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REVIEW ARTICLE

Bispecific Antibodies (bsAbs): Promising Immunotherapeutic Agents for Cancer Therapy

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Abstract: Antibodies have become the preferred therapeutic treatment option for cancers. Antibody therapy is associated with low toxic profile and specific in its activity, unlike chemotherapy and radiotherapy. Types of tumor are known to express multiple receptors that cross-talk to activate perpetual growth, proliferation and metastasis, and inhibit apoptosis in such tumors. Bispecific antibodies (BsAbs) are therefore the preferred agent for the treatment of such cancers due to its unique characteristics. This review discusses up to date therapeutic potentials of BsAbs.

Keywords: Antibody, bispecific antibody (BsAb), immunotherapeutic agent, cancer therapy, diabody, antitumor.

1. INTRODUCTION

Monoclonal antibody (mAb) therapy has become arguably the mainstay of modern medicines with proven potency and better safety profile, which has considerably improved outcomes of disease management and benefited millions of patients with serious conditions such as cancer [1].This has generated a lot of interest in its construction, development and use in recent times. The advancement in antibody engineering has resulted in the generation of different types of antibodies. These include fusion antibodies with bispecific properties. Bispecific antibodies are recombinant molecules, capable of binding two different targets simultaneously [2, 3]. They can therefore be applied to redirect effector cells such as Natural Killers (NK) cells and T-cells to cancers [2, 3]. Developing recombinant bispecific antibodies has become imperative, especially, in cancer therapy, because looking at it from the perspective of cancer biology, many cancers rely on multiple pathways for survival through crosstalk between signaling cascades. There is therefore the need to simultaneously target the different signal pathways to fully inhibit cancer proliferation and growth [4, 5]. Single targeting does not appear to be the most efficient strategy to destroy such cancer cells. Thus, treatment of cancer with mAbs has not been as successful as expected due to some of these functional limitations. Therefore, producing bispecific recombinant antibody could be the way forward in improving cancer therapy. The quest to improve the treatment outcomes has generated an interest in the development and usage of bsAbs [6, 7]. The review therefore seeks to discuss the possible techniques that can be employed to design and construct bsAbs to improve their antitumor efficacy.

2. STRUCTURE AND FUNCTION OF THERAPEUTIC ANTIBODIES

Antibodies, produced by B cells, are involved in the humoral branch of the adaptive immunity. They recognize pathogens or foreign molecules through specific binding to antigens expressed on them [8, 9]. Specific interaction can neutralize the threat pose by these pathogens or foreign molecules or trigger effector functions of the immune system to eliminate them. Examples of the effector functions are opsonization, activation of complement and antibody-dependent cell-mediated cytotoxicity (ADCC) [9, 10]. Antibody is made up of two identical light (L) chains and two identical heavy (H) chains (Figure **1**), with the molecular weight of the light and heavy chain being about 25 and 50 kDa respectively [11, 12]. Each light chain (L) is joined to a heavy chain via a disulfide bond and a combination of non-covalent interactions such as hydrogen bonds, salt bridges and hydrophobic interaction [13]. The parts of an antibody have specific functions. The arms of the Y shaped antibody

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consist of the sites that can bind two antigens and, therefore, recognize specific foreign molecules [14]. This region of the antibody is referred to as the fragment antigen binding (Fab) region. It is made up of one constant and one variable domain from each heavy and light chain of the antibody, with VH, VL, CH and CL representing variable heavy chain, variable light chain, constant heavy chain and constant light chain respectively [14]. The amino-terminal regions of the V_H and V_L vary greatly among antibodies with different specificities [15]. Within the variable regions, sequence variability is concentrated in several hyper-variable regions. These hyper-variable regions constitute the antigen-binding site of an antibody, and are called complementarity-determining regions (CDRs) [16]. Remaining domains of V_L and V_H, which exhibit far less variation, are called the framework regions (FW). The Y shaped antibody base performs an important role in controlling immune cell activity and the region is known as the Fragment crystallizable (Fc) region. This region is made up of two heavy chains that contribute two or three constant domains depending on the class of the antibody [16]. For a given antigen to bind to a specific protein, Fc fragment guarantees that the antibody generates appropriate immune response [17]. Additionally, the Fc fragment can bind to different kinds of cell receptors, for example, Fc receptors, and other immune related molecules, such as complement proteins to mediate different physiological effects such as opsonization, cell lysis, and degranulation of mast cells, eosinophil's and basophils [18]. Fc and Fab fragments can be generated from an antibody in the laboratory. Thus, the enzyme papain can be employed to cleave an immunoglobulin into two Fab fragments and an Fc fragment. Additionally, the enzyme pepsin can cleave below the hinge region to produce a F(ab')2 fragment and a pFc' fragment. Employing mild reduction, the F(ab')2 fragment can be split into two Fab' fragments. (Figure **1**) [19]. This implies that the structure of a therapeutic antibody can be re-engineered using modern technology to improve its efficacy, and has actually informed the trend of research in this field of study in recent times.

