CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3548242

Available online at: http://www.iajps.com *Review Article*

A REVIEW ON BISPECIFIC ANTIBODIES

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Abstract:

Antibodies are widely recognized for their therapeutic potential and subsequently have prompted a lot of interest into their development and application. The structure of monoclonal IgG antibody is represented, with the heavy chains in rose and the light chains in green. The constant domain is represented in bright colours and the variable domain in darker colours. Bispecific antibodies are artificially designed molecules, capable of simultaneously binding two different antigens, hence they can be applied to redirect effector cells to tumor cells. Currently, two large classes of bispecific antibodies have been developed : immunoglobulin – like and small bispecific antibodies

Keywords: *bispecific antibodies, monoclonal, immunoglobulin*

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Please cite this article in press Pooja Nair K R et al., ***A Review On Bispecific Antibodies.,*** *Indo Am. J. P. Sci, 2019; 06(11).*

INTRODUCTION:

Antibodies are widely recognized for their therapeutic potential and subsequently have prompted a lot of interest into their development and application. Progress in antibody engineering has led to the generation of many different types of antibodies that differ in size and shape, including bispecific antibodies.

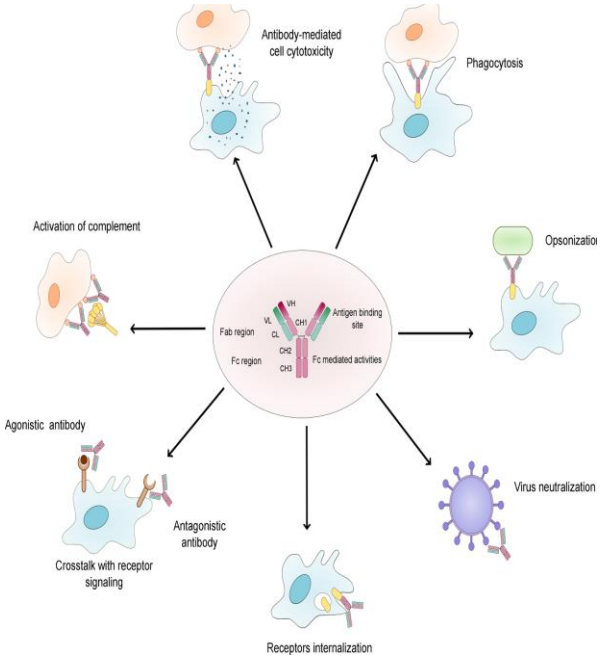

Figure. I.I. Overview of the natural function of antibodies.

The figure shows the main functions of antibodies. The structure of monoclonal IgG antibody is represented, with the heavy chains in rose and the light chains in green. The constant domain is represented in bright colours and the variable domain in darker colours.

Bispecific antibodies are artificially designed molecules, capable of simultaneously binding two different antigens, hence they can be applied to redirect effector cells to tumor cells. Currently, two large classes of bispecific antibodies have been developed : immunoglobulin – like and small bispecific antibodies.[2]

Even though bispecific antibodies have similar characteristics as monoclonal antibodies, they remain an artificial molecule that cannot be produced by normal B-cells. Bispecific antibodies was initially developed through hybrid-hybridoma chemical linkage or renaturation from purified recombinant Fab from bacterical inclusion bodies.(3) The three methods by which bispecific antibody can be obtained. The first generation of bispecific antibody was produced either by chemical coupling of different immunoglobulin Fab' fragments at the hinge region or by cell fusion of two hybridoma cell lines resulting in a quadroma cell which secretes among other immunoglobin combinations also bispecific antibody. Advance in genetic engineering has facilitated the creation of second-generation bispecific molecules of different size and binding strengths. (4)

The antihuman construct was generated by means of agarose immobilized pepsin digestion of mouse anti-human IgE and mouse anti-human CD300a, according to the manufacturer's instructions. The resulting $F(ab')^2$ fragments were reduced to Fab' by means of 18 hours of incubation in reduction buffer. The product of each stage was filtered and purified by means of centrifugal gel filtration, and the entire process was sampled to be monitored by means of SDS-PAGe and spectrophotometry. (5)

