Bispecific Monoclonal Antibodies for Targeted Immunotherapy of Solid Tumors: Recent Advances and Clinical Trials

Seyed Samad Hosseini[1], Saeed Khalili[2], Behzad Baradaran[3], Mohammad-Ali Shahbazi[4,5,6], Jafar Mosafer[7,8], Mahmoud Hashemzaei[9], Ahad Mokhtarzadeh[3]*, Michael R Hamblin[10,11,12]**, Hélder A. Santos[4,13]***

[1] Department of Biotechnology, Higher Education Institute of Rab-Rashid, Tabriz, Iran
[2] Department of Biology Sciences, Faculty of Sciences, Shahid Rajaee Teacher Training University, Tehran, Iran
[3] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[4] Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki FI-00014, Finland
[5] Zanjan Pharmaceutical Nanotechnology Research Center (ZPNRC), Zanjan University of Medical Sciences, 45139-56184 Zanjan, Iran
[6] Department of Pharmaceutical Nanotechnology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran
[7] Department of Medical Biotechnology, School of Paramedical Science, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran.
[8] Nanotechnology Research center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
[9] Department of Pharmacodynamics and Toxicology, School of Pharmacy, Zabol University of Medical Sciences, Zabol. Iran
[10] Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, 02114, USA.
[11] Department of Dermatology, Harvard Medical School, Boston, MA, 02115, USA
[12] Laser Research Centre, Faculty of Health Science, University of Johannesburg, Doornfontein 2028, South Africa
[13] Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki FI-00014, Finland

Corresponding authors:
* mokhtarzadehah@tbzmed.ac.ir (A. Mokhtarzadeh)
Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
** Hamblin.lab@gmail.com (M.R. Hamblin).
Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, 02114, USA
*** helder.santos@helsinki.fi (H.A. Santos)
Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki FI-00014, Finland

Abstract

Bispecific antibodies (BsAbs) combine two or more epitope-recognizing sequences into a single protein molecule. The first therapeutic applications of BsAbs were focused on cancer therapy. However, these antibodies have grown to cover a wider spectrum, including imaging, diagnosis, prophylaxis, and therapy of inflammatory and autoimmune diseases. BsAbs can be categorized into IgG-like formats and non-IgG-like formats. Different technologies have been used for the construction of BsAbs including "CrossMAb", "Quadroma", "knobs-into-holes" and molecular cloning. The mechanism of action for BsAbs includes the induction of antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), apoptosis, recruitment of cell surface receptors, as well as activation or inhibition of various signaling pathways. The first clinical trials included mainly leukemia and lymphoma, but solid tumors are now being investigated. The BsAbs bind to a tumor-specific antigen using one epitope, while the second epitope binds to immune cell receptors such as CD3, CD16, CD64, and CD89, with the goal of stimulating the immune response against cancer cells. Currently, over 20 different commercial methods have been developed for the construction of BsAbs. Three different BsAbs are currently clinically approved and marketed, and more than 85 clinical trials are in progress. In this review, we discuss recent trends in the design, engineering, clinical applications, and clinical trials of BsAbs for the treatment of solid tumors.

Keywords: Bispecific antibodies, Solid tumor, Immunotherapy, Bispecific T cell engagers, clinical trials

1. Introduction

Monoclonal antibodies (MAbs) are monovalent antibodies capable of specific binding to unique epitopes, which were first generated from a single B-cell clone. In 1975, Köhler and Milstein were the first to produce anti-sheep red cell monoclonal antibodies. They developed a method to produce monoclonal antibodies via a method called the hybridoma technique [1]. In recent years, MAbs have remarkably improved the treatment outcomes for a wide variety of human diseases, including inflammatory and neoplastic diseases [2]. Nevertheless, despite being widely employed for cancer immunotherapy, MAbs have not been as effective as they were first expected to be. Clinical cancer treatment strategies using monospecific therapy suffer from disadvantages, including a large number of patients with poor response, and individuals who develop resistance to the therapy and suffer tumor relapse [3]. Given these problems, MAb-based bispecific antibodies (BsAbs) have been developed to overcome some drawbacks of MAb-based therapy [4]. BsAbs can simultaneously bind to two different antigens (Figure 1). This advantage has led to a wide range of applications, and to a multitude of technologies to produce them. Technologies such as "Quadroma", "CrossMAb", "knobs-into-holes", molecular cloning and short peptide linkers have been widely used for the production of BsAbs [5]. Therapeutic antibodies have been reported to utilize different mechanisms of action to combat various diseases, such as cancer, autoimmune diseases, infections, transplantation rejection, and inflammatory disorders. These mechanisms include the induction of antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), induction of cell death (apoptosis), and engagement of cell surface receptors leading to inhibition or activation of signaling pathways [2]. Two different receptors or ligands on the surface of the same cell could be simultaneously targeted by BsAbs, which could lead to inhibition or

stimulation of two different signaling pathways. The consequences of these changes in cell signaling could include the inactivation of inflammatory-related pathways or proliferation pathways. Moreover, BsAbs can engage immune effector cells to target cancer cells, via binding to one surface antigen expressed on cancer cells, and a second antigen expressed on specific immune cells, including natural killer (NK) cells or effector T cells [6]. The fusing of the antitumor binding domain with the Fc receptor (FcR) or the anti-CD3 binding domain, is one popular strategy to produce BsAbs capable of recruiting immune cells. There are two major formats of BsAbs: a non-IgG-like format and an IgG-like format. The IgG-like BsAb format triggers Fc domain-mediated functions, such as ADCC, CDC, and ADCP. The IgG-like BsAb format also preserves the physical properties imparted by the FcR, including the use of standardized purification methods and maintains serum stability. On the other hand, non-IgG-like formats, such as "TandAbs", "BiTE", "Nanobodies", and "Diabody" lack the entire Fc region. These BsAb formats enhance efficient tissue penetration, reduce the activation of non-specific immune cells, and show higher tumor specificity due to the smaller size. However, the lack of the Fc region results in a shorter circulation half-life for this BsAb format [7]. Among the clinically approved BsAbs, Catumaxomab and Hemlibra (emicizumab-kxwh) are in the IgG-like format, while Blinatumomab is in non-IgG-like format. Catumaxomab can simultaneously bind to two different antigens (anti-CD3 and anti-EpCAM) via its antigen-binding domains. It can also interact with dendritic cells, NK cells, and macrophages through its Fc domain [8]. Catumaxomab was reported to have a prolonged serum half-life. However, the Fc domain of Catumaxomab could trigger immunogenicity against the BsAb and compromise the effectiveness in patients [9]. Catumaxomab was approved as the first anti-EpCAM antibody in 2009 to combat malignant ascites in patients who suffer from EpCAM-positive cancer. Catumaxomab was voluntarily

withdrawn from the US market in 2013 and from the European market in 2017 for commercial reasons. The product had not been marketed in Europe since 2014 [10]. Blinatumomab is a non-IgG-like molecule and lacks the Fc region. The US Food and Drug Administration (FDA) approved Blinatumomab in 2014 for the treatment of relapsed B-precursor acute lymphoblastic leukemia (ALL) [11]. Emicizumab-kxwh (HEMLIBRA), is a humanized modified bispecific monoclonal IgG4 antibody. It lacks any homology to factor VIII, binds to factor X and factor IXa, and mimics the function of the coagulation factor VIII. On 4 October 2018, the FDA approved emicizumab-kxwh administration for routine prophylaxis in patients with hemophilia A (with or without factor VIII inhibitors) [12]. Currently, more than 85 BsAbs are in preclinical studies or clinical trial phases. About two-thirds of these BsAbs have been designated for cancer therapy (Table 1) [13]. Autoimmune, infectious, hemophilia, and Alzheimer's diseases are among the conditions for which BsAbs are being developed as therapeutics [14]. In this review, we discuss recent advances in the therapeutic application of BsAbs for cancer therapy and the most recent clinical trials.
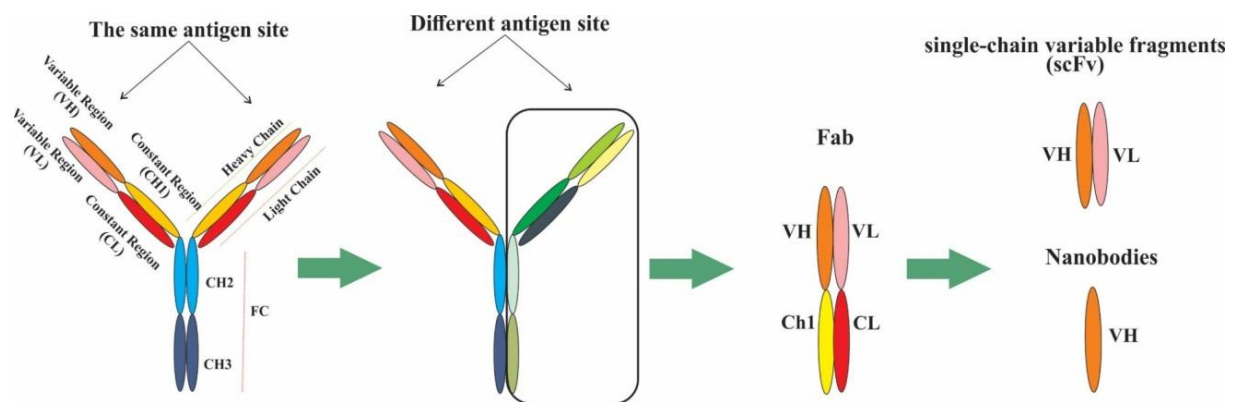

Figure 1. The differences between a standard antibody against bispecific antibody and Fab, scFv, and Nanobody structure.

2. The design and engineering of BsAbs

The first study on the production of antibodies with mixed specificities was initiated in the 1960s by Alfred Nisonoff [15]. In 1985, Staerz and Bevan used the hybridoma technology to produce the first BsAb, capable of T-cell recruitment [16]. The advent of hybridoma technology was the starting point for the growing use of BsAbs, between 1985-1995, that was called the "bispecific explosion" [17]. Three methods have been generally used for the production of BsAbs, including: (1) quadroma technology; (2) chemical conjugation; and (3) genetic approaches (recombinant DNA) [18].

2.1. Quadroma technology

Quadroma technology employs the somatic fusion of two different hybridoma cell lines for the production of BsAb. Each type of hybridoma cell expresses a murine monoclonal antibody with its own specificity. The quadroma cell lines, obtained from the fusion of the two hybridoma cell lines, produce IgG-like BsAbs with two different arms and, therefore, two specificities [19]. BsAbs produced by quadroma are similar to normal antibodies with the same Fc-mediated effector function. BsAbs with IgG-like formats have a longer circulation half-life, which is a consequence of FcRn-mediated recycling and their larger size. Moreover, the Fc region of BsAbs can be employed for their purification, and also increases their solubility and stability [20]. Non-functional antibodies are also produced by this method, due to the random pairing of the light chains and heavy chains from two distinct antibodies within the resulting quadroma cell line. The mispaired by-products of the quadroma cell line severely reduce the production yields [21]. To solve the random chain association problem, a chimeric quadroma cell line was constructed combining both rat hybridoma and murine hybridoma cell lines [22]. This chimeric quadroma