3. THERAPEUTIC ANTIBODY AND CANCER MANAGEMENT

Therapeutic Antibody and Cancer Management Antibodies have been used in several roles in cancer management. These include diagnosis, monitoring, and treatment of the disease [20, 21]. In diagnosis, flow cytometry has been used in the identification of different subsets of non-Hodgkin's lymphoma. Antibodies (Abs) have also been employed to monitor cancer progression, via the carcinoembryonic antigen measurement in colon cancer [22-24]. Essentially, Abs have been applied directly as therapeutic agent in cancer treatment [25, 26]. In relationship to treatment, Abs can bind to particular antigens on tumor cells to enhance the patient's immune surveillance. More interestingly, Abs can be programmed to inhibit growth factors which promote cancer proliferation and growth, thereby blocking cancer cell growth [27]. Additionally, Abs can carry chemotherapy drug or radioactive particle. These categories of Abs are called conjugated antibodies. These Abs are used as a homing device to transport one of these substances directly to the cancers. The Abs moves through the body until it finds and hooks onto the target. It then delivers the drug or radioactive substance to where it is mostly needed. This obviously reduces the toxicity to normal cells in other parts of the body because of the specific nature of the conjugate monoclonal antibodies [28]. Conjugated Abs are also sometimes referred to as tagged, labeled, or loaded antibodies because of their well-defined activity. Ab could also be used to select normal stem cells from bone marrow or blood in preparation for a hematopoietic stem cell transplant in cancer patients [29]. There are a number of things to consider when using Ab for any of the above stated cancer management applications. First, a target antigen on the cancer must be selected. It is imperative that this antigen is expressed uniquely on the tu-

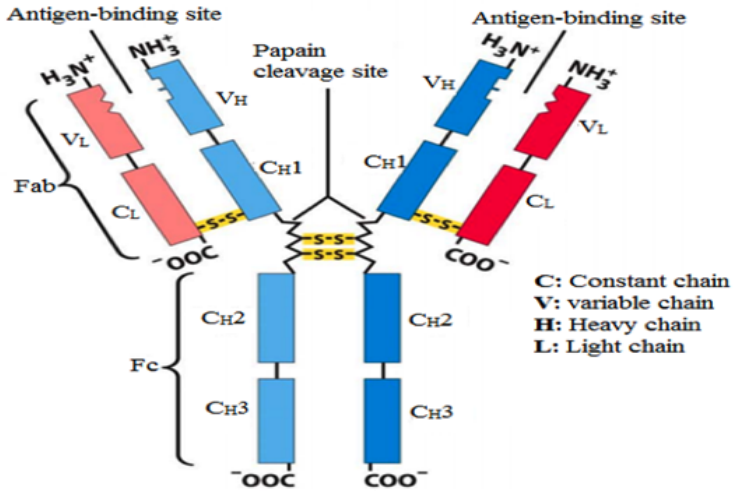

Figure 1. A schematic diagram of an IgG1 showing the various components of the heavy (H) and light (L) chains which constitute the IgG1. As indicated on diagram, the whole structure is divided into two main parts; fragment antigen binding (Fab) and crystallizable fragment (Fc). Fab is made up of variable light chain (V_L), variable heavy chain (V_H), constant light chain (C_L) and constant heavy chain (C_H) coupled with two binding antigen sites. The Fc is made up of four constant heavy chains (C_H) linked with the Fab with disulfide bonds.

mor cells and not on normal cells. Thus, the selected antigen must be over-expressed on the cancer but not/under-expressed by normal cells. The immunogenicity of the Ab should also be considered. These therapeutic antibodies are often derived from non-human sources, therefore, they are capable of eliciting an immune response themselves. Another important factor to consider is the Half-life. The Half-life should be long enough to produce the expected result [30]. Finally, logistical challenges such as cost and availability of the therapeutic antibody, and also the decision as to whether or not the mAb should be used alone or in combination with other agent(s) should be considered in order to derive the desired effect.