The key function of a bispecific antibody for tumor therapy is of course the recruitment of immune effector cells to the tumor site Depending on the type of effector cell additional functional requirements have to be fulfilled under optimal conditions, the tumor cell will be finally destroyed by the common mechanism of necrosis or apoptosis. Bispecific antibody from hybrid hybridoma display the same size and high stability as IgG antibodies. Thus their serum halflife and tissue penetration is comparable. To avoid interaction with Fc receptor bearing cells and to increase tumor accessibility $F(ab')^2$ fragments were produced by enzymatic digestion.(6)

In general bispecific antibody designed to redirect the immune system against cancer have an extensive history marked by several short coming. Whereas some alternative bispecific antibody constructs have resulted in prohibitive toxicity owing to non-specific T cells activation, others have been hampered by low potency.

With the advances in genetic engineering bispecific antibodies have experienced a revival and regained the attention of biopharmaceutical industry. The current engineering methods have yielded over 20 bispecific antibodies in clinical trials and only one has been approved for cancer immunotherapies. The majority of the bispecific antibodies in clinical trails are triomabs or small biles. Most of the developed bispecific antibody in clinical trails have very efficient anticancer effect. [7]

The main interesting bispecific antibody is due to their portential to engage immune effector cells such as NK cells and T cell, to promote tumor cell destruction. T cell are known to play a key role in the immunosurveillance and anti tumor immunity thus their engagement is essential in the cancer immunotherapies. This is an important advantage of bispecific antibody over classical IgG antibodies, since T cells do not possesa Fc receptor and they cannot be recruited by MABs. Further advantages of bispecific antibody ove monoclonal antibody is blocking two pathways in parallel to impair resistance formulation. In addition to the purpose of bispecific antibody for cancer immunotherapies, bispecific antibody can be used for virus neutralization (HIV), and as a treatment for inflammatory diseases. Recently new application for bispecific antibodies were established such as gene mediated therapy and immunodiagnostic application.[8]

BISPECIFIC ANTIBODY

Bispecific antibodies are artificially designed molecules, capable of simultaneously binding two different antigens, hence they can be applied to redirect effector celles to tumor cells. Currently, two large classes of bispecific antibodies have been developed: immunoglobulin-like and small bispecific antibodies [1,9]

The most widely used application of this approach is in cancer immunotherapy, where bispecific monoclonal antibodies are engineered that simultaneously bind toa cytotoxiccell (using a receptor like CD3) and a target like a tumour cell to be destroyed.

STRUCTURE OF BISPECIFIC ANTIBODY

A first-generation bispecific monoclonal antibody, called trifunctional antibody, has been developed It consists of two heavy and two light chains, one each from two different antibodies. The two Fab regions (the arms) are directed against two antigens. The Fc region(the foot) is made up from the two heavy chains and forms the third binding site; hence the name.

Other types of bispecific antibodies have been designed to overcome certain problems, such as short half-life, immunogenicity and side-effects caused by cytokine liberation. They include chemically linked Fabs, consisting only of the Fab regions, and various types of bivalent and trivalent single-chain variable fragments(scFvs), fusion protein smimicking the variable domains of two antibodies. The furthest developed of these newer formats are the bi-specific T- cell engager (BiTEs)[10] and mAb2's, antibodies engineered to contain an Fcab antigen-binding fragment instead of the Fc constant region.