expressed both the rat IgG2b and mouse IgG2a chimeric antibodies. Catumaxomab was the first approved BsAb produced by this approach for the treatment of ascites [23]. Similar quadroma techniques have been used for the development of other BsAbs. For example, ertumaxomab (anti-CD3 and anti-HER2) and FBTA05 (anti-CD3 and anti-CD20) [24] have been produced by this method, and now are in various phases of clinical trials for solid tumors and B-cell lymphoma (Figure 2) [25].
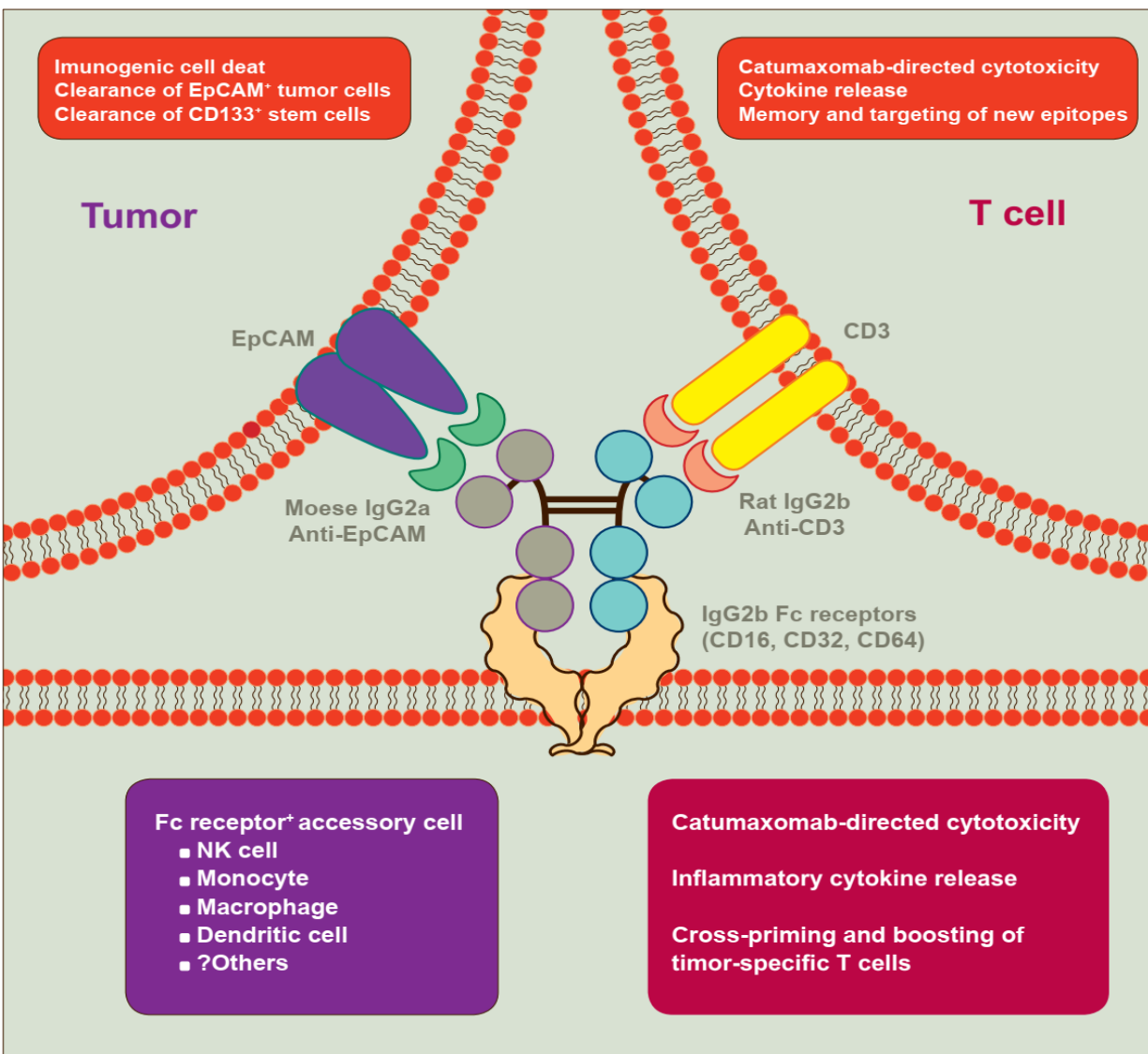
Figure 2. Catumaxomab is a trifunctional BsAb that has an affinity for accessory immune cells, T-cells, and tumor cells. It binds to EpCAM on the tumor cells, the Fc receptor on accessory immune cells, and CD3 on T lymphocytes. Catumaxomab kills the tumor cells by inducing ADCC, CDC, and ADCP.

2.2. Knobs-into-holes (KiH) technology

The production of IgG-like BsAbs is associated with problems such as the production of undesirable entities, including the mispaired molecules. These challenges could be overcome using the KiH technology that employs an engineered CH3 domain for heterodimerization. The "Knobs" are constructed by substitution of small amino acids with bigger ones at the interface across the CH3 domain of one heavy chain, while the "holes" are created by substituting large amino acids with smaller ones in the other heavy chain [26]. Although this approach enables the correct heavy chain heterodimerization, it cannot prevent the random pairing of the two different heavy chains with light chains. Some alternative approaches have been devised to deal with this problem. One of these methods produces BsAbs with common light chains. Several antibodies, which bind to different antigens while sharing identical variable light chains, have been engineered to form BsAbs without the mispairing problem involving light chains. However, this strategy is limited to antibodies that share common light chains with the ability to bind different antigens [4]. An alternative strategy to prevents the mispairing problem of light chains is the separate expression of the knob and hole as half proteins in different bacteria (Figure 3) [27]. However, the lack of mammalian glycosylation modifications may affect the functions of the produced antibody [28].
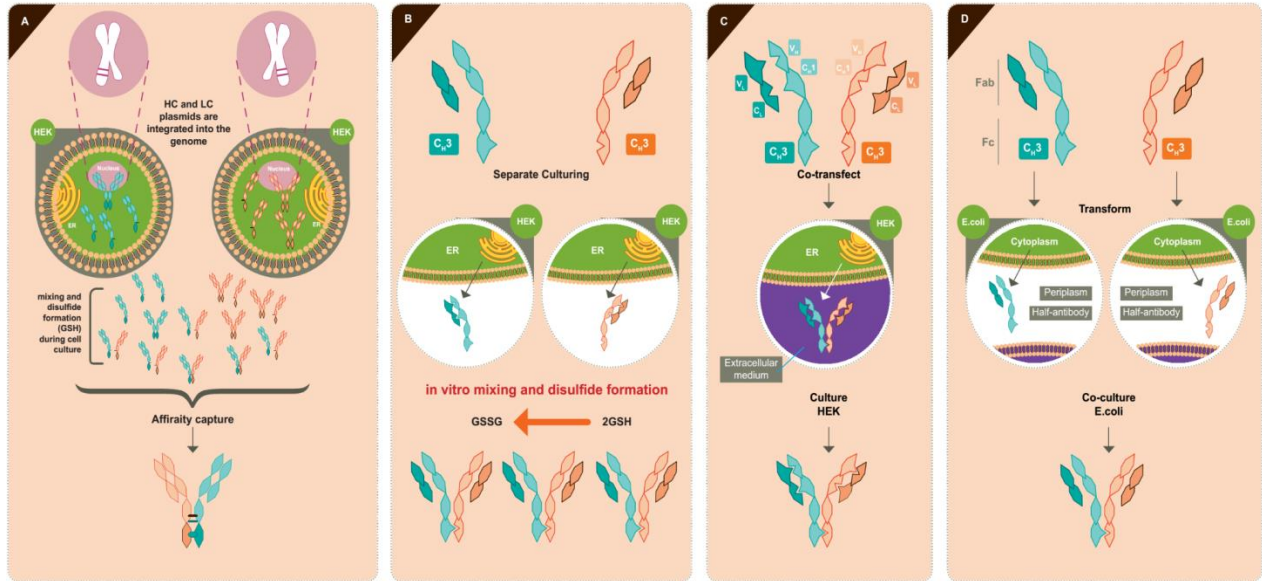
Figure 3: Alternative strategies to prevent the mispairing problem of light chains. (**A**) In the co-culture method for mammalian cell lines, two stable cell lines are grown in the same production flask. Half-antibodies are secreted and recombine to form homodimers and heterodimers. The addition of reduced glutathione helps disulfide formation and correct pairing. The correct BsAbs are then purified from the media [29]. (**B**) Using transient transfection, the plasmids for each heavy chain and light chain fragment are transfected into two mammalian cell lines and cultured separately. Two independent half-antibody molecules are secreted into the media. Antibody folding and assembly is accomplished by in vitro combination followed by treatment with reduced glutathione to catalyze disulfide bond formation. (**C**) In mammalian cells, light and heavy chains of one half-antibody are designed with complementary mutations in the CH1-CL and VH-VL interfaces that result in better pairing between two desired chains [30]. (**D**): Co-culture of *E. coli* bacterial cells that have been transfected with half-antibody plasmids. During cell lysis, the heavy and light arm fragments dimerize and inter-chain disulfide bonds are formed. After cell lysis, the assembled BsAbs can be purified from the supernatant.

2.3. CrossMAb technology

CrossMAb technology, when used in combination with the KiH technology, offers an approach to ensure accurate light chain association in IgG-like BsAbs. The BsAbs consist of one modified arm (both light chains and heavy chains), and the other is an unmodified arm. The modifications can be confined to the CL-CH1 domain, the VL-VH (light and heavy chain) domain, or the entire Fab region [31]. As a result of these alterations, the unmodified heavy chain can no longer associate with the modified light chain; therefore, the desired chain association is enforced. Among the three possible modifications, the CL-CH1 CrossMAb showed the best composition and purity (Figure 4) [32]. Generation of different BsAbs formats such as trivalent, tetravalent, and multispecific antibodies can be achieved using the CrossMAb technology. Several BsAbs have been created by CrossMAb technology and are currently being evaluated in clinical trials [33]. The BsAbs that have been generated with CrossMAb technology so far are CC-93269 (CD3, BCMA) for relapsed and refractory multiple myeloma; RO7082859 (CD3, CD20) for relapsed/refractory B-Cell Non-Hodgkin's lymphoma; RO6958688 (CD3, CEA) for carcinoembryonic antigen (CEA)-positive solid tumors; RG7221 (Ang2, VEGF), RO5520985 (ANG2, VEGF) and RO7121661 (PD-1, TIM3) all for solid tumors [34].
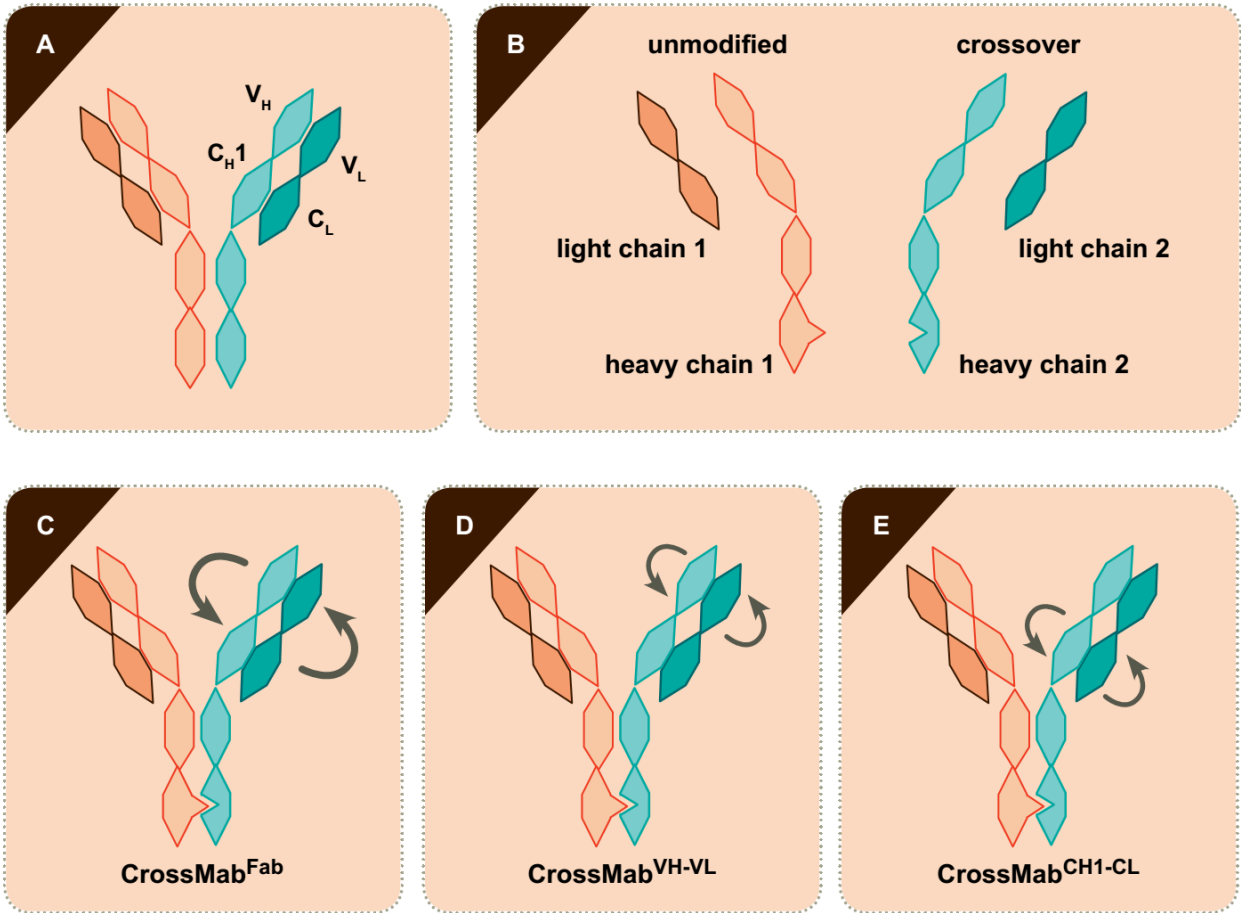
Figure 4. CrossMAb technology. Light colors indicate light-chain domains, and dark colors indicate heavy-chain domains. **(A)** Both arms of the intended bispecific antibody. **(B)** Design of the four chains of the bispecific antibody. KiH technology is applied for heavy-chain hetero-dimerization. **(C)** The VL-CL and VH-CH1 domains are cross-associated with each other. **(D and E)** Only the VL and VH domains are cross-associated (D), or the CL and CH1 domains (E) within the Fab region of one half of the bispecific antibody.

