REVIEW ARTICLE

Engineering antibodies for cancer therapy

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In the past two decades, monoclonal antibodies have become one of the important therapeutic modalities for the treatment of cancer. Antibody engineering has immensely contributed to the development of antibody-based cancer therapy. The use of recombinant DNA technology has aided the molecular engineering of antibodies in various ways to improve their efficacy, and to counter various challenges posed in the rational designing of high-impact therapeutics for the treatment of a wide spectrum of malignancies. Such engineering of antibodies has been possible mainly due to their inherent plastic and multidomain structure, which makes it amenable to various modifications. This review highlights the advances in the field of antibody engineering which have enabled the use of antibodies for cancer immunotherapy.

Keywords: Antibodies, cancer, genetic engineering, immunotherapy.

THE discovery of hybridoma technology by Kohler and Milstein[1] paved the way for the production of monoclonal antibodies against a single antigen. Antibodies are gamma globulin proteins which are used by the immune system to identify and neutralize antigens. Antibodies recognize a particular antigen by virtue of their unique specificity, and tag the antigen for attack by other components of the immune system. Recognition of the antigen follows a series of reactions which destroy the antigen or the infected cell that presents the antigen. Antibodies achieve their toxic effects through antibody-dependent cell-mediated cytotoxicity[2] (ADCC) and complement-dependent cytotoxicity[3] (CDC). In ADCC, the antibody first binds to its target antigen present on the surface of tumour cells and the Fc portion is then recognized by Fc receptors present on the effector cells such as monocytes, macrophages and natural killer cells. Natural killer cells are the principal effectors of ADCC. ADCC is mediated by the release of cytotoxic granules such as perforin, granzyme, granulysin, etc., which form pores in the infected cells and cause apoptosis. Additionally, the release of cytokines and chemokines inhibits cell proliferation and angiogenesis. Macrophages bearing Fc receptors on their surface bind and phagocytose antibody-coated tumour cells and promote ADCC through the release of proteases, reactive oxygen species and cytokines[4]. The outcome of the ADCC depends upon the isotype of the antibody; IgGs 1 and 3 bind strongly to the Fc receptor, whereas IgGs 4 and 2 display weak binding[5]. CDC is characterized by the activation of the complement pathway, also known as the complement cascade. The series of reactions in this cascade leads to the formation of a membrane attack complex which forms pores in the cell leading to cell death. CDC also depends upon the isotype of the IgG. IgG3 followed by IgG1 are the most effective isotypes for stimulating the classic complement cascade. IgG2 antibodies are less efficient in activating the complement cascade, whereas IgG4 is unable to do so[6].

Antibodies used in immunotherapy mainly exert their activity by blocking ligand–receptor interactions and thereby triggering an intracellular signal which may lead to cell death. Alternatively, binding of an antibody to a target may block a signalling event crucial for metastasis. The recombinant DNA technology has facilitated the engineering of antibodies in a variety of ways. These include engineering to modify their serum persistence, effector mechanisms, introducing new effector functions, humanization and altering size. We discuss, in this review, the various antibody engineering methodologies along with their applications.

Modification of antibody-mediated effector mechanisms

Antibody mediated effector mechanisms involve ADCC, CDC, phagocytosis and serum clearance. In a unique approach to augment CDC, Natsume *et al.*[7] shuffled the constant domains of IgG1 and IgG3 to generate a comprehensive set of mixed chimeric isotypes of anti-CD20 antibodies. They found that one of the variants, namely 1133 consisting of the CH1 and the hinge each from IgG1 and Fc from IgG3, showed strong CDC that exceeded wild-type levels[7]. In another study, it was demonstrated that removal of fucose from the Fc region of IgG increases ADCC[8]. Zhou *et al.*[9] have developed a rapid and simple method of producing non-fucosylated antibodies with increased effector function using kifunensine, a potent alpha-mannosidase I inhibitor. Using computational design, Lazar *et al.*[10] generated a battery of Fc variants with optimized Fc gamma receptor affinity. The designed variants displayed enhanced *in vitro* and *in vivo* cytotoxicity in a preclinical model[10]. McEarchern *et al.*[11] engineered a murine anti-CD70 mAb to contain human constant IgG1 domain. The engineered

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antibody induced Fc-mediated effector functions, including ADCC, CDC and antibody-dependent cellular phagocytosis *in vitro*. Administration of this engineered antibody prolonged the survival of SCID mice bearing $CD70^+$ disseminated human non-Hodgkin lymphoma xenografts. In a recent report, Zhukovsky *et al.*[12] employed the Xmab antibody engineering technology to a humanized anti-CD19 antibody to engineer a variant with 10- to 100-fold enhanced ADCC, which could be used in the immunotherapy of non-Hodgkin lymphoma. The Xmab technology employs the use of computational tools to engineer Fc domains which can be inserted in monoclonal antibodies raised against any antigen. Such antibodies exhibit increased stability and half life, and improved antibody-mediated tumour cell lysis. It is therefore amply clear that it has been possible to modify the antibody-mediated effector functions employing modern technologies for exploring these molecules for defined functions.

Introducing new effector functions

Antibodies can be conjugated or linked to cytotoxic drugs, toxins, radioisotopes and drug-activating enzymes to impart specificity to these molecules. In such molecules, antibodies act as carriers and deliver the cytotoxic molecule to the target cell without being invasive to the normal tissues. The idea behind linking antibodies to a toxic molecule is to endow the antibody with new effector functions, and impart specificity to the toxic component.

