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Chapter

Targeted Cancer Therapy Using Nanoparticles and Antibody Fragments

Sankha Bhattacharya and Kapil Gore

Abstract

Cancer is caused by an uncontrolled cell division, forming a tumor capable of metastasis. Cancer is the second leading cause of death worldwide. Conventional treatments kill healthy cells, causing side effects. Recently, nanomaterials are explored due to properties such as as- nano-size, high loading, and ligands' attachment for a selective delivery. Apart from normal body cells, cancer cells express many receptors in excess, which serve as 'targets' for attacking the cells. Various ligands like proteins, peptides, polysaccharides can be attached to nanoparticles to allow proper and specific reach to the tumor. Such nanoparticles go to their desired site and stick onto the receptors, taken inside the cells by various methods. Antibodies are natural proteins that bind to foreign substances and remove them. IgG being the most explored antibody, suffers from many disadvantages such as non-specificity for required antigen, limited binding sites, low tumor penetration. Hence many researchers experimented by removing and adjusting the binding sites, using only the binding sites, enhancing the valency of naturally available IgG. It gave many benefits such as enhanced penetration, reduced immunogenicity, better delivery of drugs with fewer side effects. Continuing advancements in the field of protein engineering will help scientists to come up with better solutions. The properties allow easy surface interaction and entry, achieve better biodistribution, and reduce the amount of drug required. Targeting is based on Paul Ehrlich's 'magic bullet, 'where the therapeutic moiety has two parts-one to identify the target and the second to eliminate it. This concept is revised to incorporate a third component, a carrier. Many nanocarriers can be used to target cancer cells containing ligands to identify malignant cells. Approaches to targeting are passive, active and physical targeting. Many such nanoparticles are in clinical trials and can be a better solution to cancer therapy.

Keywords: nanoparticles, cancer immunotherapy, targeted drug delivery, cancer, targeting, nanomedicine, antibody fragments

1. Introduction

For the innovative treatment of cancer, it is necessary to boost target-based cancer therapy, ensuring that it could differentiate between normal and cancer cells while targeting cells [1]. Targeted cancer therapies are far better than the conventional method [2]. Therefore, targeted cancer therapy enjoys lesser unwanted side effects and an excellent molecular mechanism, which promotes minimum toxicity

caused by chemotherapeutic drugs [3]. The rapid clearance from the body can be seen when the drug was administered in a higher tolerable dose, which ultimately leads to higher toxicity [4]. During targeted therapy, the drug could be modified to target biological transduction pathways and cellular factors. It also targets angiogenesis and apoptosis inducing molecules [5]. In recent years, several studies have been designed to investigate the effects of nanosized medicines inoperative targeting and diagnosis of cancer cells. Nanoparticles can possibly entrench drugs, theranostic agents, and genes [6]. It was also observed from the various research findings that, nanoparticular approach while drug targeting improves drug tolerability and bioavailability [7]. In formulation drug delivery, anchoring, fabricating, protection of payload from getting degradation by enzymes are possible [8]. The anchored nanoparticles can able to deliver a higher dose into tumor cells while bypassing the normal cells. The modified scaffold integration of nanoparticles facilitates biodistribution of specific drug delivery, which conjugates with ligands and eventually binds with tumor biomarkers [9]. Paul Ehrlich recently suggested a magic bullet, where two different targetings are possible with consistent therapeutic action [10]. In recent research articles and patents, it was often observed that many pharmaceutical carriers such as liposomes, micelles, polymeric nanoparticles designed from natural or synthetic sources were used to target chemotherapeutic medicaments in different cancer cells [11]. Many nanoparticles have passed phase II of the clinical trials stage. This suggests that effective active and passive targeting is possible, due to which greater specificity while selecting cancer target is achieved [12]. Nowadays, conjugation of antibodies, peptides, small chemical entities are versatile in delivering anticancer agents in the form of nanoparticle composite [13]. However, tumor targeting is not an easy job! Scientists are targeting tumors in three different mechanisms; (a) Where nanoparticles were pre-exposed with leaky vasculature of tumor cells and encountered with the reticuloendothelial system (RES) or enhanced permeability and retention (EPR) effects [14]. However, (b) active targeting is more advantageous, as inactive targeting, uncontrolled cell proliferative targeting of tumors, and pH and temperature-dependent targeting is possible. In physical targeting (c) pathological conditions such as pH and temperature play a key role. Nevertheless, targeting the tumor side also depends on the size of the nanoparticles. The nanoparticles, which are less than 7 nm, come under hydrodynamic diameters, easily passing through renal excretion [15]. The nanoparticles that are larger than 100 nm are eventually cleared from the circulation by the phagocytic system [16]. The nanoparticles' surface charge also plays a pivotal role, as the particles' cationic charge helps to facilitate internalization [17]. Sometimes surface addition of poly (sarcosine) and poly (ethylene glycol) [18] enhances the circulating half-life of the particles, on the other hand, preventing nanoparticles from getting engulfed by the reticuloendothelial system; by which accumulation of a certain amount of nanoparticles on the outer surface of the cancerous tissue is possible. To make nanoparticles more advanced, hooking ligands onto the nanoparticles' body facilitates internalization into cancer cells.