2.4. Dual Variable Domain immunoglobulin (DVD-Ig)

A BsAb format composed of two different light and heavy chains has recently been produced by the DVD-Ig approach. Both light and heavy chains of the DVD-Ig possess an additional variable domain. A linker sequence is used to connect the extra variable domain to the VL and VH

domains of the existing MAb. DVD-Ig possesses four antigen recognition sites; therefore, each single Fab of the DVD-Ig molecule can bind to two targets [35]. A DVD-Ig molecule could be produced by using a pair of different MAbs. Therefore, to construct molecules with high specificity and variable valence, the specific antibodies could be further modified. This approach improves the production yield, homogeneity, and stability through avoiding the mispairing of different light or heavy chains [36]. ABT-165 and OMP-305B83 are BsAbs that were generated using the DVD-Ig approach. Both of these BsAbs have been designed to target vascular endothelial growth factor A (VEGFA) plus angiopoietin 2 (ANG-2) in the vasculature of solid tumors. Currently, a phase I clinical study of ABT-165 in patients with solid tumors is ongoing, while the phase I clinical study of OMP-305B83 was completed (Figure 5) [37].
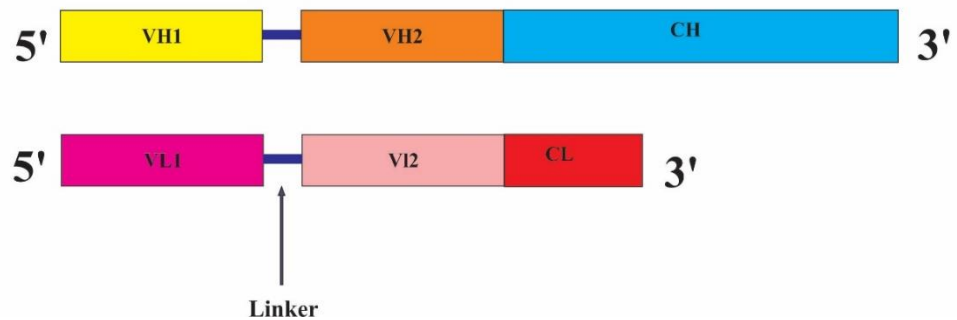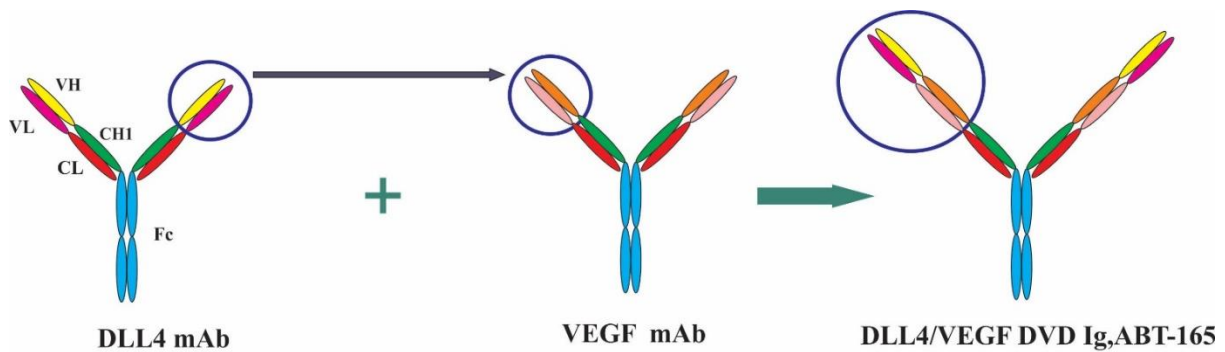

Figure 5: Production of ABT-165 BsAb with dual binding activity to VEGF and DLL4. ABT-165 DVD-Ig with an anti-VEGF and an anti-DLL4 variable domain are connected in tandem using light and heavy chains.

2.5. Dual action Fab (DAF) BsAbs

The two-in-one or dual-action Fab (DAF) is an alternative approach for *de novo* generation of BsAbs against two distinct antigens [38]. Each antigen-binding region in DAF BsAbs is able to detect two different antigens. The pattern of antibody binding to each intended antigen should be fully understood. To recognize a second antigen in addition to the first, a mutation must be introduced into the antigen-binding region, and further engineering within the variable domain is required to achieve maximum dual affinity [39]. The DAF platform employs the advantage of differential yet overlapping complementarity-determining regions as the recognition motif for each antigen [40].

3. Non-IgG-like formats

Progress in recombinant DNA technology has made it possible to produce small recombinant BsAbs composed of two different variable fragments, such as variable fragments (Fv) and single-chain Fv (scFv). Small BsAbs can be constructed using quadroma technology or chemical conjugation. These BsAbs are endowed with high target affinity and a smaller size for rapid tissue penetration [41]. A short peptide linker sequence is currently employed for the fusion of the two different scFvs (in either the VL-VH or VH-VL orientation) to construct small BsAbs [42]. Recombinant DNA technology allows researchers to generate diverse formats of BsAbs with a high degree of flexibility. Several formats of BsAbs have already been constructed using this approach. These small BsAb formats include TandAbs, BiTE, Nanobodies, dual-affinity-retargeting format (DART), and Diabody [43].

3.1. ScFv-based BsAbs

ScFvs are a very useful recombinant antibody format. For various clinical applications, this antibody format has been engineered as larger, multivalent, bispecific, and conjugated forms. ScFv molecules have the antigen-binding site comprised of the VL and VH domains of an antibody, without the FC region. The VL domain is attached to the VH domain via a polypeptide linker. These domains can be associated with each other using non-covalent interactions, disulfide bonds, or both to form monovalent antibody fragments [44]. A short circulation half-life and poor binding affinity are the limitations of these monovalent recombinant antibodies, including scFvs. To overcome these limitations, multi-specific and multivalent molecules have been designed based on ScFv domains as their fundamental antigen-binding units. The ScFvs and ScFv-based bispecific molecules have been fused to a wide range of cargo molecules, including toxins, radionuclides, drugs, liposomes, viruses for gene therapy, and biosensors [45]. ScFv molecules are produced by the genes of mouse hybridoma spleen cells. The ScFv molecule has the ability to bind to its antigen with the same affinity as the parental mouse MAb [46]. In comparison with normal IgGs, scFvs display more efficient tissue penetration and higher specificity for tumor cells; Given these facts, in comparison to other BsAbs, scFv-based BsAbs could be more generally applicable and have many clinical applications [47]. For instance, the bispecific T-cell engager (BiTE) is a class of artificial BsAb, which acts as a bridge between tumor cells and T cells. Its administration could lead to stimulation of the cytotoxic activity of T cells against tumor cells by secretion of enzymes like granzyme and perforin in the absence of MHC I recognition or expression of co-stimulatory molecules. Each BiTE molecule contains two ScFvs (with different amino acid sequences) or sequences from four different genes, which are linked by a glycine-serine rich 5-amino-acid linker peptide. One of the arms in ScFvs binds to T

cells via the CD3 receptor, and the other arm binds to a specific epitope on the surface of the tumor cell. Different BiTE antibodies can be engineered to target different types of cancers by altering the tumor-binding arm. These types of antibodies are among the most popular BsAb formats, and are currently in pre-clinical studies and clinical trials [48].

Tandem ScFvs are another class of bispecific antibody fragments, which are created by a fusion between two ScFv fragments. These fragments are joined together by a flexible peptide linker in tandem orientation. ScFvs can also be fused together by a helical linker or a flexible linker. The format of tandem ScFvs can be distinguished from each other by the direction of the VL and VH domains in the ScFvs, and the connecting linker sequence. The lack of the Fc fragment endows the tandem ScFv with a smaller size and a shorter half-life. Unlike MAbs, which are mostly expressed in mammalian cells, ScFv molecules are more often expressed in bacterial cells such as *Escherichia coli*. However, tandem ScFvs are also expressed by mammalian cells, which confers appropriate flexibility to each of the scFv fragments. BiTE technology is based on the tandem ScFvs format [49].

Decreasing the size of the peptide linker (to about five amino acids) between the variable domains could help the correct pairing of the domains from two different polypeptides. This approach can lead to the construction of compact BsAbs, called bispecific Diabody [50]. To favor heterodimerization over homodimerization, mutations can be introduced into the VL–VH interface to improve the yield of the desired format. To improve the stability of the produced Diabody, an interdomain disulfide bond could be engineered into the Diabody structure. The molecular weight of a Diabody is approximately ~50 kDa, and it can be expressed in bacterial cells, like the tandem ScFv fragments, Diabodies have short circulation half-lives, small size, better tissue penetrations and high specificity against the target antigen [51].

The correct chain pairing of the Diabodies can be ensured by fusing the two variable chain domains within a single-chain Diabody molecule (scDb). The scDbs are constructed by the expression of a single molecule, either in the opposite light-heavy chain configuration or in the format of VHA-linkerA-VLB-linkerM-VHB-linkerB-VHA (heavy-light configuration). To assemble the functional Abs, the A and B linkers should be five amino acids in length, and the M linker should be 15–20 amino acids. These scDbs have the same size as a tandem scFv constructed via the fusion of two scFvs. However, they differ in the length of the linkers and the arrangement of the domains. Therefore, a tandem scFv could be considered as two separate scFvs connected by a linker, whereas the scDb is a dense structure that is constructed from four variable domains. The scDb has a more compact and less flexible structure compared to the tandem scFv. To restore the Fc-mediated biological activity and/or to extend the half-life, the scDb can also be fused with additional Fc or CH3 domains [52].

Dual-affinity retargeting molecules (DARTs) are Diabody-like molecules created via the association of a VL region from one chain, with a VH region from a second chain in a VLB-VHA plus VLA-VHB configuration. After the introduction of disulfide bonds, the DARTs can be further stabilized [14]. The small size and the lack of the Fc fragment in DART molecules make them subject to rapid elimination. DART molecules can be tailored to incorporate an Fc fragment to enhance the half-life by FcRn-dependent recycling [53].
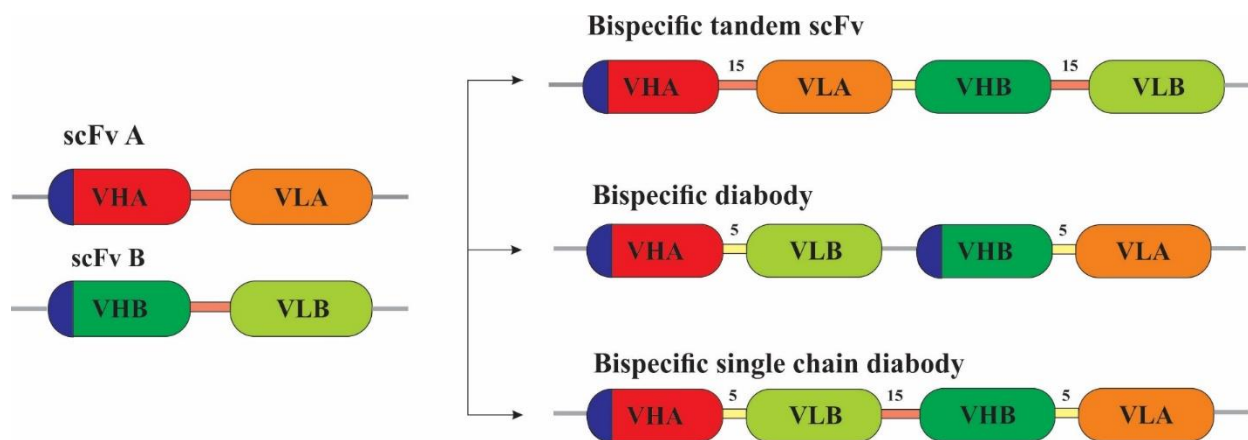
Figure 6. Formation of BsAbs from the variable chain domains of two antibodies.