4. SUITABLE CANCER ANTIGENS FOR ANTIBODY TARGETED THERAPY

One way the immune system attacks foreign substances in the body is by the production of large numbers of antibodies. These antibodies circulate through the body until they find and attach to a target. Once attached, they are capable of recruiting other relevant parts of the immune system to attack the cancer cells [31]. Researchers are now able to design and produce antibodies that specifically target specific antigens, such as those found on cancers. Many copies of these antibodies are now produced in laboratories for such purposes. The key factor that is considered when engineering or producing Ab is to identify the antigen the antibody will attack. Using Ab for cancer treatment is not always easy, because they have proven to be more useful against some cancers than others [32]. The efficacy and safety profile of Ab in oncology vary depending on the nature of the target. More importantly, the target should be abundantly and homogeneously expressed. Additionally, the target antigen should be accessible, consistently and exclusively expressed on the target cancer cells [33]. Secretion of the target antigen must be minimal as possible to derive the desire effect. This is because secreted antigens could possibly bind to the antibody and inhibit sufficient antibody from binding to the target antigen on the tumor surface [34]. Therapeutic Abs targeted antigens on tumor can be categorized into different groups. Abs target antigens associated with hematopoietic differentiation are glycoproteins usually associated with cluster of differentiation (CD) groupings which include CD20, CD30, CD33 and CD52 [35]. Cell surface differentiation antigens which are a diverse group of glycoproteins and carbohydrates found on the surface of both normal and tumor cells can also be targeted with antibodies in cancer therapy [36]. Additionally, growth factors and their receptors involved in growth and differentiation signaling could be targeted by Abs as suitable antigens for cancer therapy [36]. These growth factors includes: epidermal growth factor receptor (EGFR) [36], ERBB2 [37], ERBB3 [38], MET [39], insulin- like growth factor 1 receptor (IGF1R) [40], ephrin receptor A3 (EPHA3) [41], tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor 1 (TRAILR1), TRAILR2 and the receptor activator of nuclear factor-κBligand (RANKL) [42, 43]. Angiogenesis related antigens are mostly growth factors that support the formation of new microvasculature and therefore are also suitable candidates for Abs targeted therapy. These include vascular endothelial growth factor (VEGF) and the receptor VEGFR, integrin αVβ3 and integrin α5β1 [44]. Finally, antigens of the stromal and extracellular matrix could serve as therapeutic targets for cancer treatment, and these include fibroblast activation protein (FAP) and tenascin [45].

5. ANTIBODY ENGINEERING AND DEVELOPMENT

Advent of new techniques in molecular biology in the early 1990s made it possible to clone genes of IgG molecules and their derivatives in eukaryotic expression vectors [46]. This has paved way to develop and produce recombinant types of Ab from different cell lines in a reproducible fashion, and has undoubtedly solved some of the production challenges that come about as a result of the instability associated with most hybridoma lines [47]. Cloning of genes of antibody allowed for antibodies modification through sub cloning, random or directed mutagenesis and molecular evolution procedures. This has made it probable to augment the production of recombinant antibodies and kicked started antibody engineering [48]. Antibody engineering application has resulted in generation of chimeric antibody, which is made up of fully human Fc portion and therefore about 70% human (Figure **2**). Chimeric antibody is created by fusing murine variable domains, responsible for binding antigens, with human constant domains responsible for binding effector cells [49] resulting in the development of a new generation of therapeutic candidates [50]. In humans, humanized Fc portion renders chimeric antibodies substantially less immunogenic. With significant progress in antibody engineering methods, it has become probable to reduce further the murine part of mAbs by replacing the hyper-variable loops of the murine antibody by hyper-variable loops of a fully human antibody, employing a technique known as complementarity-determining region grafting [51]. These antibodies are therefore 85–90% human and have a reduced amount of immunogenic tendencies than chimeric antibodies (Fig. **2**). Notwithstanding, the complementarity-determining region grafting require special expertise to successfully carry out, compared to the mere fusion. For instance, directed mutagenesis approach is often required to restore the affinity in the murine parental antibody. An antibody generated with this technology is called humanized antibodies, and most of the clinically approved Abs currently in use are either chimeric or humanized.