2. Molecular targets in cancer

To target cancerous cells, it is essential to target molecular aberrations. Effective nanoparticular therapy for cancer targeting relies on the ability to targets such genetic alterations to provide significant clinical benefits [19]. Nowadays, scientists are more focused on targeting p53, ALK PIK3CA, KRAS, G-NAQ, MET, BRAF, EGFR, CKIT genes, and certain pathways, i.e., PI3K/Akt/mTOR, etc. [20].

3. Ligands for cancer targeting

Ligands are a prerequisite for cancer. Recently, immunotoxin has obtained clinical approval from USFDA, and more than 100 ligand-targeted therapies are under clinical trials [21]. Newly developed phase-display techniques allow selective targeting with higher affinity. The bispecific antibodies and fusion proteins have been used for therapeutic purposes. Mostly the nanoreservior systems viz., niosomes, and polymeric nanoparticles are most suitable for ligand-based targeting [22]. However, pharmacokinetic behaviors and bio-distribution understanding of the molecules are still unknown. The principles of Ligands for cancer targeting can also be applied to the targeted delivery of gene medicines such as antisense oligonucleotides [23].

4. Attachment of ligands to carriers

Most of the nanoparticles as specially lipid-based formulations and polymeric nanoparticles are emerging as the best carrier system to deliver the molecule in cancerous tissues [24]. Monoclonal antibodies and peptides are possibly the best carriers. The surface-bound ligands specifically bind to the target cells. The various techniques viz., covalent and non-covalent techniques help in effective active targeting of cancer cells (**Figure 1**).

5. Receptor approaches of drug targeting in cancer

Receptor based targeting is being focused on ensuring the accurate delivery of carriers to their desired location [25]. It allows targeting not only to a localized tumor but also to traveling cancerous cells. This ensures precise delivery. Due to the excessive expression of receptors, targeting becomes easy. Ligands carry out the functionality through active targeting [26].

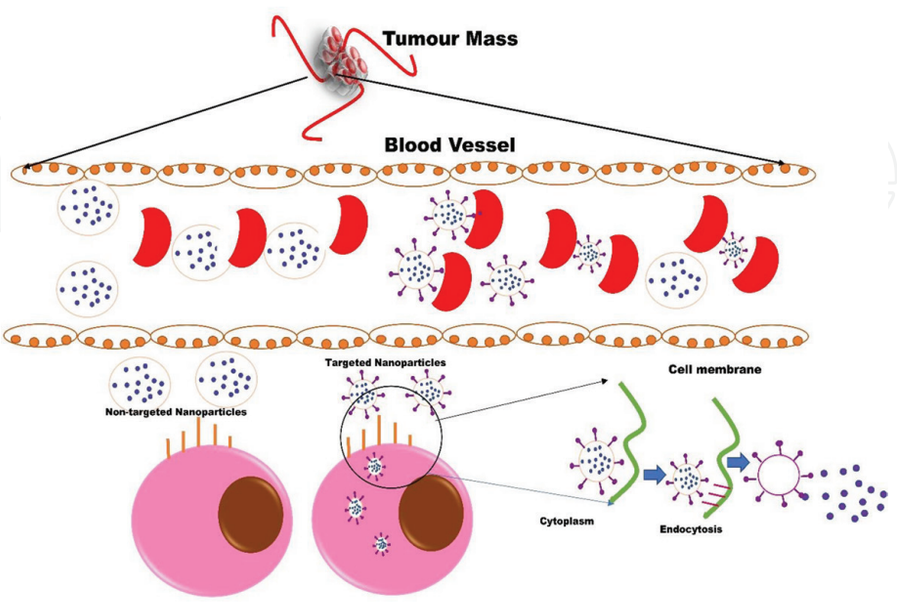

Figure 1.
Due to the active targeting, the nanoparticles are getting accumulated in the tumor site. Compered to non-targeted nanoparticles, actively targeted nanoparticles reach the tumor site with higher efficiency and through the endocytosis process, the nanocomposite triggers cancer cells death.