3.2. Nanobodies

Nanobodies are another type of antibody-based platform, with the smallest (12–15 kDa) molecular weight (Figure 1). These proteins are considered to be a new class of antibody molecule, originating from single-domain antibody (sdAb) fragments. They possess unique functional and structural properties. A sdAb is an antibody fragment containing a single monomeric variable antibody domain [54]. The first sdAbs were composed of the variable region of heavy chain antibodies (12–15 kDa) found in camelids (also known as V_HH fragments). Nanobodies are fully functional antibodies, which lack light chains and hence include only heavy chains. These heavy-chain-only antibodies possess two constant domains and a single variable domain. Owing to the constant domains, nanobodies have a relatively long half-life [55]. Nanobodies with various binding specificities (isolated from camels and llamas) have been fused to each other using short linker sequences, to construct BsAbs. They can be used to target hidden antigens or employed as primary building blocks to create multi-specific molecules [56].

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3.3. Dock and lock method (DNL)

The DNL method combines site-specific conjugation with recombinant engineering. This method has the capability to generate bioactive molecules displaying multi-functionality and multi-valency. DNL is a new technique to develop targeting molecules for advanced cancer therapy and imaging [57]. The DNL technology platform exploits the human-specific protein/protein interactions normally occurring between the anchoring domain of A-kinase anchoring proteins and the regulatory subunits of cAMP-dependent protein kinase A. Protein kinase A was isolated from lapin skeletal muscle for the first time in 1968 [58]. It has an important role in signal transduction pathways via binding the secondary messenger cAMP to the regulatory subunits. The DNL method is a powerful tool to develop diagnostic agents and novel therapeutics for unmet medical needs [59].

Figure 7. Formats for BsAbs subdivided into five main classes. BsAb fragments, BsIgG (bispecific IgG), appended IgG, BsAb conjugates, and bispecific fusion proteins. Adapted from ref [42].

4. BsAb mechanism of action

The main justification for the therapeutic application of BsAbs in diseases such as cancer, autoimmune and inflammatory disorders is their ability to allow selective engagement of effector immune cells against a specified disease-related antigen. BsAbs serve as adaptors or linkers between the target and the effector cell. Many effector mechanisms can be envisaged for clinical application, and many of these have already been evaluated. The mechanisms of action for a BsAb can include the induction of CDC, ADCC, ADCP, apoptosis, and the engagement of cell

surface receptors to either inhibit or activate signaling pathways (Figure 8) [60]. BsAbs can block two signaling pathways by simultaneous binding to two different targets. This could occur via simultaneous targeting of two different ligands or two different receptors on the same cell; thus induce changes in cell signaling [6].
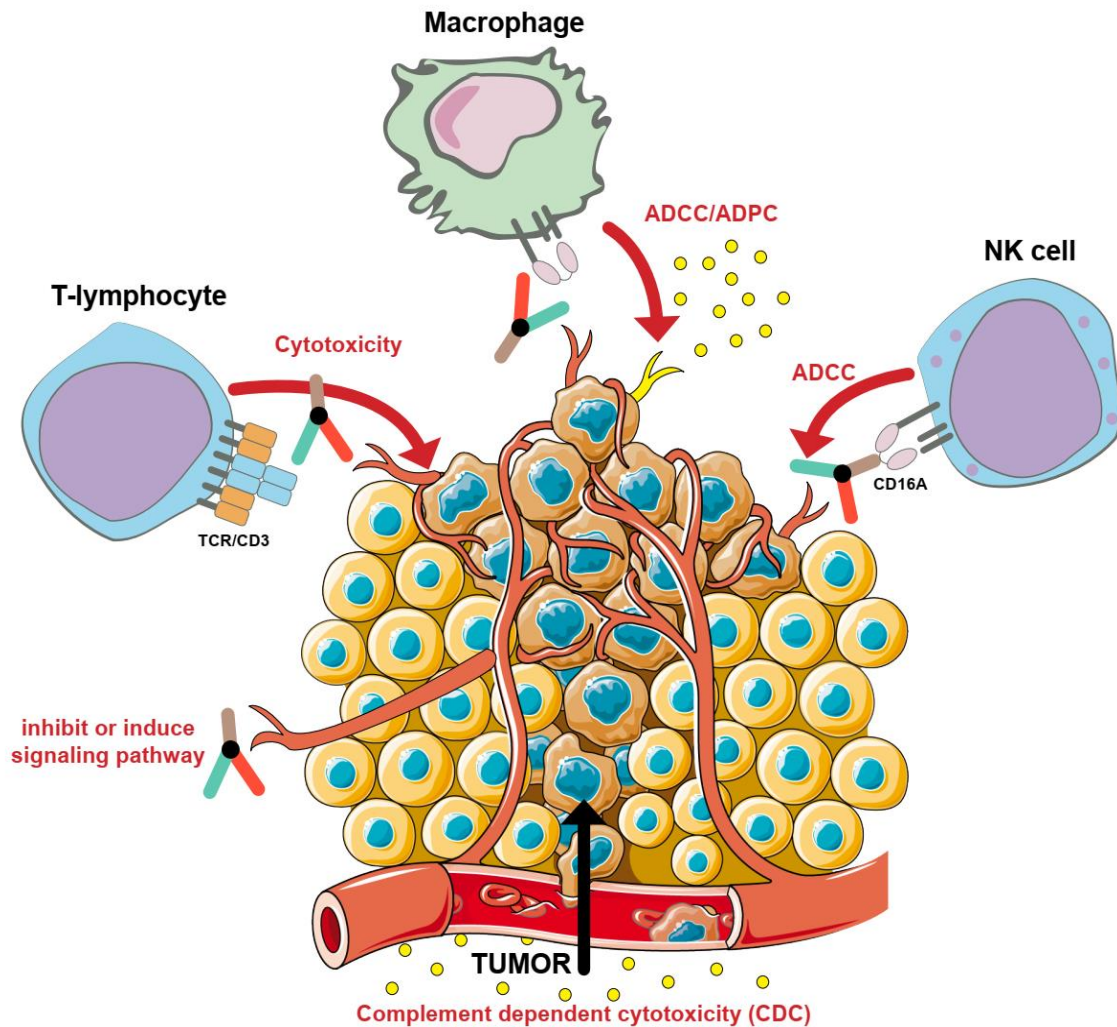

Figure 8. Mechanism of action for Fc domain of IgG-like BsAbs. BsAbs can induce ADCC, CDC, and ADCP in tumor cells. BsAbs can simultaneously bind to two different antigens or receptors and induce cytotoxicity or block two signaling ligands.

4.1. Immune cell engagement by BsAbs

A cytotoxic T cell (CTL) is a type of T lymphocyte cell that is able to kill cancer cells, virus-infected cells, or other damaged cells that require elimination. The CTLs express T-cell receptors (TCRs) containing CD3 subunits that can bind to a unique specific antigen. Since the CD3 binding domain can bind to cancer-specific antigens and redirect CTL response against cancer cells, CD3 was engineered into the structure of BsAb [61]. Due to the immune escape mechanisms employed by tumor cells during the immunoediting process, naturally-occurring tumor-specific T lymphocyte responses are often limited in cancer patients. Some of the recent advances in immunotherapy approaches have the possibility to overcome this problem, and the use of BsAbs is a good example. Multiple BsAb molecules have been designed and produced to redirect immune cells to attack cancer cells. Aside from T cells, the other effector cells of the immune system, such as monocytes, NK cells, macrophages, and granulocytes, have also been harnessed for tumor-killing effects [62].

4.2. BiTE

BiTEs are single-chain antibodies that engage T cells against cancer cells. The BiTE format has been used as an improvement on tandem scFv BsAbs. BiTEs are fusion proteins consisting of two scFvs from different antibodies, or the amino acid sequences from four different genes, expressed in a single peptide chain of about 55 kDa. One of the scFv arms of the BiTE binds to T cells via the CD3 receptor, while the other binds to a tumor cell via a tumor-specific antigen [63]. Blinatumomab links T-cells to normal and malignant B lymphocytes via simultaneous binding to the invariant CD19 molecule on B-cells and the CD3ε T cell receptor subunit. These interactions lead to the stimulation of T-cell activation, the formation of cytolytic synapses, and subsequently

target cell lysis. T-cell activation up-regulates the expression of CD25, HLA-DR, CD69, and cell adhesion molecules; it also causes the transient release of inflammatory cytokines. Memory T-cells appear to contribute to the redirected cell lysis. The lysis by activated the T-cell-Blinatumomab complex requires granzyme, perforin, and activation of cell death signaling pathways in the target cells [64]. BiTEs have great potential to activate T lymphocytes. It should be noted that effective interactions between BiTEs, T cells, and the target cells are essential. The small size of BiTE antibodies is optimal for effective interaction between both cell types [65]. Blinatumomab is a first-in-class BiTE antibody against CD19/CD3, which was developed against B cell malignancies. At only picomolar concentrations, Blinatumomab is able to retarget the T lymphocytes against the CD19+ve lymphoma cells [66]. Blinatumomab leads to an expansion of CD8+ T cells, dominated by the cytotoxic CD8+ T effector memory sub-type [67]. Malignant B cells mostly express CD19, and therefore they are suitable targets for non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL) and acute lymphoid leukemia (ALL). The high specificity and sensitivity of Blinatumomab against CD19 have been demonstrated by in vitro studies. It has been shown that cells lacking the CD19 antigen were not killed in the presence of Blinatumomab and T cells [68]. Activated T cells increase the expression of CD69 and CD25 antigens, inflammatory cytokines such as IL-2, IL-4, IL-6, IL-10, IFN-γ, TNFα, and show increased T cell proliferation. Inflammatory cytokines play a pivotal role in the innate immune response by promoting inflammation [69]. Blinatumomab was approved by the FDA in December 2014 and is administered as a continuous infusion for the treatment of Ph-chromosome negative relapsed or refractory B-cell precursor ALL, because it has a short half-life (only around 2 h). Patients receive Blinatumomab by an implanted venous access port system [70]. Administration of Blinatumomab in recurrent ALL led to complete positive responses

in 72% of patients, minimal residual disease (MRD) in 88% of patients and lengthened the average life expectancy to 9 months [71]. Therapy with Blinatumomab in patients with refractory and recurrent ALL, which are characterized by loss of CD19 expression on B lymphocytes due to extramedullary hematopoiesis (EH), is ineffectual [72]. Treatment of non-Hodgkin's lymphoma with Blinatumomab showed good effect in monotherapy clinical trials at much lower blood concentrations. The results of Blinatumomab therapy remarkably exceeded the results of conventional anti-CD19 MAb therapy [73].

AMG 330 is a novel BiTE, which was developed for the treatment of acute myelogenous leukemia (AML). AMG 330 has dual specificity for CD33 plus CD3, which are mostly expressed on the leukemic blasts and AML stem cells [74]. MEDI-565 (MT111) is another BiTE, which is in clinical trials against tumors expressing carcinoembryonic antigen (CEA) [75]. The MT110 BiTE binds to CD3/EpCAM, and directs T cells to kill EpCAM expressing tumor cells [76].

4.3. Diabody BsAbs engaging immune cells

The Diabody format appears to be more efficient than quadroma technology-derived BsAbs for the in vitro engagement of T-cells and NK-cell cytotoxicity against cancer cells. However, even though BsAbs had a much longer serum half-life, they showed comparable in vivo antitumor activities. A synergistic antitumor effect was indicated for some diabodies both, in vitro and in vivo studies, by engaging both NK cells and T cells. Diabodies can also be used to enhance the activity of dendritic cell-induced T-cells. Tandem diabodies (TandAbs) contain four antigen-binding regions without a Fc domain. The molecular weight of TandAbs is about 115 kDa, and

therefore they are larger than BiTEs. TandAbs are twice as the size of Diabodies, and bind bivalently to both target and effector cells, with improved pharmacological characteristics [77].

AFM13 is a tetravalent TandAb against CD16A plus CD30. CD16 is a receptor for the IgG Fc domain and is involved in ADCC. CD16 has two isoforms, CD16A and CD16B. The CD16A isoform is one of the most highly expressed receptors on macrophages and NK cells. Patients with Hodgkin's lymphoma (HL) over-express CD30 mostly on Hodgkin's and Reed-Sternberg cells. The AFM13 molecule has four binding sites, two of which bind to immune cells, and the other two bind to tumor cell antigens [78]. AFM13 activates NK cells to attack CD30+ve cancer cells. Reusch et al. demonstrated that AFM13 showed strong cytotoxicity against CD30+ tumor cells, while no NK cell cytotoxicity was observed in the absence of CD30+ cells [79].