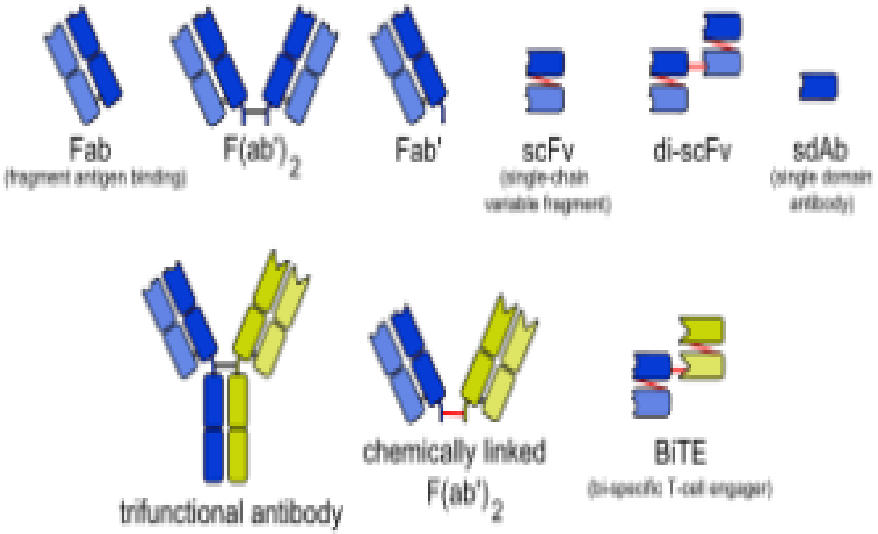

Fig: 1.2: Three types of bispecific antibodies: trifunctional antibody, chemically linked Fab and bi-specific T-cell engager (bottom row). Parts of the two different antibodies are coloured blue and green, respectively.

MECHANISM OF BISPECIFIC ANTIBODY

Of the two paratopes that form the tops of the variable domains, one can be directed against a tumour antigen and the other against a T-lymphocyte antigen like CD3. In the case of trifunctional antibodies, the Fc region additionally binds to a cell that expresses Fc receptors, like a macrophage, a natural killer cell or a dendritic cell. In sum, the tumour cell is connected to one or two cells of the immune system, which subsequently destroy.[11]

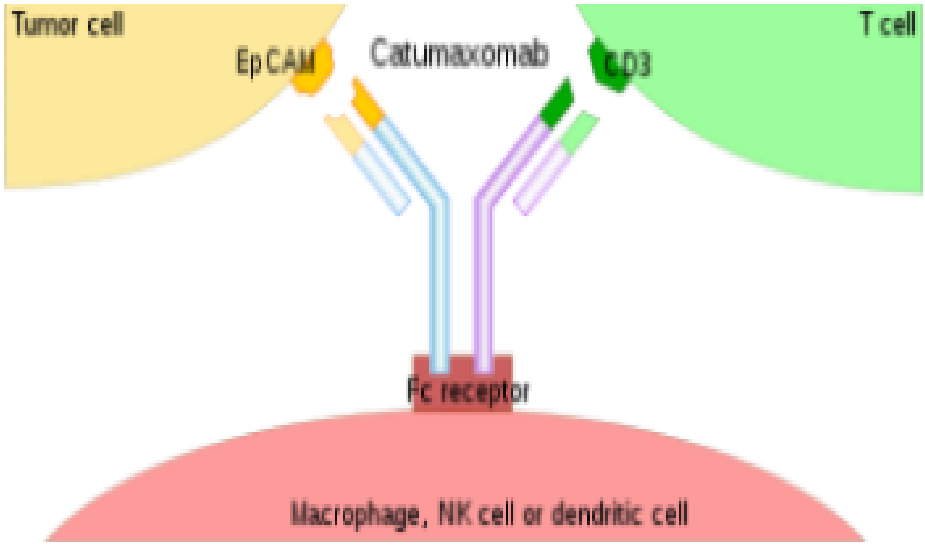

Fig: 1.3: The mechanism of action of a Bispecific monoclonal antibody, exemplified by catumaxomab, representing the first approved bispecific trifunctional antibody.

