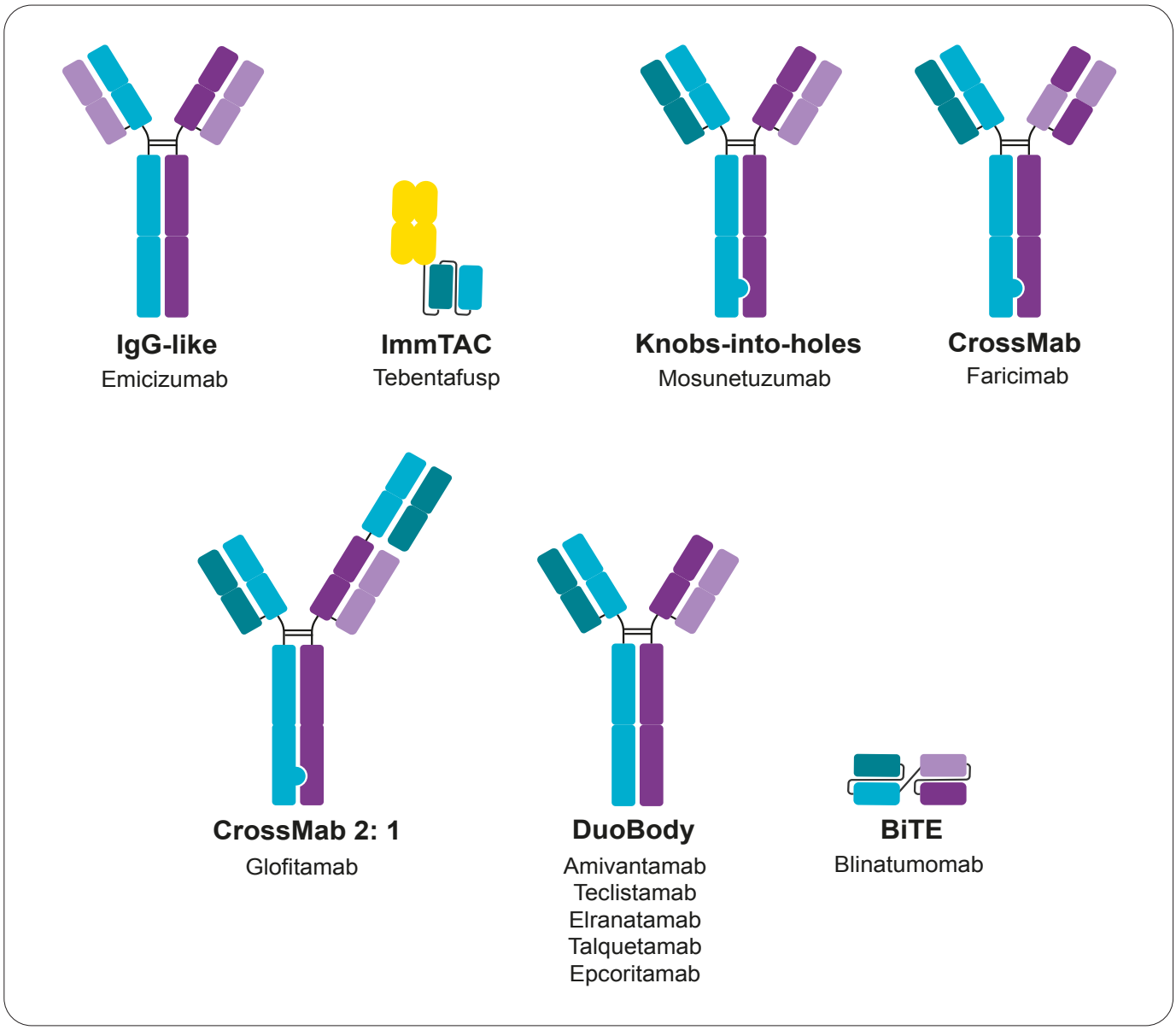

Figure 4. Overview of structures of bispecific antibodies currently FDA approved.

However, two bispecific antibodies have also been approved for conditions outside of cancer:

- Emicizumab (Hemlibra) was approved in 2017 to treat hemophilia A. Emicizumab is an IgG-like bispecific antibody that acts as a replacement for factor VIII (which is deficient in hemophilia A patients) by binding and linking factor IXa and factor X.[37,38]
- Faricimab (Vabysmo) was approved in 2022 to treat neovascular age-related macular degeneration, retinal vein occlusion, and diabetic macular edema. Faricimab is a CrossMab bispecific antibody that binds and neutralizes Ang-2 and VEGF-A.[5,39,40]

By multispecific antibodies being able to target multiple substrates, these constructs can widen the therapeutic applications of traditional antibody-based therapies, such as by improving delivery and access of antibodies to the target cell/site and targeting multiple disease pathways.[41] Consequently, while cancer is the main therapeutic focus so far, a variety of other diseases are being explored in preclinical and clinical studies—such as infections (e.g., HIV, COVID-19, hepatitis B, and human cytomegalovirus)[21,27,42] and autoimmune (e.g., rheumatoid arthritis)[43], vascular (e.g., atherosclerosis)[45], and neurodegenerative (e.g., Alzheimer's disease)[46] conditions.

Impacts of multispecific format on safety and development

Despite the strengths of multispecific antibodies, the complex and multicomponent structures of multispecific antibodies can impact their safety and development in clinical use, which is discussed below.