The *in vitro* selection techniques development, especially, the phage display is a major improvement in the development of therapeutic antibodies. The synthesis of repertoires of antibody fragment genes are made possible via the improved antibody engineering from immunized or non-immunized animals, including humans. This therefore implied that a powerful selection technique was required to isolate antibodies from this large number of potential ligands, capable of binding specific antigen. This selection method like most *in vitro* methods relies prominently on the ability to establish a physical connection among a particular protein and the gene encoding it. Thus, establishing a link between a protein fused to a filamentous phage capsid protein (p3 or p8) displayed on the phage M13 surface and the matching gene confined in the encapsidated DNA [52]. Immunopurifying the said molecule via conjugating to antigen of interest makes the gene of interest readily accessible and therefore allows for sequencing and further multiplication of

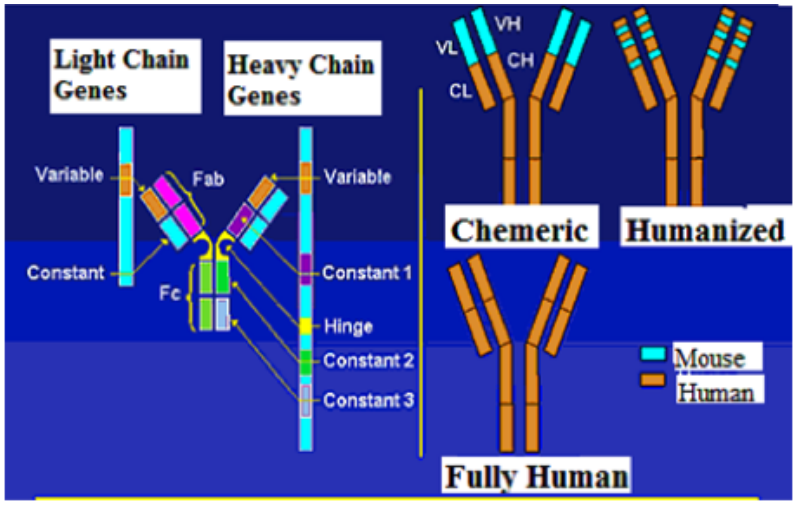

Figure 2. Schematic diagram of the construction of monoclonal antibody (mAb) from the light and heavy chain gene, and the possibility of generating chemeric, humanized and fully human mAbs.

the particular clone. One advantage of *in vitro* selection techniques is that, it has made it probable to efficiently select fully functional human antibody fragments against virtually any antigen, using large non-immunized libraries [53]. Additionally, *in vitro* selection techniques can be used to improve the affinity of a therapeutic antibody through the creation of a secondary library comprising of first candidate of mutants and carrying out vigorous *in vitro* specificity against the antigen of interest [54]. More importantly, Phage display and equally efficient ribosome display have successfully been used to produce ligands with higher affinities for the antigen of interest compared to most of the conventional Abs [55]. The quest to generate fully human antibodies in the last decade has led to the development of a complementary approach in the production of therapeutic antibodies. The idea was that creating 8 humanized mice could provide the vehicle for the production of fully human antibodies. Transgenic 'humanized' mice were therefore generated by substituting the whole mouse IgG repertoire with a human repertoire [56]. These humanized mice produce human IgGs upon immunization (Figure **2**). The already popular hybridoma technique can then be used to clone the human antibodies with the mandatory properties [57]. Notable advantages of this approach are that, it yields already matured *in vivo* antibodies, and therefore require no additional affinity maturation. More importantly, it directly yields full-length IgG, the preferred format for therapy. Nevertheless, one major limitation to this technique is that the humanized mice cannot be used efficiently when the immunogen has high toxicity or if the antigen of interest demonstrates high degree of homology sharing with its murine ortholog [57].