ADVANTAGES OVER ORDINARY MONOCLONAL ANTIBODIES

Cancer immunotherapy with ordinary monoclonal antibodies does not activate T-lymphocytes because this type of cell does not possess Fc receptors, so the Fc region cannot bind to them, and the Fab regions are already used for binding the tumour cells. Bispecific antibodies have a higher cytotoxic potential. They bind to antigens that are expressed relatively weakly. The effective dose is around 0.01 mg m-2 d -1 (milligrams per square metre body surface area per day), several orders of magnitude lower than with ordinary antibodies. Additionally, targeting more than one molecule can be useul to circumvent the regulation of parallel pathways and avoid the appearance of resistance to the treatment.[11]

Under the optimal conditions, the tumor cell will be finally destroyed by the common mechanisms of necrosis or apoptosis. Bispecific antibody from hybrid hybridoma display the same size (MW approximately 150kDa) and high stability as conventional IgG antibodies. Thus, their serum half life and tissue penetration is comparable. To avoid interaction with Fc receptor bearing cells and to increase tumor accessibility $(ab)^2$ fragments were produced by enzymatic digestion.[11]

PRODUCTION AND PURIFICATION

The design and Engineering of IgG like Bispecific Antibodies

- **Quadroma (Hybrid Hydromas) approach**

The quadroma technology consists in constructing and producing bispecific antibodies bysomatic fusion of two hybridoma cells (obtained by a somatic fusion of an antibody producing lymphocyte and a myeloma cell). Both hybridoma cells express monoclonal antibodies with different specificities, the ones desired for the bispecific antibody. The quadromas cell line from the two fused hybridomas secretes the two antibodies including the bispecific antibody with two distinct arms. However, this method has not been adequate to produce bispecific antibodies in a quality and quantity required for there therapeutic use, because of random chain association. The random pairing of the light and heavy Ig chains, resulted in less then 1/10 of functional bispecific antibody, hence, the bispecific antibody purification has been complicated. To overcome this chain

association issue, a chimeric quadroma was created from a murine and a rat hybridome cell line.

- **"Knobs into Holes" approach**

The "knobs-into-holes "approach is based on a transfection of modified human genes coding for theantibodies, in mammalian cells. The advantage of this method is that the resulting bispecific antibodies are of human instead of murine/rat nature, and consequently they are less immunogenic. The production of bispecific antibodies with the "knobs into-holes" strategy is based on a single amino acid substitution in the opposite CH3 domains, that Promotes heavy chain heterodimerization. In one of the heavy chains referred as a "knob" variant, a small amino acid has been replaced with a larger one (T366Y) in the CH3 domain. Subsequently, in the other heavy chain, a large amino acid has been replaced with a smaller one (Y 407T). A sort of a hole is formed, permitting the interaction with the 'Knob' variant miming a key-lock concept. Furthermore, to optimize the heavy chains association as well as the stability of bispecific antibodies, six mutations were introduced two in the "knob" heavy chain (S354A, T166W) and four in the "hole" heavy chain (Y349C, L366S, L368A, Y407V).Moreover, the heterodimeric Fc part can be further stabilized by an additional disulfide bridge created artificially. The co-expression of the two variants of the heavy chains is sufficient to allow efficient production of bispecific antibodies, with more than 90% correct chain association. While homodimerization between the "hole" variants occurs, no homodimerization between the "knobs" variants can be observed. Problems arising from the fact that the light chains have no preference for one of the two heavy chains can be solved by applying antibodies with a common light chain sequence.

- **Cross Mab approach**

The Cross Mab approach has been described as an option to ensure correct light chain association in bispecific IgG-like antibodies when combined with the "knobs-into-holes" approach. In this structure, one of the antibody's arms is untouched, whereas in the opposite hand the heavy and the light chain are modified. Three different modifications are proposed:

(1) The whole Fab region
(ii) The VL-VH region
(iii) The CL-CH1 region.

Consequently, the modified light chain can no longer associate with the unmodified heavy chain hence, the correct chain association is enforced. From the three suggested models, the CL-CH1 bispecific antibody showed the best profile and purity. Importantly, with the CrossMab method, there is no modification of the antigen-binding specificity, since the only difference from a conventional antibody is the connection to the Fc part.