Safety of multispecific antibody formats

Many multispecific antibody formats can involve structures that are not typically exposed to or encountered by the immune system, such as antibodies containing single or non-human domains.[47] These structures can create immunogenicity issues in humans by the immune system detecting these constructs as foreign, triggering anti-drug antibodies (which can clear or neutralize the antibodies) or toxic responses.[27,48,49]

Among the most severe immunological responses is cytokine release syndrome (CRS)—where a substance can trigger a strong systemic inflammatory response that can be fatal. CRS results after strong activation of immune cells and is linked with high levels of cytokines, such as interleukin-6 (IL-6) and interferon γ.[50] Immunotherapies – such as antibody-based therapeutics – cause a strong stimulation of the immune system compared to the levels found under normal conditions, which can lead to adverse immunological responses after treatment. CRS has become a particular problem facing T-cell engaging therapies, including multispecific antibodies that recruit and activate T cells for targeted cell killing. T-cell engager bispecific antibodies, catumaxomab and blinatumomab, have both been linked with CRS—catumaxomab has been voluntarily withdrawn from the market (due to commercial reasons).[51]

However, the multicomponent aspect of multispecific antibodies also allows researchers to develop formats that can mitigate the risk of immunogenicity issues like CRS. For example, Carrera et al. designed a multispecific antibody that binds four different antigens: CD3, IL-6 receptor, EGFR, and PD-L1. This multispecific antibody was able to recruit and activate T cells (through CD3 binding) for selective T cell killing of cancer cells (through EFGR and PD-L1 binding). By binding to the IL-6 receptor, the multispecific construct also reduced IL-6 signaling after immune cell activation, which reduced – but not completely blocked – interferon γ levels. Cytokine levels were further controlled by the CD3-binding ligand, which had attenuated binding for CD3, modulating stimulation of T cells.[51] Alternatively, different immune cells can be recruited for immune-cell engager multispecific antibodies, such as natural killer (NK) cells, which are associated with a better safety profile than T cells.[42] In addition, the multicomponent structure of multispecific antibodies aims to enhance their selectivity for target cells, which can reduce off-target toxicity by restricting action on non-target cells.[52]

Manufacturing, design, and development of multispecific antibody formats

The complex nature and wide diversity of multispecific antibody structures can create manufacturing challenges, such as inefficient assembly or misassembly and aggregation.[41,47] For example, some fragment-based multispecific antibodies can aggregate easily.[49] Multispecific antibodies may need specialized expression vectors to express complex structures and to successfully assemble. And once multispecific antibodies are expressed, variations can be found in their structures (such as glycosylation patterns and misassembled structures) and contaminants may be generated that have similar biophysical features to the desired multispecific antibody product. The complexity

of these structures and potential variations or contaminants can make purifying and analyzing multispecific antibodies challenging—particularly as conventional methods have been developed to handle monoclonal antibodies.[47,49] These manufacturing challenges can restrict multispecific antibody scalability, testing in high-throughput studies, and consequently, their development.[47,49]

However, research is underway to improve design, production, purification, and analysis of multispecific antibodies to aid development. For example, new resins can help purify multispecific antibodies and new computational methods are being developed to help better predict multispecific structures that have a more desirable drug-like profile.[41,53] And, despite the challenges facing some multispecific antibodies, other formats can display excellent properties. For example, some Fabs-in-tandem, involving two Fabs linked together through a variable region of one Fab heavy chain to a constant region of the other Fab light chain, can show low aggregation and favorable expression and stability.[47] In addition, multispecifics can be designed to improve their biophysical properties, such as introducing disulphide bonds in some fragment-based multispecific antibody formats to improve stability and reduce aggregation.[49]

The half-life is another important consideration when designing a multispecific antibody. With fragment-based mutlispecifics lacking an Fc domain, their half-lives are typically much shorter than IgG-based multispecific antibodies. The Fc domain interacts with the neonatal Fc receptor, stopping degradation of the antibody construct.[49] While a shorter half-life can reduce the extent of adverse events – as antibodies with a shorter half-life are cleared quicker from the body than those with a longer half-life – designing formats with extended half-lives can reduce dosing frequency, improving patient convenience.[49]

Summary

Multispecific antibodies represent a promising and diverse class of therapeutic antibodies, with 11 members already FDA approved for treating various cancers, eye conditions, and blood disorders. These antibodies feature multicomponent structures designed to enhance specificity and efficacy compared to traditional monoclonal antibody-based treatments. Additionally, they address drug resistance that can arise with monoclonal antibody therapies. Despite manufacturing challenges posed by their complex structures, ongoing scientific advancements in designing, purifying, and analyzing multispecific antibodies hold great potential for advancing this exciting field of research.[41]

About Revvity

Revvity – a science-based solutions company – is committed to advancing healthcare by expanding the boundaries of human potential through science. Revvity offers a variety of immunoassays and reagents to aid monoclonal antibody-based therapeutics research, such as:

- Toolbox screening reagents – reagents that can aid characterization of protein–protein interactions (such as antibody–antigen binding efficiency). For example, Revvity offer reagents that can help run affinity maturation experiments and screen medium to large biologics libraries.
- Potency assays – bioassays, including cell-based assays, to help determine the potency of the drug substance and evaluate the reproducibility and stability of drug products.
- *In vitro* assays to assay immune response – a range of highly sensitive immunoassays – including Cr51, TRF-based assay technology, and next-gen no-wash assays – to characterize the cytokine response, measure signaling events, and understand the antibody-dependent cell-mediated cytotoxicity (ADCC) of biologics.
- Fc gamma receptor (FcgR) and neonatal Fc (FcRn) assays – binding assays, cellular assays, or biochemical binding assays to measure FcgR-specific antibody binding. These assays are ideal for predicting antibodies with strong ADCC activity and longer circulating half-lives.
- Contamination assays – ready-to-use assays to detect host cell contaminants (such as from CHO and HEK293 cell lines, residual dsDNA, and mouse or human albumin), which is critical to obtaining reliable research results and ensuring the efficiency and safety of a drug product.

To find out more about Revvity's research solutions, visit www.revvity.com.

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