6. BISPECIFIC ANTIBODY: THE PREFERRED AGENT FOR CANCER THERAPY

Bispecific antibody derivatives (bsAbs) have come to be accepted over time as the next generation of targeted drugs for cancer therapy. The general concept underlying the development of bsAbs is about the physical connection of the recombinant antibody-derived moieties with at least two binding specificities [1]. Thus, bsAbs bind to two antigens or epitopes simultaneously, which is not the case in the conventional antibodies, it is therefore an improvement over the conventional antibodies. The bsAbs combine specificities of two different antibodies or proteins and concurrently address different antigens or epitopes (Figures **3** and **4**). Aside its ability to interact with multiple cell surface receptors and ligands, bsAbs can also place target and effector cells in close proximity to trigger cytotoxicity in the target cell [58].These therapeutic bsAbs are mostly generated as recombinant proteins which are either large IgG-like proteins with Fc moieties or as smaller moieties with bispecific antigen binding fragments but without Fc moieties. The first bsAb was approved in 2009 after many years of research, and as recent as December 2014, another bsAb was introduced into the market coupled with the fact that many of these bsAbs are at different stages of clinical trials [1, 59]. Basically, bsAbs can be grouped into two main classes based on the absence or presence of Fc moiety. The bsAbs without Fc moiety are smaller than those with Fc moiety (Figure **4**). BsAbs with Fc fragment present with certain advantages which include the Fc region's ability to facilitate its purification, employing the already established protocol for the purification of IgG molecule [60]. Additionally, bsAbs with Fc moiety are relatively large and as a result has lengthy half-life which could enhance its potency as drug (Figure **3**). Furthermore, Fc moiety mediated effector functions such as antibody-dependent mediated cytotoxicity (ADCC) and complement fixation (CDC) are associated with bsAbs carrying Fc moiety [1]. This bispecific IgG molecule is commonly developed via two diverse light and heavy domains expressed in the same cell. However, due to the random assembly of the different chains that constitute the molecule, it leads to the formation of substantial amount of IgG molecules which are nonfunctional [61].

This bottleneck could be resolved by fusing another binding moiety such as single chain fragment Fv to the N or C terminal of the light or heavy chains, resulting in the formation of tetravalent molecules with two different reacting sites for particular antigen. Also a second VH and VL can be fused to the VH and VL respectively, forming double vari-

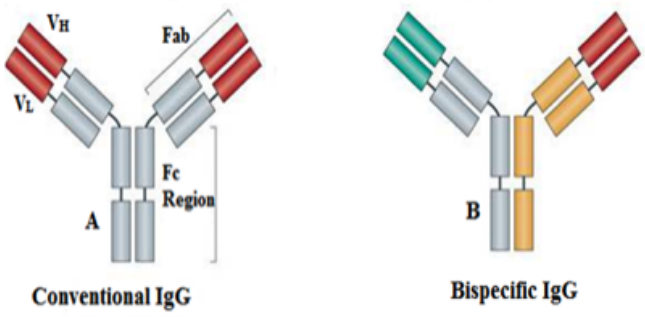

Figure 3. A schematic diagram of conventional IgG and bispecific IgG. (A) Conventional IgG with the same antigen binding sites; (B) BispecificIgG with different antigen binding sites.

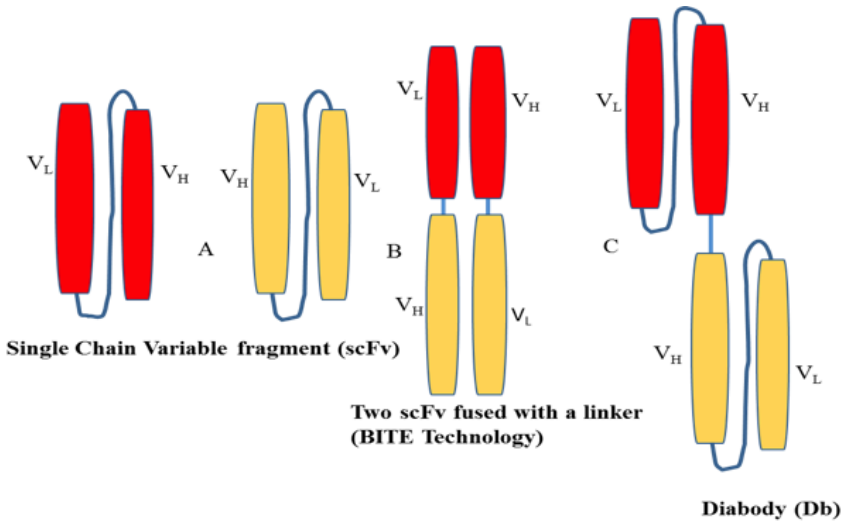

Figure 4. Schematic diagram of bispecific antibody fragments without Fc moiety. (A) Single chain variable fragment (scFv); (B) Two single chain variable fragments fused with a linker employing BiTE technology, which is bispecific in function; (C) Bispecificdiabody (Db).

able domain (DVD) IgG [62]. Additionally, the knob into holes technology has been used in the generation of bivalent bispecific IgG. Thus, heavy-chain heterodimerization was generated by the introduction of different mutations into the two CH_3 moieties, leading to the formation of asymmetric antibodies [63]. There could be variation in this approach which includes the usage of alternative mutations, hybrid CH_3 moiety obtained from IgG and IgA antibodies or electrostatic steering effect [64]. Nevertheless, these technologies or approaches are faced with random pairing challenge commonly referred to as light chain problem, which is random combination of the two chains of the heavy chain heterodimer with the two different light chains [65]. Reportedly, this problem can be prevented by using common light chains instead of the different light chains, which allows binding to both antigens [66]. Although this approach has proved efficient in resolving the light chain problem, it has not been successful for many antibodies. In situation like this, bacterial expression containing light and heavy chain combination can be employed [1].