- **Dual – Variable-Doman Immunoglobulin approach**

The dual –variable-domain immunoglobulin bispecific antibodies are generated by combining the variable domains of two pre-existing monoclonal antibodies with different specifities. The variable domains of the two monoclonal antibodies are fused in tandem via naturally occurring linkers, allowing to create a dual specific IgG-like molecule. The DVD-Igbispecific antibody preserve the affinities of both monoclonal antibodies, indicating that each antigen binding site can function independently without posing significant steric hindrance. In addition, despite the cell surface molecules, the DVD-Igbispecific antibody can target soluble molecules, such as interferons, interleukins, chemokines. Moreover, with the DVD-Ig technology, it is possible to combine monoclonal antibody of different nature (human, chimeric, murine). An optimized DVD-Igbispecific antibody has many desirable properties such as easy purification to homogeneity using standard approaches, good pharmacokinetic properties, and amenability to large scale manufacturing.

Diabodies are group of small bispecific antibodies generated with DNA recombinant technology, which consist of two VH and VL domains of two different antibodies. In this design, each VL domain is cross linked via short peptide linkers with the VH domain of the other antibody. Since, chains from the same antibody tend to dimerize when co-expressed in the same cell, the production of bispecific diabodies requires the following arrangements :VHA-VLB and VHB-VLA (VH-VL configuration) or VLA-VHB and VLB-VH(VL-VH configuration). Moreover, the linker should be of small size (five amino acids), positioned between the VH and VL domains in a way that these domains will be forced to associate with the complementary domains of the second antibody. Thus, a diabody is created with two antigen-binding sites. By over expression of VH-VL fused domains in bacteria (E.Coli), soluble diabodies can be produced.

- **Bispecific T-cell Engager Antibodies**

Bispecific T-cell engager antibodies(BiTEs) are single chain antibodies designed for polyclonal activation and redirection of cytotoxic T-cells to tumor cells. BiTEs combine the minimal antigen-binding domaiof two monoclonal antibodies, fused with a short flexible linker. One of the antibody's

arms recognize CD3, a cluster of differentiation for T-cells, and the other one detects tumor cells.

BiTEs Antibodies have a high potential to activate T-cells. However, in order to fully activate the T-cell, an interaction between the T-cell and a cluster of BiTes the surface of the target cell is necessary. The small design of BiTEs antibodies is optimal to enable an interaction between both cells, ensuring the formation of a lytic immunological synapse. Using this approach Blinatumamab (M103), CD19-specific BiTE antibody has been developed for treatment of B cell malignancies. Currently in phase II of clinical trial, Blinatumamab has shown outstanding results for the treatment of patients with non-Hodgkin's lymphoma.[12]

THERAPEUTIC BENEFITS OF BISPECIFIC ANTIBODY

There are a variety of potential mechanism for the use of antibodies as therapeutic specific components of tumor development, such as angiogneneis, growth factor receptors, or ligand-receptor interaction, or via directly killing tumor cells by activating death receptor pathways. Additionally, antibodies can invoke an immune response by inducing complement-cytotoxicity or antibody dependent cellular cytotoxicity. They can also inhibit tumor progression by directly inducing apoptosis or by preventing the expression of proteins that are necessary for tumor development. For example, the cd4 antigen has been used to block the function of specific molecule without killing the cells. This co receptor, along with the T cell receptor complex, can bind MHC class 2 molecules, which initiate the immune response. Antibodies against CD4 can promote the tolerant state in T cells to suppress immune aggression and control T cell during the post transplantation period. The antibodies against interleukin and transferrin receptors were also able to inhibit growth in factor dependent myeloma cells.[13]

Monoclonal antibodies to a human cell surface antigen and anti APO-1 that gave a cytotoxic effect similar that of and tumor necrosis factor. The cell killing was independent of complement, and relied on the activation of the Fas/CD/APO-1 death receptor. The FAS antigen is part of the TNF receptor family.