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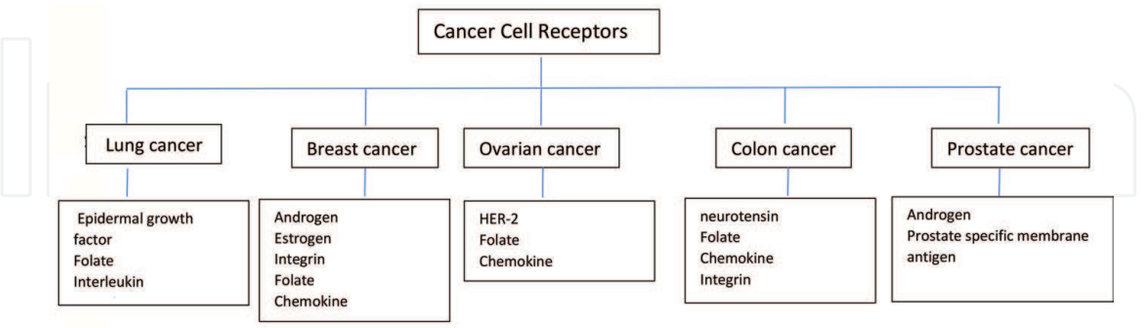

6. Receptors

6.1 EGFR

EGFR is a glycoprotein spanning across cell membranes. It is a target for Epidermal Growth Factor [28]. It initiates a signaling pathway leading to cell proliferation and mitosis. This is overexpressed in breast, colon, head and neck, ovarian tumors, and renal and glioma. The binding of antibodies against this receptor reduces cell proliferation. Many researchers prepared drug-loaded vesicles to target EFGR, which increased the drug's efficacy more than 10-fold [29]. Recently, cancer varieties like colorectal, lung, head, and neck carcinoma showed resistance to EGFR targeting due to mutations in the gene causing inhibition of activation and hence signaling.

6.2 Interleukin receptors

As interleukin receptors are majorly expressed, they are essential as a target for delivery. Among many varieties, types-3, 4, 6, 11, 13 & 16 were investigated as a target. The complex of ligand and receptor is taken inside the cell and hence helps cell growth and proliferation. But an overexpression may cause resistance to apoptosis and malignant growth (**Table 1**).

Interleukin type	Overexpressed in
IL-6	Ovarian cancer
IL-4	Glioblastoma, ovarian cancer, lung cancer, breast cancer
IL-3	Chronic Myelogenous leukemia, acute myeloid leukemia blast
IL-13	Head and neck cancer

Table 1.
Type of interleukin.

Various researchers used many ways to target IL receptors and obtained positive results such as reduced proliferation, enhanced uptake of carriers, decreased tumor volume, as well as accumulation and entry into the tumor as well as increased survival. This is not a full-proof strategy as blocking signaling of one type may be compensated with another type. And if the interleukins are blocked, it hinders the functioning of the immune system.

6.3 Folate receptors

Folate receptor is a glycoprotein present on the external cell surface. It is rich in cystine content. It has three subtypes- α, β and γ [30]. It is responsible for the entry of folic acid into cell, which is important for nucleic acid synthesis. The α subtype is overexpressed in breast, lung, colon cancer, and mesothelioma [31]. According to studies from many researchers, positive results such as a reduced tumor volume, nuclear delivery of therapeutic moieties, increased survival of cells, upregulation of cell death were observed. As folate receptors are present in the body on abundant sites, the selectivity may not always be achieved. Also, there are no animal models for therapeutic advantages or toxicity profiling.

6.4 Integrin receptors

Integrins are proteins that bind to the extracellular matrix and promote cell binding to tissues. Due to this action, this receptor is essential in the progression and metastasis of tumors. 'RGD motif' is a polypeptide chain important for binding and has been majorly exploited as a drug target [32]. Targeting the RGD motif has advantages such as improved survival, reduced tumor mass, and tumor growth inhibition. A major drawback of integrin targeting is diseases such as progressing lymphadenopathy [33].

7. Receptors to inhibit metastasis

7.1 The cluster of differentiation receptors

CD receptors initiate tumor development and can self-renew, overexpression of ABC, dormant. These features give tumor characteristics such as resistance, recurrence, and metastasis. Blocking these receptors prevents metastasis. CD subtypes 14, 22, 36, 44, 133 have been explored as drug targets for delivery. Targeting CD-44 receptors on cells allowed delivery into even the resistant tumors and showed enhanced cytotoxicity [34].