In contrast, the therapeutic potentials of bsAbs without Fc moiety depend solely on their antigen-binding capacity. However, due to their relatively smaller molecular size they are able to diffuse faster and therefore reach the target cells easily [1]. Production of this antibody is mostly done in either yeast or bacterial expression factory, which are known to be relatively cheaper but robust. In view of this, purification cannot be done employing the already established technology for the purifying full IgG. As result, tagged amino acid such as His-tag which facilitates protein purification is added to the original bsAb during its construction for purification purposes. Small bsAbs without Fc moiety are either made up of variable VH and VL domains of two different antibodies or Fab moieties. Utilizing for instance the Bispecific T cell Engager (BiTE) technology, two scFv moieties are fused genetically, resulting in the formation of tandem scFv molecule. Two scFv fragments form independent folding molecules and are linked with flexible peptide linker [67]. Alternatively, the diabody technology can be employed. Thus the variable domains VH and VL from two antibodies (A and B) are expressed as two polypeptide chains VLB -VHA and VHB-VLA (Fig. **4**). These domains are linked via short flexible peptide linkers, resulting in heterodimerization of the two chains forming the diabody [68]. The diabody can further be modified to improve its therapeutic activities by transformation into a solitary chain to form scDb and dimeric tetravalent byproducts with two reacting sites for each antigen [1], and also the dual- affinity retarget-

ing molecules DART which are disulfide-stabilized variants [1].

Construction and utilization of bsAbs became imperative when it became evident through research that growth, proliferation, metastasis of cancer cells can be attributed to more than one tumoral receptor (commonly two), which simultaneously contribute to the survival of the cancer cells through cross-talk [69]. This situation is a common place in most cancer conditions. This has rendered most conventional antibodies ineffective in the treatment of cancers with these characteristics. In such cases, the best therapeutic option is to co-target the two receptors to derive the optimum benefit of the treatment. The suitable antitumor agent for this purpose therefore is undoubtedly bsAb and its derivatives. A case in point is the VEGFR2-EGFR (Vascular endothelial growth factor receptor2-Endothelial growth factor receptor) pathway cross-talk which plays critical role in the angiogenesis of the cancer cells. The extensive degree of EGFR-VEGFR pathway cross-talk has provided a rationale for joint targeting of these two pathways [70]. Reports from other studies elsewhere have also demonstrated that, combining EGFR and VEGFR2 antagonists increased the antitumor effect, and combined EGFR and VEGFR2 targeted therapy demonstrated significant therapeutic potential [71, 72]. My research team has successfully constructed and produced both recombinant human IgG-like bispecific antibody (Bi-Ab) and bispecificdiabody (Db), targeting both VEGFR2 and EGFR [72, 73]. Our Bi-Ab exhibited improved antitumor activity and demonstrated slightly superior activity over their combination (cetuximab and mAb-04) treatment. It has shown promise as a therapeutic agent, especially, to treat cancers co-expressing EGFR2 and EGFR. Also, the Db demonstrated similar antitumor trend. Thus, the Db exhibited enhanced antitumor activity compared to their combination (anti-VEGFR2 scFv and anti-EGFR scFv) treatment and the treatment with individual components (anti-VEGFR2 scFv or anti-EGFR scFv) of the Db. Reports from these two studies and others elsewhere give credence to the fact that, bsAbs are the preferred candidates for cancer therapy, especially tumor cells that express more than one tumoral receptor [72,73].