The concept of using bispecific antibodies for cancer immunotherapies was conceived more than 20 years ago, when it became apparent that single targeting is often insufficient to enhance tumor cells destruction. However, the initial clinical studies with bispecific antibodies were rather disappointing due to the low efficacy, stability and immunogenicity. The deeper understanding in cell biology and immunology and the concomitant development of antibody engineering led to the production of new classes of bispecific antibodies with better pharmacokinetic properties. The major issue that has been overcome in these past few years have been the ability to produce pure product on a large scale - sufficient for clinical testing, and with good drug-like properties and stability. Immunogenicity remains an important issue to be resolved. Regarding the IgG-like bispecific antibodies, having an arrangement that is as close as possible to conventional IgG antibodies with less linker or additional domains, is desirable. Obtaining a closer traditional IgG format should also decrease any possible secondary adverse effects. Although IgG-like bispecific antibody exhibit appropriate stability and effector functions, their large size limits tissue penetration. On the contrary, small bispecific antibodies exhibit short half-life, and therefore further work needs to be done to increase their serum half-life.[14]

With the advances in genetic engineering, bispecific antibodies have experienced a revival and regained the attention of the biopharmaceutical industry. The current engineering methods have yielded over twenty bispecific antibodies in clinical trails and only one has been approved for cancer immunotherapies. The majority of the bispecific antibodies in clinical trails are Triomabs or small BiTes. Most of the developed bispecific antibodies in clinical trial have very efficient anti-cancer effect, and are promising to receive the FDA approval.[14]

The main interest in bispecific antibodies is due to their potential to engage immune effector cells, such as NK-cells and T-cells, to promote tumor cell destruction. T-cells are known to play a key role in the immunosurveillance and antitumor immunity, thus their engagement is essential in cancer immunotherapies[15]

This is an important advantage of bispecific antibodies over classical IgG antibodies, since T-cells do not possess a Fc receptors and they cannot be recruited by mAbs. Further advantage of bispecific antibodies over monoclonal antibodies is blocking two pathways in parallel to impair resistance formation (for example, receptors tyrosine kinases and angiogenic ligands). In addition to the purpose of bispecific antibodies for cancer immunotherapies, bispecific antibodies can be used for virus neutralization (HIV), and as a treatment for inflammatory diseases. Recently, new application forbispecific antibodies were established, such as

gene mediated therapy and immunodiagnostic applications.[15]

APPLICATIONS OF BISPECIFIC ANTIBODIES

For the clinical use of bispecific antibodies, human immunoglobulins would be preferred. Human hybrid hybridoma cell lines are difficult to produce and may have the same theoretical and technical disadvantages as the murine hybridoma cells. Genetic manipulation by introducing sets of chimaeric immunoglobulin genes into myelomor hybridoma cell lines is an alternative. The new techniques of antibody engineering which may revolutionize the monoclonal antibody technology will be a powerful tool for the production of 'tailormade' bispecific molecules[15]

- **Immunoassays:**

The effector binding arm can be designed to have specificity for marker enzymes or other indicator systems. The anti-target anti-peroxidase bispecific antibodies which have been used in immunohistochemistry have led to improvements in sensitivity, signal-to-noise ratioand simplification of staining procedures with preservation of fine ultrastructural detail. These reagents may also simplify or improve diagnostic techniques, such as in single –step immunoassays and other assay systems.

- **Tumour targeting**

The use of bispecific antibodies for immune diagnosis and therapy has shown some encouraging results. They have been used for delivering effecotr substances such as toxins and cytotoxic drugs to tumours and some are now in clinical trials.