8.2 Estrogen receptors

These are nuclear receptors binding to the hormone estrogen. Estradiol acts as a transcription factor and controls cell growth. Estrogen helps in the development of sexual and reproductive functions, initiation and forming of lungs, mammary glands, and prostate gland. These receptors are excessively expressed in the majority of types of breast cancers, making it an ideal target. Initially, hormone analogs were used as ligands to target, but the binding strength was not optimal due to the modified structure of hormones. Also, another drawback was excessive RES uptake of such liposomes, which was overcome by formulating stealth liposomes. These formulations showed reduced tumor volume [35].

8. Targets for resistant tumors

8.1 Transferrin receptor

It is a receptor present on the cell membrane which is responsible for obtaining iron inside the cell. Iron binds to transferrin, and this complex binds to the receptor, which is then internalized [36]. Iron is necessary for many functions, such as DNA synthesis. This receptor can be targeted even if the tumor is Multi-drug resistant. Targeting this receptor has shown excellent results in resistant tumors such as increased formulation internalization, reduced drug required, higher cell death, and enhanced cytotoxicity [37]. This receptor's drawback is its presence at non-malignant sites such as endocrine glands may cause a loss in efficacy.

8.2 HER2

Human epidermal growth factor receptor-2 (HER-2) is a receptor spanning across the cell membrane with having a protein kinase internal subunit [38]. It is excessively expressed in gastric, lung, breast, and ovarian cancers. Overexpression of this receptor makes it challenging to forecast carcinoma. But, commercial products such as Herceptin have shown a promise targeting this receptor [39]. Targeting the HER-2 may help in cases of tumors showing resistance. This type of targeting has also demonstrated reduced cancer cell viability, enhanced cytotoxicity in resistant tumors, and increased uptake in the studies. A significant drawback is the absence of any natural ligand for the HER-2 receptor, which makes targeting difficult [39].

8.3 Antibodies for targeting cancer

Cancer cells are derived from normal body cells; hence they have a similar receptor constitution on the cell surface. Due to this lack of specialized markers, chemotherapeutic agents cannot differentiate between normal and cancer cells. Hence, they show toxicity. Keeping a low dose may result in resistance [40]. Therefore to achieve a specific targeting, antibodies specific for antigens presented by cancer cells can be targeted.

8.4 Engineering of antibodies

IgG is the majorly used antibody for targeting cancer [41]. Antibodies have 'Y' shaped structures where two arms have the sites for antigen binding. Monoclonal antibodies which are derived from a single clone of cells themselves or only the targeting fragments, can be used for cancer therapy. These agents bind the antigen and cause cell death by antibody-dependent toxicity, complement activation, or blocking signal transduction inside the cell. The antibody has a high affinity for its specific antigen and has excellent binding strength due to the presence of two binding sites. Entire antibodies may cause activation of the immune system inside the body. And due to their long half-lives, detection also becomes an issue. Using antibodies from species other than humans may cause severe allergic reactions. Hence to combat this situation, researchers have developed various molecules based on antibodies. Antibodies have been developed where binding sites from mouse have been attached to human antibody (chimeric antibody), humanized or human antibodies have also been developed. Researchers have separated the F_c fragments responsible for binding and have used them for targeting.

9. Effector mechanism of antibodies

Antibodies usually target antigens that are unique to cancer cells or which are excessively expressed on the cell surface. The majority of antibodies induce cancer cell death by binding to their target receptor, either blocking the receptor or changing the receptor's activation requirements [42]. They disturb signaling pathways responsible for cell growth and survival; hence, they end up killing the cells [43]. For example, Cetuximab is an antibody directed against EGFR. Epidermal growth factor upon binding to EFGR causes tumor growth, proliferation, and migration. EFGR is seen in many cancers. Cetuximab binds to EGFR and blocks the receptor hence preventing ligand binding and subsequent receptor dimerization. This process leads to apoptosis. Human epidermal growth factor receptor 2 is a member of the tyrosine kinase family. It is overexpressed in breast and ovarian cancers. The unique feature of this receptor is the absence of any known natural ligand. Instead, it forms dimers with other growth factor receptors and exerts its effects. Hence antibodies that are made to target this receptor prevent its dimerization with any other receptor and prevent survival.