7. MODE OF ACTION OF bsABs

The mode of action of bsAbs on cancers can be categorized into three, based on the probable operational therapeutic paths. These are: recruitment of the appropriate immune cells, blocking receptor signaling and inactivating of ligand signaling, and targeted or pretargeted payload delivery bsAbs as summarized in Figure **5** [1]. As demonstrated in Table. **1**, recruitment of immune cells such as an NK and T cell to the site of the cancer is an important step towards the elimination of the cancer cells. Although the ability of bsAbs to effectively recruit appropriate immune cells to eliminate or reduce the cancer cell burden in patients, only a few are already in the clinic with majority at various stages of preclinical trials. Anti-CD3 bsAb called catumaxomab was the first to received approval for clinical use [74]. It was developed by a German Pharmaceutical Company called Trion Pharma as a trifunctional bsAb, a hybrid of murine IgG2a and rat IgG2b. The bsAb functionally targets a tumor antigen, and recruits T effector cells through CD3 binding and possibly activates NK cells, monocytes, macrophages and dendritic cells via its Fc moiety [75]. Catumaxomab has proven potent for the treatment of malignant ascites, and

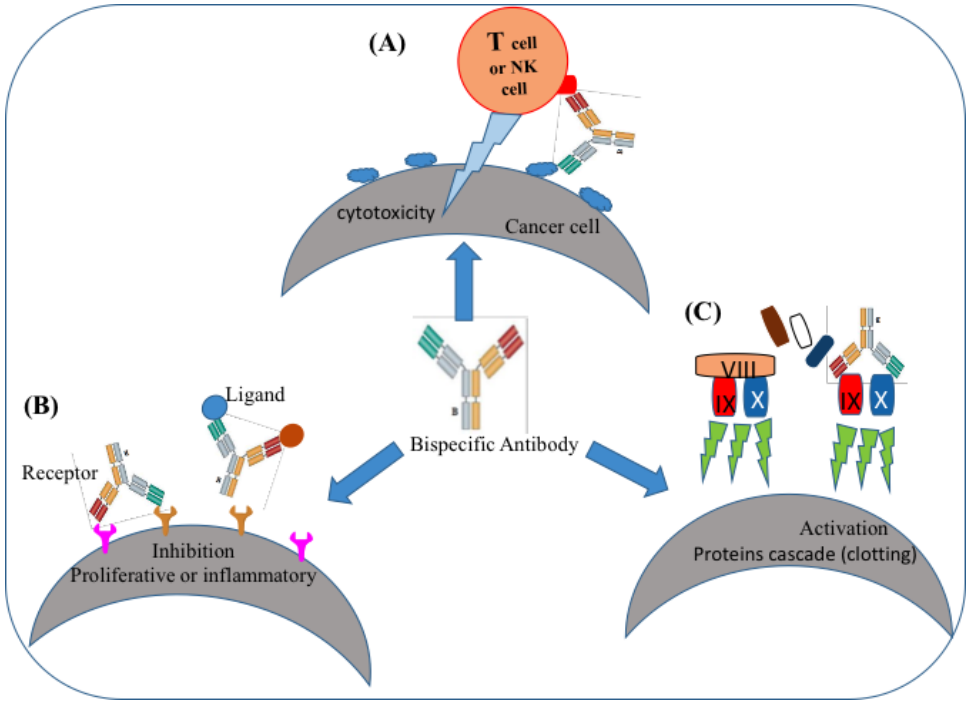

Figure 5. Mode of action of bispecific antibodies: (A) recruitment of the appropriate immune cells, (B) Blocking receptor ligand signaling pathway, and (C) Forced protein association (application of bsAbs in antibody mediated forced assembly of the coagulation Xasc complex).

Table 1. Some common bsAbs in clinical development.

bsAb	Format	Biological Function	Target	Status and Cancer type
Catumaxomab	TrioMab	T cell recruitment, Fc-mediated effector functions	EPCAM x CD3	Market: Malignant ascitis; phase II: ovarian, gastrointestinal, breast cancer; phase I/II: peritoneal carcinomatosis
Blinatumomab	BiTE	T cell recruitment	CD19 x CD3	Phase II: non Hodgkin lymphoma, acute lymphomatic leukemia
Ertumaxomab	TrioMab	T cell recruitment, Fc-mediated effector functions	HER2 x CD3	Phase I: metastatic breast cancer
Lymphomun	TrioMab	T cell recruitment, Fc-mediated effector functions	CD20 x CD3	Phase I-II: BCL
MGD006	DART	T cell recruitment	CD123 x CD3	Phase I: AML
AFM13	TandAb	NK cell recruitment	CD30 x CD16	Phase I: Hodgkin Disease
MM111	scFv2-HSA	Two-RTK inactivation	HER2 x HER3	Phase I: breast, stomach and esophageal cancer
MM141	IgG-scFv	Two-RTK inactivation	IGF1R x HER3	Phase I: solid cancers
RG7597	DAF-IgG	Two-RTK inactivation	HER1 x HER3	Phase II: head and neck cancer, metastatic colorectal cancer
TF2	D&L-Fab3	Payload delivery	CEA x Hapten	Phase I: colorectal cancer