4.4. Triomab BsAbs for immune cell recruitment

Triomab molecules are a class of BsAbs composed of two half antibodies of the rat IgG2b and the mouse IgG2a chain. Triomab BsAbs have three different binding sites, including a specific binding site for CD3 expressed on the surface of T cells, a specific binding site for a tumor-associated antigen, and a chimeric rat IgG2b × mouse IgG2a Fc region. The Fc region binds to the existing Fcγ receptors (including subtypes I, IIa, and III) on human immune cells such as dendritic cells, macrophages, and NK cells [22]. A well-known rat-mouse trifunctional hybrid monoclonal antibody was named Catumaxomab (anti-EpCAM/anti-CD3). It contains an Fc fragment to bind to FcRs on immune cells, such as macrophages, dendritic cells, and NK cells, one arm to bind to EpCAM on tumor cells, and a second arm to bind to CD3 on T cells. Catumaxomab was the first BsAb to receive regulatory approval in Europe on 20 April 2009 [80]. EpCAM or CD326 (epithelial cell adhesion molecule) is a single-span transmembrane

glycoprotein (39–42 kDa) expressed on healthy epithelial cells. Over-expression of EpCAM is related to cancer progression and poor outcomes in several tumor types, including pancreatic and gastric cancer [81]. The specific cancer cell killing is due to the activation of T cells and other immune cells, and their reciprocal stimulation [82]. This activation could also induce killing of tumor cells in ovarian carcinoma, and reduce or prevent the accumulation of ascites. Catumaxomab has been used to treat malignant ascites [83]. Catumaxomab is a potent BsAb with a good safety record, which is administered intraperitoneally in small doses four to five times [84]. Some other BsAbs have also been constructed using the quadroma technology. One of them is a BsAb molecule that targets human epidermal growth factor receptor 2 (HER2) as well as the CD3 and CD20 antigens. Ertumaxomab (anti-HER2 /anti-CD3) is a trifunctional BsAb that binds to HER2, Fcγ, and CD3. It creates a ternary complex between accessory cells, tumor cells, and T cells. The cellular and humoral immune responses induced by this BsAb lead to the killing of HER2 expressing tumor cells [85]. Bi20 (FBTA05, anti-CD20 × anti-CD3), is a triomab BsAb that targets CD20 on B cells and CD3 on T cells by its two arms and also engages FcγRI+ve accessory immune cells by its Fc domain. Bi20 has been reported to trigger specific and effective lysis of B cells derived from CLL patients even with low levels of CD20 expression [86].

4.5. BsAb interactions with signaling receptors

Signaling pathways control the fundamental processes of cells and regulate the functions of most different cell types. Disturbances in cellular information processing and signaling interactions are the reason behind many diseases such as autoimmunity, cancer, and diabetes. Multiple signaling pathways have been recognized as being genetically changed in cancer, including the PI3K/Akt signaling pathway, RTK/RAS/MAP-kinase pathway, and others. Analysis of these pathways has

demonstrated how they are dysregulated in cancer, and govern many properties of malignant cells [87]. Signaling pathways are not only involved in cancer but have also been found to be important therapeutic targets in inflammatory disorders, such as rheumatoid arthritis (RA). ABT122 is a dual-variable domain BsAb that binds to interleukin 17 (IL-17) and human tumor necrosis factor-α (TNF). Both IL-17 and TNF contribute to inflammatory disorders, such as RA [88]. TNF is produced by immune cells as a cytokine capable of inhibiting tumor cell proliferation and inducing tumor regression [89]. IL-17A induces the secretion of a wide range of other chemokines and proinflammatory cytokines and is involved in cartilage destruction [90]. In a phase-II multicenter trial using ABT122 in RA, 150 patients completed the 6-month treatment. In this study, ABT122 showed passable tolerability and a sustained therapeutic effect in patients with RA [91].

Table 1. Bispecific antibodies in clinical trials (ClinicalTrials.gov).

Names	Sponsor	Format	Targets	Diseases	Development stages	Code
Bispecific antibodies in clinical trials for hematological cancer						
FBTA05	Technische Universität München	TrioMab	CD20 × CD3	B-cell Lymphoma	Phase I/II	**NCT01138579**
Blinatumomab	Amgen Research	BiTE	CD3 × CD19	Acute lymphoblastic leukaemia	Approved	
AMG 330	Amgen Research	BiTE	CD33 × CD3	Acute myeloid Leukemia	Phase I	**NCT02520427**
AMG420	Amgen	BiTE	BCMA × CD3	Multiple myeloma	Phase I	**NCT03836053**
AMG 701	Amgen	BiTE	CD3 × BCMA	Multiple myeloma	Phase I	**NCT03287908**
CC-93269	Celgene	BiTE	CD3 × BCMA	Multiple myeloma	Phase I	**NCT03486067**
AMG562	Amgen	BiTE	CD3 × CD19	Haematological malignancies	Phase I	**NCT03571828**
AMG 673	Amgen	half-life extended BiTE	CD3 × CD33	Acute myeloid leukemia	Phase I	**NCT03224819**
rM28	University Hospital Tuebingen	Tandem scFv	CD28 × HMVMAA	Malignant melanoma	Phase I/II	**NCT00204594**
MGD006	MacroGenics	DART	CD123 × CD3	Acute myeloid leukemia	Phase I	**NCT02152956**
MGD010	MacroGenics	DART	CD32B × CD79B	Healthy subjects	Phase I	**NCT02376036**
MGD009	Macrogenics	DART	CD3 × B7-H3	B7-H3 expressing tumor	Phase I	**NCT02628535**
				Relapsed/Refractory Cancer	Phase I	**NCT03406949**

DT2219ARL	Masonic Cancer Center	2 scFv linked to diphtheria toxin	CD19 × CD22	Leukemia- lymphoma	Phase I	**NCT00889408**
REGN1979	Regeneron Pharmaceuticals	unclear	CD20 × CD3	CD20+ B cell malignancies	Phase I	**NCT02290951**
BTCT4465A	Roche	CrossMAb	CD3 × CD20	B-Cell non-Hodgkin lymphoma	Phase I/II	**NCT03677141**
				Diffuse large B-cell lymphoma	Phase I/II	**NCT03677154**
				B-Cell non-Hodgkin lymphoma	Phase I	**NCT03671018**
				Non-Hodgkin lymphoma; chronic lymphocytic leukemia	Phase I	**NCT02500407**
RO7082859	Roche	F(ab)3 CrossMab	CD20 × CD3	B-cell non-Hodgkin lymphoma	Phase I	**NCT03467373**
					Phase I	**NCT03533283**
					Phase I	**NCT03075696**
GBR1342	Glenmark Pharmaceuticals	scFv-IgG	CD3 × CD38	Multiple myeloma	Phase I	**NCT03309111**
AFM13	University of Cologne	TandAb	CD30 × CD16A	Relapsed or refractory Hodgkin lymphoma	Phase II	**NCT02321592**
AFM11	Affimed GmbH	TandAb	CD30 × CD19	Relapsed and/or refractory CD19- positive B cell NHL	Phase I	**NCT02106091**
AMV564	Amphivena Therapeutics	Tandem diabodies	CD3 × CD33	High-risk myelodysplastic syndromes	Phase I	**NCT03516591**
				Acute myeloid leukemia	Phase I	**NCT03144245**
AMG427	Amgen	Tandem scFv-	CD3 × FLT3	Hematological	Phase I	**NCT03541369**

		scFc		malignancies		
RO7187797	Genentech	Tandem scFv	CD3 × CD307	Relapsed or refractory multiple myeloma	Phase I	NCT03275103
GTB-3550	GT Biopharma	Tandem scFv fusion protein	CD16 × CD33	Hematological malignancies	Phase I/II	NCT03214666
XmAb-13676	Xencor	XmAb	CD3 × CD20	CD20-expressing hematologic malignancies	Phase I	NCT02924402
XmAb-14045	Xencor	XmAb	CD3 × CD123	CD123-expressing hematologic malignancies	Phase I	NCT02730312
AMG424	Amgen	XmAb	CD3 × CD38	Multiple myeloma	Phase I	NCT03445663
SAR440234	Sanofi	DVD-Ig	CD3 × CD123	Acute myeloid leukemia; myelodysplastic syndrome	Phase I/II	NCT03594955
JNJ-64007957	Janssen	DuoBody	CD3 × BCMA	Multiple myeloma	Phase I	NCT03145181
JNJ-63709178	Janssen	DuoBody	CD3 × CD123	Hematological malignancies	Phase I	NCT02715011
GEN3013	Janssen	DuoBody	CD3 × CD20	Hematological malignancies	Phase I/II	NCT03625037
JNJ-64407564	Janssen	DuoBody	CD3 × GPRC5D	Hematological malignancies	Phase I	NCT03399799
GEM333	GEMoaB Monoclonals	Diabody	CD3 × CD33	Acute myeloid leukemia	Phase I	NCT03516760
MCL A-117	Merus	Biclonics	CD3 × CLEC12A	Acute myeloid leukemia	Phase I	NCT03038230
Bispecific antibodies in clinical trials for solid tumors						
MT110	Amgen Research	BiTE	CD3 × EpCAM	Solid tumors	Phase I	NCT00635596

BAY2010112	Bayer	BiTE	CD3 × PSMA	Prostate cancer	Phase I	**NCT01723475**
Catumaxomab	Neovii Biotech	Triomab	CD3 ×EpCAM	Ovarian cancer	Phase II	**NCT01815528**
				Malignant ascites	Phase II	**NCT01065246**
				Gastric cancer	Phase II	**NCT00464893**
				Gastric adenocarcinomas	Phase II	**NCT01504256**
Ertumaxomab	Krankenhaus Nordwest	Triomab	CD3 × HER2	Solid tumors	Phase I/II	**NCT01569412**
				Breast cancer	Phase II	**NCT00452140**
					Phase II	**NCT00522457**
					Phase II	**NCT00351858**
RG7221 (Vanucizumab)	Hoffmann-La Roche	CrossMab	Ang2 × VEGF	Solid tumors	Phase I	**NCT02665416**
					Phase I	**NCT02715531**
					Phase I	**NCT01688206**
					Phase I	**NCT02141295**
					Phase I	**NCT02304393**
RO6958688	Hoffmann-La Roche	CrossMab	CEA × CD3	(CEA)-positive solid tumors	Phase I	**NCT02650713**
					Phase I	**NCT02324257**
				Non-Small Cell lung cancer	Phase I/II	**NCT03337698**
OMP-305B83	OncoMed Pharmaceuticals, Inc.	DVD-Ig	DLL4 × VEGF	Colorectal cancer	Phase I	**NCT03035253**
				Ovarian cancer	Phase I	**NCT03030287**
				Solid tumors	Phase I	**NCT02298387**
Anti-CEA × anti– (DTPA)	Nantes University Hospital	scFv-IgG	CEA × DTPA	Medullary thyroid carcinoma	Phase II	**NCT00467506**
MT111	Med Immune LLC	scFv-IgG	CEA × CD3	Gastrointestinal adenocarcinomas	Phase I	**NCT01284231**
MM141	Merrimack Pharmaceuticals	scFv-IgG	IGF1R × Her3	Solid tumors	Phase I	**NCT02538627**
					Phase I	**NCT01733004**