Indirect action of antibodies involves host immune system participation and causes cytotoxicity by activation of complement system, antibody-dependent phagocytosis, and antibody-dependent cytotoxicity. Most antibodies are able to activate the complement system, which targets and destroys the cancer cell. Ofatumumab, an anti-CD20 antibody, intensifies the process of cytotoxicity through complement activation. Antibody-dependent phagocytosis occurs after a cancer cell opsonized by mAb attaches to a macrophage FcyRI glycoprotein. Then macrophage consumes such a marked cell [44].

10. Resistance to mAb

Even if mAb therapy is successful, many patients show resistance to it. The resistance may be innate or acquired after exposure to antibodies. Natural resistance is already present in mutations in cancer cells prior to the therapy, and acquired resistance is received after the exposure to therapy [45]. Another limitation is the dependency of the therapy on the overexpression of receptors. Mutations of receptors and the components in the signaling pathway may decrease the efficacy of antibody-targeted therapy. If cells express a variant of the receptor, therapy's potency may still decrease even if the binding site does not change [46].

11. Antibody fragments

Advancement in protein sciences has enabled scientists to produce antibody fragments with a smaller size but the same efficacy. The ideal characteristics of an antibody fragment are discussed in **Figure 2**.

In the beginning, proteolysis was the method of choice to produce smaller antibody fragments [47]. These fragments had a molecular weight of around 54 kDa–100 kDa (Fab, Fab_2). In the later stages, recombinant DNA technology was used to prepare univalent and bivalent fragments which had heavy and light chains of a variable section of antibody [48]. Such a structure was the smallest targeting unit to be generated. The two chains were joined with a flexible polypeptide linkage giving a 'single chain variable fragment' (scFv). It was convenient to use because of its small size and easy production.

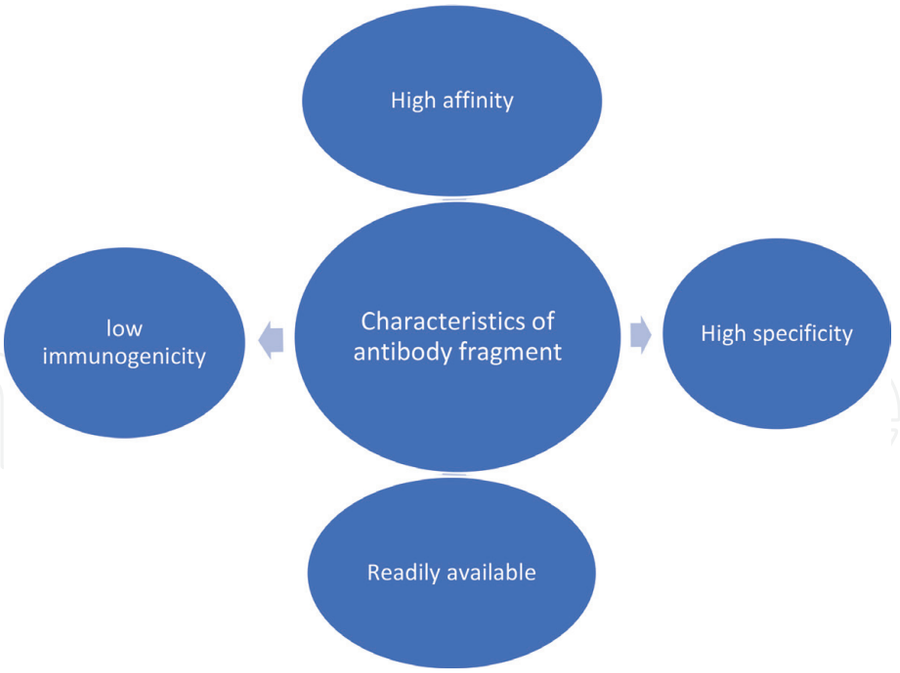

Figure 2.
Different characteristics of antibody fragments.

In the 1980s, researchers isolated and screened a heavy chain of the murine antibody for its binding to lysosomes [49]. It was called a single domain antibody' (dAb) as it contained only a heavy or light chain and had a meager molecular weight (15 kDa). However, it had drawbacks like poor solubility and aggregation, and a major issue was that the fragments did not retain the original's binding efficacy [49]. Components from animals such as camels, llamas, and fish such as sharks were used as more soluble, but they suffered from immunogenicity issues. Efforts like immunization and bioengineering to reduce agglomeration were carried out for effective use in therapy [50].