therefore, it is being tried on ovarian, epithelial and gastric cancers. In addition to catumaxomab, there are other bsAbs with Fc moiety which have similar mode of action as catumaxomab which were successful at the preclinical trials. Out of these, ertumaxomab which targets the cancer cells via HER2 and recruits T cells via its anti-CD3 arm, and lymphomun that targets CD20 antigen on B cell lymphoma and recruits T cells through its anti-CD3 arm are currently being tried clinically [75, 76]. Additionally, there are bsAbs without Fc moieties such as blinatumomab (BiTE) which targets CD19 on acute lymphoblastic leukemia has demonstrated potency in the treatment of this cancer [76]. Another common bsAb without Fc moiety which recruits immune cells is MGD006. MGD006 is DART that targets CD123 on hematological cancers and recruits T cells via CD3 [77]. Unlike the other bsAbs without Fc, AFM13 which is a TandAb recruits NK cells or macrophages via CD16A on these effector cells [78, 79].

The second mode of action of bsAbs is by blocking receptor and inactivation of ligand signaling that promotes tumor growth. Receptor tyrosine kinases (RTKs) such as HER family which is made up of four members: HER1/EGFR, HER2, HER3 and HER4 which promote cancer growth when activated [80]. They are therefore target for cancer therapy. Thus, mAbs such as panitumumab and cetuximab which target HER1/EGFR as well as pertuzumab and trastuzumab that target HER2 are known cancer therapeutic agents [80]. Notwithstanding, most cancers are able to express more than one tumoral receptor which crosstalk to promote cancer growth, therefore using monospecific RTKs targeting antibody may not be the appropriate strategy to completely inhibit cancer growth. The bsAbs which are capable of binding these receptors simultaneously are the best agents to completely inhibit the growth of such cancers. For instance, using pertuzumab and trastuzumab do not completely inhibit ligand induce HER3 activation, however targeting both HER3 and HER2 simultaneously with bsAb completely inhibits cancer growth [80, 81]. Notable examples of these kinds of bsAbs are MM111 (phase I trial) that targets HER2 and HER3, MM141 (phase I trial) that targets HER3 and insulin-link growth factor (IGF-IR) which is not a member of the HER family, and RG7597 (phase II trial) that binds HER1 and HER3 [1, 79].

Targeted or pretargeted payload delivery bsAbs is employed to deliver payloads such as drugs, radiolabels and nanoparticles to the tumor site. The attached drugs or nanoparticles are usually too powerful to be used directly, because of their high toxic level. Additionally, bsAbs can be used to enrich payloads in tumor sites which prolong the serum retention time and improves the tumor/blood ratio [79, 82]. The use of radiolabel bsAbs is an efficient strategy employed in diagnosing tumor or locating tumor site [83]. Thus, used for tumor imaging and radioimmunotherapy. The bsAb TF2 is a notable example of targeted delivery bsAbs, designed for such purpose. It works by binding to CEA and ^{99m}T-labeled hapten histamine-succinyl-glycine (HSG) which is found on the surface of most solid tumors [84]. TF2 is currently undergoing phase I trial in patients with colorectal cancer [84]. Other possible applications TF2 include binding to Lutetium-177-labeled HSG or Indium-111 HSG and CEA for radioimmunotherapy in patients with advanced colorectal tumor, and it is in the phase I of the clinical trial [79, 84].

CONCLUSION

This review has focused mainly on the therapeutic potential of antibody for cancer treatment. The advent of new powerful antibody engineering has resulted in a generation of bispecific antibodies capable of simultaneously binding

multiple targets on cancer cell to inhibit its progression. This unique characteristic has been found to be very crucial in controlling cancers possessing multiple pathways for survival and proliferation. Apart from its acceptable toxic profile, the agent has been specific in its activity, which accounts for its potent anti-tumor activity. Due to the proven therapeutic potential of bsAbs compared to the conventional Abs, a number of bsAbs are in clinical trials, given credence to the fact that bsAbs are gradually becoming the preferred therapeutic agents for the treatment of cancers. Nevertheless, identifying optimal application modes and potentially modulating the PK properties of bsAbs, especially small sized bsAbs molecules are some of the important aspects that can be addressed to further improve the efficacy of these cancer therapeutic agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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