				Pancreatic cancer	Phase II	**NCT02399137**
ES414	Emergent Product Development Seattle LLC	scFv-IgG	CD3 × PSMA	Prostate cancer	Phase I	**NCT02262910**
MM111	Merrimack Pharmaceuticals	scFv2-HSA	CEA × CD3	Breast cancer	Phase I	**NCT01097460**
				Heregulin positive cancers	Phase I	**NCT00911898**
				HER2 positive cancer	Phase II	**NCT01304784**
TF2	Garden State Cancer Center at the Center for Molecular Medicine and Immunology	Dock and lock	CEA × HSG	Colorectal cancer	Phase I	**NCT01273402**
					Phase I	**NCT00860860**
				HER2 negative breast carcinoma	Phase I/II	**NCT01730612**
				Medullary thyroid carcinoma	Phase I/II	**NCT01730638**
				Small cell lung cancer	Phase I/II	**NCT01221675**
				Head and Neck Cancer	Phase I	**NCT03640195**
PF06671008	MacroGenics, Pfizer	DART	P-cadherin × CD3	Solid tumors	Phase I	**NCT02659631**
Indium-labeled IMP-205xm734	Radboud University	unclear	CEA × in-labeled Peptide	Colorectal cancer	Phase I	**NCT0018508**
BTRC4017A	Genentech	unclear	CD3 × HER2	Locally advanced or metastatic HER2-expressing cancers	Phase I	**NCT03448042**
JNJ-61186372	Genmab	Duobody	EGFR × cMet	Non-Small Cell lung cancer	Phase I	**NCT02609776**
RG7386	Roche	CrossMAb	FAP × DR5	Solid tumors	Phase I	**NCT03448042**
MGD007	MacroGenics	DART-Fc	GPA33 × CD3	Colorectal cancer	Phase I	**NCT02248805**
					Phase I/II	**NCT03531632**
LY3164530	Eli Lilly and Company	OrthoFab-IgG	MET × EGFR	Neoplasm metastasis	Phase I	**NCT02221882**

RG7813	Hoffmann-La Roche	ScFv-IgG	CEA × IL2	Advanced and/or metastatic solid CEA+ tumors	Phase I	**NCT02004106**
GBR1302	Glenmark Pharmaceuticals	scFv-Fab IgG	CD3 × HER2	HER2 positive cancers	Phase I	**NCT02829372**
				HER2-positive Mmetastatic breast cancer	Phase I/II	**NCT03983395**
MDX-447	Medarex	2 (Fab') crosslinked	CD64 × EGFR	Glioblastoma multiforme	Phase I	**NCT00005813**
AMG757	Amgen	Tandem scFv-scFc	CD3 × DLL3	Small cell Lung cancer	Phase I	**NCT03541369**
AMG596	Amgen	Tandem scFv	CD3 × EGFRvIII	EGFRvIII + glioblastoma	Phase I	**NCT03296696**
TargomiRs	University of Sydney	unclear	EGFR × EDV	Recurrent MPM and NSCLC	Phase I	**NCT02369198**
JNJ-61186372	Janssen	DuoBody	EGFR × MET	Solid malignancies (NSCLC)	Phase I	**NCT02609776**
MCLA-128	Merus	Biclonics	HER2 × HER3	Solid tumors	Phase I	**NCT02912949**
				Breast cancer	Phase I/II	**NCT03321981**
MCLA-158	Merus	Biclonics	Lgr5 × EGFR	Solid tumors	Phase I	**NCT03526835**
MCLA-129	Merus	Biclonics	EGFR × c-MET	Solid tumors	Pre-Clinical	**-**
MCLA-145	Merus	Biclonics	PD-L1× CD137	Solid tumors	Phase I	**NCT03922204**
ERY974	Chugai	ART-Ig	CD3 × GPC3	Solid tumors	Phase I	**NCT02748837**
ABT165	AbbVie	DVD-Ig	DLL4 × VEGF	Solid tumors	Phase I	**NCT01946074**
				Metastatic colorectal cancer	Phase II	**NCT03368859**
MEHD7945A	Genentech	DAF	EGFR × HER3	Neoplasms	Phase I	**NCT01986166**
				Malignant epithelial tumors	Phase I	**NCT01207323**
				Head and neck cancer	Phase I	**NCT01911598**
					Phase II	**NCT01577173**

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				Colorectal cancer	Phase II	NCT01652482
Bispecific antibodies in clinical trials for other diseases						
MEDI3902	MedImmune	ScFv-IgG	PsI × PcrV	Pneumonia	Phase II	**NCT02696902**
AMG 570	MedImmune	ScFv-IgG	BAFF × B7RP1	Systemic lupus erythematosus	Phase I	**NCT02618967**
				Rheumatoid arthritis	Phase I	**NCT03156023**
MEDI7352	MedImmune	ScFv-IgG	NGF × TNF	Painful diabetic neuropathy	Phase II	**NCT03755934**
				Painful osteoarthritis of the knee	Phase I	**NCT02508155**
MSB0010841	Merck KGaA	Nanobody	IL-17A/F × HSA	Psoriasis	Phase I	**NCT02156466**
ALX-0061	Ablynx	Nanobody	IL-6R × HSA	Rheumatoid arthritis	Phase I/II	**NCT01284569**
Ozoralizumab (ATN-103)	Ablynx	Nanobody	TNF × HSA	Rheumatoid arthritis	Phase II	**NCT01063803**
BI1034020	Boehringer Ingelheim	Nanobody	Aβ40 × Aβ42	Alzheimer's disease	Phase I	**NCT01958060**
ABT122	AbbVie	DVD-Ig	IL1α × IL1β	Rheumatoid arthritis	Phase II	**NCT02433340**
				Rheumatoid arthritis	Phase I	**NCT01853033**
				Psoriatic arthritis	Phase II	**NCT02349451**
				Active rheumatoid arthritis	Phase II	**NCT02141997**
				Active psoriatic arthritis	Phase II	**NCT02429895**
ABT-981	AbbVie	DVD-Ig	TNF × IL17A	Erosive osteoarthritis of the hand	Phase II	**NCT02384538**
				Osteoarthritis of the knee	Phase I	**NCT01668511**
				Knee osteoarthritis	Phase II	**NCT02087904**
SAR156597	Sanofi	DVD-Ig	IL4 × IL13	Systemic scleroderma	Phase II	**NCT02921971**
				Idiopathic pulmonary fibrosis	Phase II	**NCT01529853**
				Idiopathic pulmonary fibrosis	Phase II	**NCT02345070**
GSK2434735	GlaxoSmithKline	DVD-Ig	IL13 × IL4	Asthma	Phase I	**NCT01563042**

RG7716	Hoffmann-La Roche	CrossMAb	VEGF × Ang-2	Wet AMD	Phase II	NCT02484690
				Neovascular age-related macular degeneration	Phase II	NCT03038880
				Central diabetic macular edema	Phase II	NCT02699450
				Diabetic macular edema	Phase III	NCT03622593
				Age-related macular degeneration	Phase III	NCT03823300
				Age-related macular degeneration	Phase III	NCT03823287
				Diabetic macular edema	Phase III	NCT03622580
COVA322	Covagen	IgG-fynomer	TNF-α × IL17A	Plaque psoriasis	Phase I/II	NCT02243787

5. BsAbs for solid tumor treatment

A solid tumor is a malignant mass of proliferating cancer cells, which are organized in an organ-like structure, usually without cysts or liquid areas. The tumor cells can spread to various parts of the body, including the liver, brain, lungs, or bones. The population of cells in the tumor mass can interfere with surrounding healthy cells and prevent the organ from carrying out its normal function. Common types of solid tumors include carcinoma, sarcoma, neuroblastoma, and Wilms tumor [92]. A solid tumor is a complex tissue consist of stromal cells (such as mesenchymal stromal cells, fibroblasts, occasionally adipocytes, pericytes, blood, and lymphatic vascular networks), inflammatory cells, and extracellular matrices (ECM) that all of them create tumor microenvironment (TME) [93]. The TME prevents effective lymphocyte priming, suppresses infiltrating effector cells, and reduces lymphocyte infiltration, which leads to a defeat of the host to effective attack tumors. There are various mechanisms for the resistance to immunotherapy which consist of 1) biological impediments around tumor tissues that lead to low numbers of immune cells have ability to migrating into tumor sites; 2) an inhibitory microenvironment or absence of antigen stimulation for immune cells, mainly T cells, within the TME which promote tumor growth and increase immune escape; 3) short-lived activation or exhausted of antigen-specific T cells via restricted repertoires which fail to suppress tumor growth; 4) indirect or weak direct antigens presentation in lymphoid tissues which lead to a lack of lymphocytes priming due to inadequate release of tumor-specific antigens to the draining lymph gland by the TME [94].

The most important function of BsAbs in cancer treatment is the recruitment of immune system effector cells to trigger tumor cell lysis. Most of BsAbs have been designed to bind to the CD3 receptor for T-cell recruitment. However, there are other BsAb constructs designed to bind to CD16, CD64, and CD89. BsAbs can also simultaneously bind to two different antigens and

activate or neutralize other receptors or their ligands. Moreover, BsAbs can restrict tumor growth by blocking signaling pathways, targeting tumor angiogenesis, and blocking the effects of cytokines [95]. Currently, a large number of antibodies are undergoing clinical trials for therapeutic applications in various types of solid tumors (Table 1). Some of these therapeutic BsAbs will be discussed in the following sections.

5.1. RO6958688 (RG7802)

RO6958688 BsAb (anti-CEA/CD3) is produced by CrossMAb technology. It is an IgG1-based heterodimeric antibody that has two Fab domains and an Fc region. One of the Fab domains binds to CEA expressing tumor cells, while the other Fab domain binds to CD3 expressed on T cells, [96]. CEA is a family of highly related glycoproteins, which are associated with cell adhesion. These glycoproteins are naturally produced in certain tissues during fetal development. However, the production of CEA naturally decreases postnatally and is reduced to a very low level in the blood of healthy adults [97]. It serves as a functional E-selectin and L-selectin ligand in the colon and may play a pivotal role in the metastasis of colorectal cancer. CEA is over-expressed in many tumors, including head and neck carcinoma, non–small cell lung cancer (NSCLC), breast cancer, gastric cancer, colorectal cancer, and pancreatic adenocarcinoma. Given its widespread expression, it can be used as a tumor marker. RO6958688 induces a potent CTL reaction against CEA-expressing cancer cells. This BsAb caused the regression of human colorectal cancer xenografts in the NOG immunodeficient mouse model [98]. A phase I clinical study (non-randomized, multicenter, open-label, dose-escalation, parallel assignment) using RO6958688 in patients with metastatic and locally advanced CEA-positive solid tumors has been completed. In this study, patients were divided into two groups (S1 and S2). In the S1 group, 80

patients were treated with RO6958688 doses ranging from 0.05 mg to 600 mg. In the S2 group, 38 patients were treated with RO6958688 doses ranging from 5 mg to 160 mg in combination with atezolizumab (1200 mg). Two patients in the S1 and S2 groups had a partial response. Preliminary tumor size reduction was observed in 4 additional patients in the S1 group and 5 patients in the S2 group. When monotherapy with RO6958688 was used, improved antitumor activity was observed with increasing doses. Moreover, the activity of RO6958688 increased when used in combination with atezolizumab (another monoclonal antibody) with a manageable safety profile (NCT02324257) [99].

5.2. MT111 (MEDI-565)

MT111 is an anti-CEA/CD3 bispecific scFv antibody. MT111 activates T cells, inhibits the growth of CEA-expressing tumor cells in various cancer models, and induces cell lysis in CEA expressing tumor cells. Preclinical trials have shown that the expression of CEA is essential for MT111 binding and for effective T cell-mediated lysis. Phase I trials using MT111 (non-randomized, open-label, dose-escalation, single group assignment) have been completed in patients with advanced gastrointestinal adenocarcinoma [100]. None of the 39 patients that received intravenous MT111 on day 1–5 ± dexamethasone (in a 28-day cycle) showed any objective responses. Five patients were reported to show symptoms of grade 3 therapy-related adverse events, such as increased alanine aminotransferase, hypertension, hypoxia, diarrhea, and cytokine release syndrome (CRS). Moreover, four patients showed dose-limiting toxicity (DLT), diarrhea, and CRS. Six patients were observed to experience therapy-related serious adverse events, such as vomiting, diarrhea, pyrexia, CRS, and hypoxia. Stable disease was reported as the best therapy response for 11 patients (28%) (NCT01284231) [101].

5.3. TF2

Dock-and-lock technology was applied to produce the TF2 BsAb as a humanized tri-Fab. This BsAb contains a third Fab fragment and two anti-CEA Fab fragments, which bind to the histamine-succinyl-glycine (HSG) peptide epitope. This antibody lacks any Fc domain, and therefore it has a short serum half-life [102]. Radioimmunotherapy (RIT) is a promising new therapeutic method, which employs radiolabeled antibodies against specific tumor antigens. The MAbs bind to tumor cells and deliver the radiation directly to the tumor. MAbs have the advantage of specificity against both unique tumor-specific antigens and tumor-associated antigens [103]. TF2 is one of the new BsAb designs which is currently undergoing phase I/II trials in radioimmunotherapy of colorectal and lung cancer. In a phase I study of CEA-pre-targeted radionuclide therapy against advanced colorectal cancer (open-label, single-group assignment), it was observed that pre-targeting with TF2 BsAb and radiolabelled peptide IMP288 was practicable and safe in patients with CEA-expressing colorectal cancer. Using this approach, tumor cells could be specifically and rapidly targeted by TF2. In this study, 21 patients were enrolled and divided into 4 cohorts. Patients first underwent diagnostic medical imaging with ^{111}In-labelled IMP288 and TF2; the following week, they have received a therapeutic dose cycle of ^{111}In-labelled IMP288 and TF2. Images of ^{111}In-IMP288 showed accurate and selective targeting of the tumors in all patients. Selective and rapid tumor targeting with radiolabelled peptides could be visualized within 1 h, giving a high tumor-to-tissue ratio at 20h-24 h. Progressive disease was observed in all patients eight weeks after the initial treatment cycle, and hence none of the patients qualified to receive the next treatment cycle (NCT00860860) [104].