Later the above types were converted from univalent to multivalent through protein engineering, which was then used to target multiple entities at once [51]. These multivalent fragments show slower dissociation from the receptor and high functionality. One great example of multivalent fragments is a 'diabody' formed by linking light and heavy chain by a single chain variable fragment to be self-assembled into a dimer [52]. Diabodies have an advantage such as moderate molecular weight, multivalency which give them characteristics like improved penetration in tissue, rapid clearance. These diabodies bind to tumor antigen as well as to CD3 cells to kill tumors through T-cell mediated toxicity. The mini body is another type of synthesized antibody fragment, which is a pair of single chains of variable fragments interconnected by-CH_3 bonds, and a variable region specific for any antigen is attached to this pair. Minibodies are more suitable for targeted radiotherapy because they show better uptake and are cleared faster as compared to other types of fragments and are cleared rapidly. In the structure of the Mini body, the single variable region can be replaced by a cytotoxic agent, radioisotope, for its delivery.

Nanobody is the shortest antibody fragment. It is isolated from camelid heavy chains of variable antibody region. It is produced by making phage viral coat cover the desired fragment. As these antibodies do not have light chains, they are structurally different than normal antibodies. They have a concave antigen-binding region larger than other antibodies. Hydrophilic structures replace the usual hydrophobic amino acid residue. Such adjustments allow antigen-binding property even in the absence of light region. Another specific property is its ability to cross blood–brain barrier.

12. Application of fragments in imaging

12.1 Cancer imaging

Antibodies that bind to the target can be coupled with a radionuclide, fluorescent molecules to obtain images of cancer. Antibodies that have longer half-life need more time for imaging and may blur the image. These fragments have a short half-life and higher permeability, which allow easy detection. Techniques such as Positron emission tomography (PET), Computed tomography (CT), Single-photon emission computed tomography (SPECT) are now being performed where the antibody fragments are employed [53].

12.2 Nuclear imaging

Nuclear imaging is essential for the detection of cancer. Using fragments reduces nuclear exposure to radiation. The bifunctional connector connects antibody fragment and radionuclide, and such complexity easily accumulates at the tumor and gives clear visualization [54]. This method can be used to measure the absorption of drugs and the expression of receptors. One issue with the complex is increased radiation in the kidney due to complex breakdown and retention of radionuclide in the kidney [37].

12.3 Dual modality imaging

It involves non-invasive assessment of disease and fluorescence imaging of tumors during surgery. This technique shows accurate and reliable results [55]. Heterodimeric antibodies are used to target two issues at once and have a stronger affinity than homodimer.

12.4 Other imaging

Antibody fragments can be coupled with microbubbles, and it enhances the targeting efficiency [56]. Photoacoustic imaging also can be performed with antibody fragments to give high-resolution images. The laser causes expansion of tissues, and it produces sound waves, which later can be converted to images by the ultrasonic transducer.

13. Application in cancer therapy

13.1 Intrinsic therapeutic effect

The selection of antigen is most important for targeting. The target antigen is highly expressed in cancer cells but not on a normal cell. Single, as well as multiple targeting, can be achieved through the use of an engineered antibody. Multivalent antibodies not only block signaling but also overcome resistance through multiple targets [57].

13.2 Targeted drug delivery

The delivery of anti-tumor drugs using antibody fragments is a common practice of targeting medicine to the tumor [58]. Such systems ensure accurate delivery and improve the pharmacokinetics of agents. Effector molecules such

as siRNA cannot target and have very low uptake. Coupling them with antibodies shows enhanced tumor uptake and reduced side effects [59]. An example is CXC chemokine receptor siRNA delivered by coupling with anti-HER2 peptide fusion protein e23sFv-9R is used to target breast cancer cells, having an excess of HER-2 receptors, which promotes apoptosis. Immunotoxins also can be targeted for effective therapy. Immunotoxins from plants and animals may show a rapid immunogenicity and have toxic side effects that may be harmful to humans. For example, an immunotoxin was synthesized by a combination of *Pseudomonas aeruginosa* exotoxin and short-chain variable fragment, which reduced tumor cells' reduced survival and improved the survival rate. An antibody fragment can also be attached to nanoparticles, which then enter the tumor cells via receptor-mediated endocytosis. An example is the anti-HER-2 short-chain variable fragment attached to the PEG-coated iron oxide particle, which shows a better targeting and improved serum half-life.

13.3 Application in immunotherapy

Tumor cells have barriers to protect them from the body's immune system, such as an impenetrable stroma and immunosuppressive cells in the tumor environment [60]. Tumor cells block antigen overexpression and increase regulatory-T cells and hence escape the immune system of the body. Immunotherapy overturns this mechanism and brings about the death of cancer cells. Bispecific antibodies can target two antigens at once, including an antigen on the cancer cell and a receptor on the cell of the immune system. Then the proteins in cell death signaling are activated by phosphorylation, which then initiates apoptosis. Bispecific antibodies link two cells hence bypassing innate as well as acquired resistance.