- **Cross-linking of cellular antigens and focusing of effector cells:**

Many efforts have been made to use bispecific antibodies to focus cytotoxic effector cell response to tumour targets. This system has been studied either invitro and invivo, in animal and human models, using both the heteroconjugates and the hybrid bispecific antibodies. Several effector binding specificities were used. These included antibodies to Fc receptor, T cell receptor/CD3 complexes, and CD2 molecules. Bispecific antibodies which bind to target cells can activate effector cells, and cross-link the targets to the effector cells. Lysis of virus-infected target cells has also been observed. The use of bispecific antibodies may not simply serve to glue the targets and effector cells together, but may also trigger the cytolytic process. Cytotoxicity has been shown not to be due to bystander lysis, since direct contact between effector and target cells is required. Most of the experiments on effector cell targeting were performed using homologous effector cell populations, such as cloned T cells. The mechanisms of cytotoxicity in vivo may be different and may involve several killing systems. Destruction of the putative effector cells, possibly due to the fact that the bispecific antibody-bound effector cells may themselves serve as targets for antibody dependent cell mediated cytotoxicity, has been observed. It seems that mixed isotype bispecific antibodies, such as ratIgG2b-IgG2c, which can mediate cytotoxicity of target cells by non-antibody-dependant celllular cytotoxicity mechanisms, may minimize this problems.[15]

- **Specific deliver of effector compounds to targets:**

Targeting toxic compounds to tumours has been investigated by using anti- CEA –anti-vinca alkaloid bybrid bispecific antibodies. Radiolabelled vinblastine sulphate was localized at the tumour sites when injected with or after the bispecific antibodies. The background radiation in other organs such as liver and spleen was low compared with the radiolabelled drug alone. Therapeutic data produced in the in vivo mouse xenografted model indicated that t his method was more effective in suppressing tumour growth than the vincaalkaloids when given as free drug. A study using anti-idiotype-anti-sapornhetero conjugates for treatment of lymphoma also showed encouraging results. Clinical studies using heteroconjugate bispecific F(ab)2 anti-CEA-anti BLEDTA IV, an In-I II benzyl EDTA derivative of cobalt bleomycin, injected into patients with colon cancer 24-120 hours before the injection of "In BLEDTA, showed good tumour targeting with low uptake by liver and bone marrow.

A multi-stage delivery system using bispecific antibodies may have a disadvantage since its effectiveness relies on the two antigen-antibody interactions, between two arms of bispecific antibodies and both target and effector molecules. This potential problem may be overcome by the use of high-affinity bispecific monoclonal antibodies. The bispecific anibody must also be accessible to the effector molecules on the surface of target cells. This problem may not occur with the monovalent bispecific antibodies. Bispecific antibodies can be used to distinguish cells that coexpress two different surface antigens. Anti-CD3-anti-CD4 and anti CD3-anti CD8 bispecific antibodies were shown to promote complement mediated lysis of target cells that express both the relevant surface antigens 25 to 3125 times more efficiently than those expressing only one of the antigens. Several other systems have also been studied, such as direct targeting of tissue

plasminogen activator (tPA) by anti tPA anti-fibrin bispecific antibody to enhance thrombolysis. Anti-interferon (IFN)-anti-target cell heteroconjugates were shown to deliver IFN specifically to target cells and also inhibit their growth in vitro. In addition to the purpose of bispecific antibody for cancer immunotherapies, bispecific antibody can be used for virus neutralization (HIV), and as a treatment for inflammatory diseases. Recently new application for bispecific antibodies were established such as gene-mediated therapy and immunodiagnostic application [15]

CONCLUSION:

Bispecific antibodies are artificially designed molecules, capable of simultaneously binding two different antigens; hence, they can be applied to redirect effector cells to tumor cells. Bispecific antibodies have similar characteristics as monoclonal antibodies, they remain an artificial molecule that cannot be produced by normal B-cells. Bispecific antibodies can be obtained by different biochemical methods such as chemical conjugation of two antibodies, fusion of two antibody producing cell lines, or genetic approaches resulting in recombinant bispecific antibody potential of bispecific antibodies, a lot of work is required to manage the increased complexity involved in their design molecules. While many challenges remain, these antibodies have promising clinical applications, especially in cancer immunotherapies. Targeting two antigens simultaneously is a promising approach in blocking tumor latent escape pathways from single target inhibition.

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