5.4. Anti-CEA × anti–diethylenetriaminepentaacetic acid (DTPA)

Medullary thyroid cancer (MTC) accounts for 5-10% of all thyroid cancers and arises from calcitonin-producing parafollicular cells (C cells). The CEA antigen has been described to be a tumor marker for MTC [105]. The RIT approach has shown promising results in MTC using anti-CEA MAbs. To deliver enhanced tumor-absorbed doses to solid tumors and to improve the therapeutic index, pre-targeted RIT (pRIT) was developed. In patients with metastatic MTC, promising therapeutic results have been shown in clinical trials employing anti-CEA × anti-DTPA-indium BsAb labeled with ^{131}I-di-DTPA-indium. Phase II studies of anti-CEA pRIT (non-randomized, factorial assignment, open-label) have been completed in patients with progressive metastatic MTC. In these studies, 54.7% of patients showed grade 3–4 hematologic toxicity. The overall disease control rate was 76.2%. After pRIT, 56.7% of assessed patients showed a significant impact on tumor doubling time. The pre-pRIT doubling time and the post pRIT doubling time were good predictors for overall survival after pRIT. pRIT showed anticancer activity with controllable hematologic toxicity in MTC. Increased overall survival was correlated with increased biomarker doubling time after treatment (NCT00467506) [106].

5.5. RG7221 (vanucizumab)

RG7221 is an IgG1-like CrossMAb that inhibits the receptor-ligand interaction between vascular endothelial growth factor A (VEGFA) and angiopoietin 2 (ANG-2); two key pro-angiogenic factors [107]. One promising approach in cancer therapy is the depletion of angiogenesis factors, because tumor neovascularization is essential for tumor growth, and is stimulated by various angiogenic factors. [108]. ANG-2 is a central regulator of angiogenesis with a context-dependent

effect on endothelial cells. ANG-2 acts as an autocrine endothelial cell-derived antagonistic receptor for blood vessel maturation, and signals via the ANG-1/tyrosine kinase 2 (TIE2) signaling axis [109]. VEGF was initially described to be an endothelial cell-specific mitogen that stimulates the formation of blood vessels. It is a signaling protein produced by various types of cells, including macrophages, tumor cells, keratinocytes, renal mesangial cells, and platelets. The functions of VEGF are not exclusive to the vascular system; it has an important role in normal physiological functions such as hematopoiesis, bone formation, wound healing, and fetal development [110]. In preclinical trials, RG7221 was shown to potently inhibit tumor growth and angiogenesis, with a higher efficiency compared to mono-specific VEGFA- or ANG-2 binding antibodies. A phase I study using vanucizumab in adults with advanced solid tumors has been completed (non-randomized, parallel assignment, multi-center, dose Escalation, open-label), and it is now being evaluated in a phase II study. In the phase I study, 42 patients were treated. Headache (31%), arterial hypertension (59.5%), and asthenia (42.9%) were the most common toxicities. Two patients achieved confirmed partial responses. Ten patients showed stable disease for more than six months (NCT01688206) [111].

5.6. OMP-305B83 (Navicixizumab)

OMP-305B83 is an IgG2 humanized BsAb, which was designed to bind to both VEGF and Notch ligand delta-like 4 (DLL4). This BsAb was designed to increase the antitumor activity, and avoid any cardiac toxicity caused by inhibition of VEGF or DLL4 alone [112]. In most multicellular organisms, Notch is an evolutionarily conserved cell signaling system. It has an important role in the determination of cell fate and differentiation during embryogenesis and postnatal development. Mammals possess four different Notch receptors (NOTCH1, NOTCH2,

NOTCH3, and NOTCH4), which can bind five different ligands, including Jagged1 (JAG1), Jagged-2 (Jag2), and Delta-like 1, 3, 4 [113]. The expression pattern and activity of DLL4 suggests there is a pro-angiogenic role for this ligand; it regulates endothelial cell migration and proliferation, and angiogenic sprouting. Therefore, DLL4 is a possible target in cancer therapy [114]. A phase I study using OMP-305B83 (single group assignment, open-label, dose-escalation) in patients with solid tumors that had failed previous treatment was completed. In this study, 71 patients were enrolled, and 66 were treated with OMP-305B83. The most common tumor types were colorectal, ovarian, pancreatic, breast, endometrial, and uterine cancers. Among the participating individuals, 17 patients showed stable disease, four patients (1 uterine, 3 ovarian) showed a partial response, 38 patients had progressive disease, and seven patients were not evaluable. The most promising outcomes were seen in ovarian cancer. In view of these results, the OMP-305B83 was suggested to have antitumor activity in numerous tumor types. These results were confirmed by a phase 1b clinical trial to further evaluate OMP-305B83 (NCT02298387) [112].

5.7. AMG 212 (BAY2010112)

AMG 212 is a single-chain (T cell-engaging) BsAb construct, which contains an anti-prostate specific membrane antigen (PSMA) scFv and an anti-CD3 scFv. Both of the scFv arms can bind to their targets in cynomolgus monkeys and in humans. This BsAb was developed to treat prostate cancer [115]. The PSMA is a transmembrane glycoprotein with an extensive extracellular domain, a transmembrane domain, and an intracellular segment. It was originally characterized using a murine monoclonal antibody that is expressed in prostate tissue, including prostate cancer. PSMA has been shown to be a compelling target in cancer therapy due to its high rate of

expression in almost all prostate cancers [116]. AMG 212 induces cytokine release from T cells, targets T-cell-dependent activation, and efficiently engages T cells for the lysis of prostate cancer cells. Different PSMA expressing cell lines, such as PCa 22Rv1, VCaP, C4-2, MDA PCa 2b, LNCaP, and PC3-PSMA were all lysed by human T cells which were engaged by AMG 212 at EC50 values (half-maximal effective concentration) between 0.1- 4 ng/mL. The degree of lysis was correlated with cell surface expression levels of PSMA. No cell lysis was observed in the PSMA-negative PCa, PC3, and DU145 cell lines. These results showed the antigen-specific activity of AMG 212. In patients with prostate cancer, a phase I clinical study of AMG 212 was completed (NCT01723475) [117].

5.8. ES414

ES414 is another BsAb for the treatment of metastatic prostate cancer, designed to target PSMA and CD3ε. The ES414 sequence was derived from the variable regions of two distinct murine antibodies specifically against CD3Ɛ and PSMA. ES414 contains an anti-PSMA scFv connected to the CH3 and CH2 domains of human IgG1, and an anti-CD3 scFv joined together via a flexible linker. This BsAb was constructed using the ADAPTIR platform. The presence of an Fc region increases the ES414 half-life and induces target-dependent effects by engaging in vitro and in vivo T-cell cytotoxicity against cancer cells expressing the PSMA antigen. A reduced cytokine release profile was observed with ES414 compared to other bispecific antibody formats. ES414 can redirect T-cell cytotoxicity and drives multiple rounds of T-cell proliferation. Administration of ES414 reduced the levels of PSMA in serum and produced remarkable inhibition of tumor growth and increased survival in mouse xenograft models of prostate cancer.

A phase I clinical trial of ES414 in metastatic castration-resistant prostate cancer is currently ongoing (NCT02262910) [118].

5.9. MM-141 (Istiratumab)

The MM-141 is a BsAb, which contains two stable scFvs linked to the Fc fragment of an IgG. It can simultaneously bind to the insulin-like growth factor I receptor (IGF-IR) and human epidermal growth factor receptor 3 (HER3). HER3 is a member of the HER superfamily of tyrosine kinase receptors, consisting of EGFR (HER1), HER2, HER3, and HER4. They are type I transmembrane growth factor receptors that activate intracellular signaling pathways in response to extracellular signals. The HER family is widely expressed in numerous cell types, such as neuronal, epithelial, mesenchymal cells, as well as tumor cells. Over-expression of HER3 is associated with several cancers, including breast, gastric, ovarian, prostate, melanoma, bladder, colorectal, lung, and squamous cell carcinoma [119]. IGF1R is a transmembrane protein that exists on the surface of human cells and is a tyrosine kinase receptor. Over-expression of IGF1R induces cancer cell growth, tumorigenesis, and neoplastic transformation [120]. In various tumor types, increased IGF1R signaling may provide a pathway for the tumor to escape from signaling blockades. Hence, a BsAb that inhibits both IGF1R and HER3 signaling could block downstream mechanisms and limit the development of resistance [121]. A phase I clinical trial of MM141 has been completed in patients with advanced solid tumors (non-randomized, parallel assignment, open-label). A phase II trial is ongoing in patients with metastatic pancreatic cancer [122]. The phase I study enrolled 15 patients with advanced solid tumors. Adverse events, regardless of causality, that were reported with a frequency >15% included, nausea, vomiting, abdominal pain, fatigue, increased AP, diarrhea, dyspnea, increased AST, anemia, and rash.

Disease stabilization was observed in patients with parotid gland carcinoma (1 patient) and Ewing's Sarcoma (1 patient) (NCT02399137) [123].

5.10. Duligotuzumab (MEHD7945A/ RG7597)

Duligotuzumab is a phage-derived humanized DAF BsAb. This BsAb blocks ligand binding to HER3 and EGFR, and inhibits downstream signaling pathways of the HER-family receptors [124]. Duligotuzumab has been suggested to bind to Fcγ receptors and activate the ADCC pathway in vitro models [38]. The EGFR (HER1) has been implicated in both head and neck and colorectal tumorigenesis and tumor progression, and its over-expression is associated with advanced disease [125]. Patients who are treated with Cetuximab (EGFR inhibitor) alone, usually develop anti-EGFR resistance. Duligotuzumab is able to bind to the EGFR/HER receptors and block their ligand binding [126]. A phase Ib study of Duligotuzumab in combination with irinotecan (FOLFIRI) and 5-fluorouracil was completed (non-randomized, parallel assignment, open-label). In patients with KRAS exon two wild-type metastatic colorectal cancer, no improvements in progression, overall survival or disease-free survival of patients were observed compared with second-line treatment with FOLFIRI plus Cetuximab [127]. As first-line therapy for recurrent or metastatic head and neck squamous cell carcinoma, Duligotuzumab demonstrated encouraging activity in a phase I b trial (NCT01911598) [128].

5.11 MM111

MM111 is a BsAb (anti HER2/HER3), which contains two scFvs fused to human serum albumin (HSA). This BsAb inhibited the signaling in HER2-amplified tumors and HER3-ligand binding via preventing the formation of HER2/HER3 heterodimers. HER2 over-expression has been

found in several tumor types, such as gastroesophageal (GOC) and breast cancer. HER3 is activated via its ligand, called Heregulin (HRG). HRG is a growth factor that is involved in cell survival, differentiation, invasion, and proliferation of both malignant and normal tissue [129]. A phase II study of paclitaxel and trastuzumab without MM-111 (Arm B), or plus MM-111 (Arm A) has been completed (randomized, parallel assignment, open-label) in patients with HER2 positive GOC cancer. In this clinical trial, 88 patients were randomized, 71 patients had received prior trastuzumab, 57 patients showed HRG mRNA expression, and 54 patients had HER2+ status confirmed. Progression-free survival (PFS) in an intent-to-treat analysis was 9.6 weeks for arm A, and 23.3 weeks for arm B. The median overall survival of intent-to-treat analyses was 32.1 and 56.1 weeks for arm A and arm B, respectively. MM-111 did not improve PFS or overall survival in HER2+ GOC in combination with paclitaxel /trastuzumab (NCT01774851) [130].