Chimeric antigen receptor on T cells (CAR-T) is being focused on as a target of antibodies as it helps in specific recognition of cancer cells [61]. Upon activation, it shows a high amount of cytokine release, which then kills cancer cells. This also can help to circumvent tumor resistance for killer T-cells.

For immunotherapy, a specific target is required for the immune system to attack or else; there can be severe side effects related to immunity. Also, the extent of immune response should also be considered for the efficacy of the treatment. An immune response, which is too low may not be able to kill the malignant cells, and a very strong immune response may cause cytokine storms and harm the healthy cells instead.

14. Antibody derivatives

IgG is the most explored antibody of choice for cancer targeting. Antigen binding if is the desired mechanism; only antigen-binding fragment can be separated and used. In such cases, the binding affinity and crystallization properties remain the same with smaller size and hybridization, which give enhanced tumor penetration. Also, modifications can be made to increase the specificity and valency of antibodies.

15. IgG formats

Involves changes in the structure of IgG to alter its natural properties to obtain desired attributes. The most common structural change includes changes in antibody binding region, e.g., ranibizumab. Right now, there are many fragment-based

therapeutics in preclinical and clinical trials. The most advanced is otlertuzumab for treating chronic lymphocytic leukemia. Fragment-based derivatives are not able to activate the immune system without a crystallization region [62]. But they show advantages like enhanced penetration, longer circulation and increased diffusion in the tumor. Hybrids can also be made with Fc from IgG and scFv from a targeting antibody. They do not penetrate in the tumor but instead retain activities of Fc such as immunization.

16. Multivalent binders

IgG can only bind a single type of antigen. Hence it is bivalent but not bispecific. Hence, to increase the specificity, the valency of antibodies is being enhanced. Many different regions from different sources are connected together to form molecules that are multivalent and can bind to more than one type of molecules.

16.1 Bispecific

This is the most used type from multispecific modified IgG. These can bind to two different antigens at once. For e.g., blinatumomab is used for some types of acute lymphocytic leukemia. It binds to CD19 protein on the cancer cell and CD3 protein on T-cell. It causes the release of cytotoxic chemicals that kill the cancer cell. Dual affinity targeting agents are made from two separate light and heavy regions linked by disulfide bonds after translation.it can be stored for a long time and should be stable for a long time [63].

16.2 Multispecific and multivalent

A combination makes multivalent antibodies of three or more antibody domains joined sequentially or in a row. These are proteins specific for two antigens containing two pairs of light and heavy variable regions connected in a single chain forming a polypeptide. By virtue of their multivalency, tandem Abs not only target tumors but also cause infiltration and destruction of tumors by killer T-cells. These have longer storage life, better pharmacokinetics, are highly potent [64].

17. Antibody conjugates and fusion

Antibodies can be linked with other molecules such as proteins and peptides to form hybrids. Such combinations give altered biodistribution by targeting a different receptor, extend the half-life of formulation, or impart a new mechanism of action [65].

18. Antibody-drug conjugates

These hybrid molecules contain antibodies linked to a therapeutic agent. They combine the selective nature of antibodies with cytotoxicity of active agents [66]. It can reduce side effects to a minimum and shows maximum therapeutic success. Conjugates such as Mylotarg have been approved by the FDA. This has caused the emergence of many compounds that differ just by changing the method and site of conjugation. The drugs or linkers are attached to free lysine or cysteine, which gives different products based on the location of conjugation. The most recent trend

involves forming a homogenous product by conjugation at particular sites only. It can be done by altering the structure of antibodies to incorporate non-natural amino acids, which act as a handle for attachment. Available tags have been discovered, such as sortase A tag, which attaches the drug to antibody at the glutamic acid site and attaches to therapeutic moiety through a polyglycine tag.

19. Antibody-fusion constructs

These are molecules synthesized using recombinant DNA technology. They combine targeting efficiency of IgG and various protein domains. This fusion affects selectivity and PK parameters extensively. Toxic proteins can be used to form immunotoxic compounds such as diphtheria toxin attached to antibodies. Dose-limiting toxicity and immunogenicity are the main challenges in the formation of such complexes. The most successful examples of protein fusion complex are

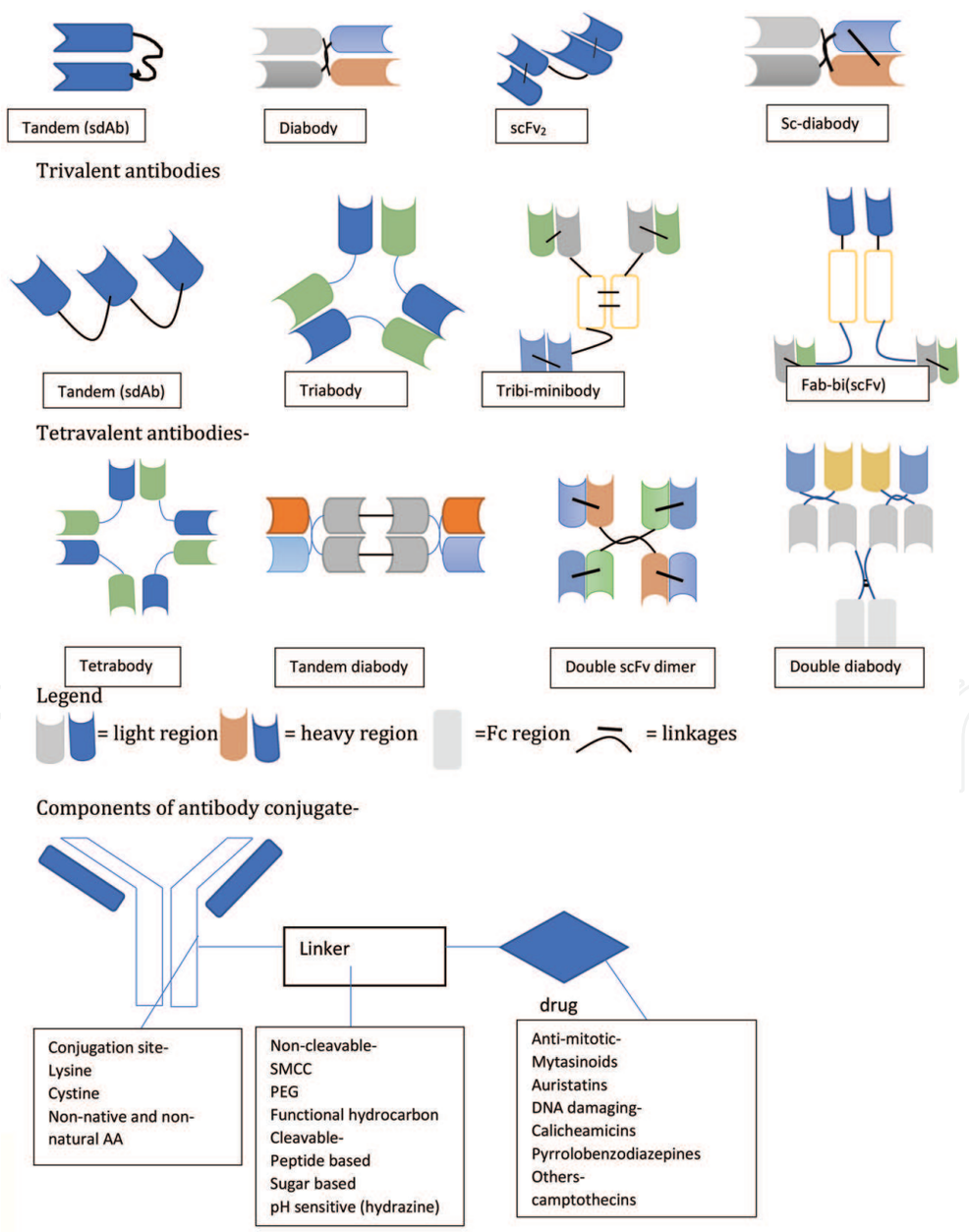

Figure 3.
Types of antibody fragments.

bioactive proteins attached to the crystallization region of IgG. Amevive and zaltrap are two such complexes approved by USFDA for release in the market. This combination shows an excellent immune response against the tumor [67].

Types of antibody fragments: Deferent type of antibody fragments are mentioned in **Figure 3**.

20. Conclusion

There is no single magic remedy that acts against all of the diseases. Antibodies are ingenious proteins targeted toward specific foreign molecules inside the body. Their therapeutic use involves their ability to disrupt signal transduction, block receptor, which further affects cell growth and cell death. They also can be used to stimulate the immune system so as to destroy any foreign or ingenious harmful materials. IgG's natural structure can be modified to remove problematic residues and add newly modified proteins for the purpose of targeting and immunostimulating. These attachments come with issues like immunogenicity, acquired resistance. But more research in field of antibody engineering is required to address this issue.

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