5.12. Ertumaxomab

Ertumaxomab is an anti-Her2/CD3 trifunctional BsAb. It also can bind to some Fcγ receptor-positive antigen-presenting cells (macrophages, dendritic cells, and NK cells) and form a ternary-cell complex between accessory cells, tumor cells, and T cells. Ertumaxomab destroys tumor cells that only display low-level HER2 expression [131]. A phase I/II clinical trial of ertumaxomab (single group assignment, open-label, dose-escalating) was completed in patients with advanced solid tumors (breast, gastric, rectal cancer). A total of 14 heavily pretreated patients were enrolled in 4 groups. Amongst them, three patients had to be withdrawn, and two patients showed serious adverse events, both reversible. No DLT was detected in any of the subjects; therefore, the maximum tolerated dose could not be determined. Adverse events were transient

and completely reversible. This study demonstrated that therapy with ertumaxomab was possible and could be studied further (NCT01569412) [85].

5.13. MDX-447

MDX-447 is a BsAb, which is directed against the Fc receptor (FcγRI /CD64) and EGFR. This BsAb can enhance immune-mediated cytotoxicity against cancer cells [132]. Four major classes of FcRs have been defined in mammalian species, namely FcγRI, FcγRII, FcγRIII, and FcγRIV. FcγRI has a high affinity for the constant region of the antibody and is expressed on activated neutrophils, hematopoietic cells, immature dendritic cells, monocytes, and macrophages. MDX-447 BsAb was tested in a study to improve the clinical effects of the H425 antibody. In vitro trials demonstrated that MDX-447 could simultaneously bind to both FcγRI and EGFR, and triggered ADCC to kill EGFR over-expressing cells. MDX-447 was constructed via cross-linking of the anti-EGFR monoclonal antibody (H425) F(ab′) fragment with the anti-FcγRI monoclonal antibody (H22) F(ab′) fragment [133]. A phase-I trial of MDX-447 was completed in patients with advanced solid tumors. Sixty-four patients were enrolled; 23 patients in group two received granulocyte-colony stimulating factor (G-CSF) + MDX-447, while 51 patients in group one received MDX-447 alone. Hypotension was the dominant DLT experienced by 7 patients in each group. The maximum tolerated dose for MDX-447 BsAb was 30 mg/m^2. Co-administration of MDX-447 and G-CSF was not well-tolerated, despite the good tolerability of MDX-447 as a single agent, and there were no objective tumor responses (NCT00005813) [134].

5.14. LY3164530

LY3164530 is a BsAb that binds to both EGFR and MET. This antibody contains two heavy chains and two light chains. LY3164530 is composed of a scFv fragment against EGFR and an IgG4 antibody against MET (mesenchymal-epithelial transition or hepatocyte growth factor), which is fused to the N- terminal of each heavy chain. LY3164530 inhibits signaling via both EGFR and MET receptors by blocking ligand binding, inhibiting internalization, and stimulating degradation [135]. MET signaling is the inverse process of EMT (epithelial-mesenchymal transition), whereby mesenchymal cells are transformed into polarized epithelial cells. Epithelial cells are characterized by expression of adhesion molecules such as epithelial cadherin (E-cadherin), apical-basal polarity, and occluding cell-cell junctions, while mesenchymal cells lack mature cell-cell contacts. They can exceed through the extracellular matrix, and they also express different markers such as zinc finger protein SNAI1 (Snail), Twist-related protein 1 (TWIST1), neural cadherin (NCAD), and fibronectin. The MET process occurs in normal development, induced cell potency reprogramming, and cancer metastasis. Therefore, in order to prevent cancer metastases, MET could be considered as an important therapeutic target [136]. A phase I clinical trial of LY3164530 in patients with metastatic or advanced cancer (colorectal cancer or squamous cell cancer) was completed (single group assignment, open-label). Most treatment-related adverse events were due to EGFR inhibition. These adverse events included hypomagnesemia, maculopapular rash/dermatitis acneiform, paronychia, fatigue, skin fissures, and hypokalemia. Partial responses were achieved in 3 patients with squamous cell cancer or colorectal cancer. The disease control rate was 51.7%, the overall response rate was 10.3%, and 17.2% of patients had stable disease for more than four months (NCT02221882) [137].

5.15. JNJ-61186372

JNJ-61186372 is an anti- EGFR /c-MET IgG-like BsAb. Both EGFR and c-MET play significant roles in the proliferation of tumor cells. A Chinese hamster ovary (CHO) cell line was used to produce this BsAb, which lacked the protein fucosylation ability. The human FcγRIIIa (CD16a) is essential for ADCC, and antibodies with low-levels of core fucosylation, show increased efficiency of ADCC and lead to the better killing of tumor cells [138]. The tyrosine-protein kinase Met (c-MET) is a single pass tyrosine kinase receptor. A wide range of cellular signaling pathways involved in proliferation, angiogenesis, invasion, motility, and migration are activated by the interaction of hepatocyte growth factor/scatter factor (HGF/SF) with c-MET. HGF/SF is the specific ligand of c-MET. One of the most important roles of MET is the control of tissue homeostasis under normal physiological conditions. Moreover, c-MET has been found to be abnormally activated in human cancers, caused by mutation, over-expression, or amplification [139]. EGFR and c-Met are often co-expressed in tumors, and the two receptor pathways converge at the PI3K/AKT/mTOR and Ras-Raf-MEK-ERK signaling pathways. Cross-talk between EGFR and c-Met has been widely reported in lung cancer [140]. NSCLC is the most common type of epithelial lung cancer and accounts for about 85% of all lung cancer. The most common types of NSCLC are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. The NSCLC usually becomes resistant against tyrosine kinase inhibitors drugs (TKI) by activation of EGFR mutations, mostly via second-site resistance mutations in EGFR (Thr790Met). The results of preclinical studies have shown that the combination of one of the several available third-generation EGFR TKI drugs with JNJ-61186372 BsAb could lead to complete and stable regression in human lung cancer xenografts. Significant toxicity was observed in cynomolgus

monkeys (*Macaca fascicularis*) treated with JNJ-61186372 BsAb. A phase I clinical trial of JNJ-61186372 BsAb is currently in progress in patients with advanced NSCLC (NCT02609776) [138].

5.16. MT110 (Solitomab/AMG 110)

MT110 is a BiTE with dual specificity for EpCAM and CD3 [141]. This BiTE molecule activates T cells and redirects the lysis of target cells showing over-expression of EpCAM [142]. MT110 has shown potent anti-tumor activity in various animal models [143]. Tumor-initiating cells (TICs) are a serious limitation for the effective therapy of cancer using conventional therapies. TICs have now been recognized and characterized in numerous human malignancies, such as colorectal and pancreatic tumors. EpCAM is frequently over-expressed on most primary and metastatic human adenocarcinomas [144]. MT110 showed a powerful effect to mediate redirected lysis of colorectal TICs. In vitro sensitivity assays and in vivo testing of survival and tumor progression in xenograft mouse models, demonstrated highly efficient elimination of TICs using MT110 [145]. A phase I clinical trial of Solitomab (MT110/AMG 110) was completed in patients with refractory solid tumors (single group assignment, Open-Label, multi-center dose escalation). Some patients showed a partial response, but dose-limiting toxicity was observed that prevented the dose being sufficiently raised to therapeutic levels. Solitomab did show primary signs of antitumor activity in the 65 patients enrolled in this trial. According to the response evaluation criteria in solid tumors (RECIST), 55 patients underwent a response assessment. Eight patients were not evaluable due to a lack of measurable target lesions. Eighteen patients had stable disease (SD) with an average duration of 84 days, and 11 patients had no follow-up tumor assessment (NCT00635596) [146].

5.17. Catumaxomab

Catumaxomab used in clinical trials for breast cancer, non-small cell lung cancer, gastrointestinal cancer, ovarian cancer, and peritoneal carcinomatosis. A phase II trial of catumaxomab in 35 patients with gastric cancer was completed. Since Catumaxomab had ceased being marketed in the European Union since 2014, patient recruitment was ended prematurely. 4 patients were excluded from analysis, and 31 patients were evaluable for the final analysis. 15 patients (arm A) were allocated to Catumaxomab plus chemotherapy with FLOT (leucovorin, 5-fluorouracil, docetaxel, oxaliplatin) and 16 patients (arm B) to FLOT only. After systemic treatment, three patients (19%) in the chemotherapy alone arm B, and four patients (27%) in arm A showed complete remission. A partial response rate of 46% was achieved in both arms. Three patients (23%) in arm A and two patients (15%) in arm B showed stable disease, and progressive disease was observed in five patients (38%) from arm B and three patients (23%) from arm A [147].

5.18. MGD007

MGD007 is a DART protein (anti-glycoprotein A33/anti-CD3) designed to engage T cells to specifically target gpA33, which is over-expressed on colon tumor cells. The attachment of the Fc region to the DART molecule prolongs the serum residence time [148]. The A33 antigen is a surface protein expressed in > 95% of normal intestinal tissue, and also in human colon tumors, but does not exist in other organs. The A33 antigen is encoded by the GPA33 gene. It is a surface glycoprotein of colonic epithelium and small intestine. It has homology to tight junction-associated proteins (TJAP) of the immunoglobulin superfamily, containing the Coxsackie adenovirus receptor (CAR) and junction adhesion molecules (JAM) [149]. MGD007 is currently in

a phase I trial in patients with relapsed/refractory metastatic colorectal cancer (NCT02248805) [150].

6. Conclusions

Despite some success with immunotherapy of cancers, many patients remain non-responsive to therapy due to various mechanisms of intrinsic or acquired tumor resistance. Blocking of angiogenesis is an important mechanism for inhibition of cancer progression. Parallel pathways of angiogenesis or inherent bypass might be responsible for tumor resistance against anti-angiogenic drugs. It can be hypothesized that the blockade of tumor resistance pathways by combinations of various drugs may inhibit resistance. Currently, an essential question facing cancer therapy is the extent to which combination drug therapies could simultaneously block tumor resistance pathways. BsAbs have shown high potential for the treatment of different diseases, such as cancer, autoimmune, and inflammatory diseases. Although BsAbs showed potent efficacy against various cancers in preclinical trials, most results did not achieve the expectations. The employed BsAbs can be cleared from both outside and inside the tumor. Plasma clearance and endocytic clearance decrease the concentration of antibody. In this regard, the non-IgG-like formats of BsAbs have been designed, which lack the Fc region and have the ability to efficiently penetrate the tissue and show higher tumor specificity due to their small size. Different factors are involved in tumor resistance against immunotherapy, and various biological parameters are still unclear in the understanding of the TME and its biology. More detailed information about the interactions between the TME and immunotherapy can provide new approaches to ameliorate the response rates of common immunotherapies. Increasing both the specificity and sensitivity of BsAb is very important in the design of novel BsAbs, and the

production yield compared to traditional hybridomas, and minimizing the cost of the production are other important factors in the design and preparation of new BsAbs. Further engineering of BsAbs would circumvent these therapeutic barriers and make them more efficient in the fight against various cancers. Demands to develop new and innovative therapeutic BsAbs are ever-growing. The number of BsAbs in development has grown significantly over the past few years. Up to now, three BsAbs have been approved, and two BsAbs (Emicizumab-kxwh and Blinatumomab) are commercially available in the market. Overall sales of these antibodies have reached approximately 1 Billion US$ in 2018. Currently, more than 85 BsAbs are in clinical trials, and it is clear that BsAbs-based therapeutics will continue to be of high interest. A further range of approved BsAbs seems to be around the corner. The market of BsAbs has been estimated to surpass 8 Billion US$ by 2025. The future for the development of BsAbs remains highly promising and profitable, and this novel immunotherapy strategy has great potential for the therapy of different diseases.

Conflicts of interest

MRH declares the following potential conflicts of interest. Scientific Advisory Boards: Transdermal Cap Inc, Cleveland, OH; BeWell Global Inc, Wan Chai, Hong Kong; Hologenix Inc. Santa Monica, CA; LumiThera Inc, Poulsbo, WA; Vielight, Toronto, Canada; Bright Photomedicine, Sao Paulo, Brazil; Quantum Dynamics LLC, Cambridge, MA; Global Photon Inc, Bee Cave, TX; Medical Coherence, Boston MA; NeuroThera, Newark DE; JOOVV Inc, Minneapolis-St. Paul MN; AIRx Medical, Pleasanton CA; FIR Industries, Inc. Ramsey, NJ; UVLRx Therapeutics, Oldsmar, FL; Ultralux UV Inc, Lansing MI; Illumiheal & Petthera, Shoreline, WA; MB Lasertherapy, Houston, TX; ARRC LED, San Clemente, CA; Varuna

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