Conjugation with therapeutic drugs and toxins

Antibodies conjugate with cytotoxic drugs and toxins are referred to as immunoconjugates and immunotoxins respectively. An example of an immunoconjugate is Mylotarg which is an FDA-approved, humanized anti-CD33 IgG4 armed with a cytotoxic antitumour antibiotic, calicheamicin (CM), isolated from bacterium, *Micromonospora echinospora* subsp. *calichensis*. It is applicable in the treatment of drug-resistant acute myeloid leukaemia[13,14]. Boghaert *et al.*[15] have reported an immunoconjugate of CM and anti-5T4 antibody, raised against the oncofoetal protein 5T4, to be more potent than either the free drug or a non-binding control CM-conjugate in an *in vitro* study. *In vivo* studies demonstrated reduction of tumour growth upon treatment with the antibody drug conjugate[15]. Oflazoglu *et al.*[16] have demonstrated antitumour activity of a conjugate of tubulin inhibitor monomethyl auristatin E (MMAE) and chimeric anti-CD30 monoclonal antibody, cAC10 in a preclinical model of Hodgkin lymphoma. In a phase 1 clinical trial, Galsky *et al.*[17] showed a novel immunoconjugate, MLN2704 comprising of an antimicrotubule drug, maytansinoid-1, and prostate-specific membrane antigen-directed monoclonal antibody MLN591 to exhibit anti-prostate cancer activity. Patra *et al.*[18] have developed a nanoparticle-based targeted delivery system in which they have employed gold nanoparticles as a delivery vehicle, cetuximab as a targeting agent, and gemcitabine as an anticancer drug. *In vivo* application of this system resulted in significant inhibition of tumour growth[18].

Recombinant immunotoxins are fusion proteins comprising an antibody and a toxin. Most of the toxins employed are derived from bacteria or plants. The commonly used bacterial toxin is the *Pseudomonas* exotoxin A (PE), whereas Ricin and Saporin are well-documented toxins derived from plants. Table 1 gives a list of some immunotoxins developed using the naturally occurring protein toxins[19–30]. PE inhibits protein synthesis by ADP ribosylating the elongation factor 2. For more than two decades, Ira Pastan's group at NIH, USA, has pioneered in the development and application of PE-based immunotoxins for cancer therapy, ranging from *in vitro* studies to clinical trials. For a detailed account, the reader may refer to the reviews mentioned in the works of Pastan and coworkers[31–34]. The toxin in an immunotoxin, by virtue of its immunogenicity may elicit a humoral response in the subjects in which it has been administered with an antibody conjugate. This usually leads to a low cytotoxicity and serum half-life resulting in the subsequent clearance of the toxin. One approach of getting around this problem is PEGylation of the toxin, which involves modification of the toxin by conjugation with polyethylene glycol (PEG). PEGylation of toxins and therapeutic proteins results in shielding of immunogenic residues. This leads to a diminished immunogenicity in an otherwise immunogenic molecule, increased circulation in the blood and reduced renal clearance[35]. Wang *et al.*[36] demonstrated PEGylation of a chimeric toxin composed of TGFα and PE to significantly decrease its immunogenicity and toxicity. Tsutsumi *et al.*[37] made a mutant of a recombinant immunotoxin composed of a single-chain Fv fragment of the anti-Tac monoclonal antibody to the human IL-2 receptor alpha subunit fused to a 38-kDa fragment of *Pseudomonas* exotoxin. This mutant when PEGylated showed increased antitumour activity compared to the wild type, accompanied by a significant reduction in immunogenicity in an animal model. Filpula *et al.*[38] have modified a mesothelin targeted immunotoxin, SS1P, employing releasable linkages between the protein and PEG polymers. The releasable PEGylated immunotoxin displayed increased antitumour activity and led to complete tumour regression of a human carcinoma in mice.

Conjugation with radioisotopes

Radioimmunotherapy involves the use of antibodies as carriers for delivering toxic doses of a radioactive material to the target tissue, without being invasive to the

Table 1. Immunotoxins used in haematologic malignancies and solid tumours

Toxin	Receptor	Type of tumour	Reference
Pseudomonas exotoxin	erbB2	Human gastric cancer	19
Pseudomonas exotoxin	Human transferrin	Human epidermoid carcinoma	20
Restrictocin	Human transferrin	Leukaemias and carcinomas	21
Pseudomonas exotoxin	Her/neu	Solid tumours	22
Pseudomonas exotoxin	IL13	Glioma and renal carcinoma	23
Blocked Ricin	CD19	Lymphoma, leukaemia	24
Diptheria toxin	GMCSF	Acute myeloid leukaemia	25
Diptheria toxin	Interleukin 2	Cutaneous T-cell lymphoma	26
Deglycosylated Ricin	CD22, CD19	B-cell lymphoma	27
Ricin	CEA	Colorectal	28
Ricin	55 kDa antigen	Mammary carcinomas	29
Restrictocin	EGF	Leukaemias and carcinomas	30

normal tissue. Specificity is achieved by conjugating the radioisotope to an antibody against tumour-specific antigen; such conjugates are referred to as radioimmunoconjugates. Apart from their usefulness in the immunotherapy of malignancies, these conjugates are also applicable in diagnostic imaging of cancer. In practice, a radioimmunoconjugate is administered as an intravenous injection to the patient, which leads to a homogeneous distribution of the conjugate over the tumour. Radiolabelled antibodies have an edge over their unconjugated counterparts as they exhibit a bystander effect, i.e. in addition to killing the cell they are targeted to, they also lyse neighbouring tumour cells and unlike immunotoxins are non-immunogenic. Furthermore, they need not be internalized to kill a target. In deciding a regimen for radio immunotherapy, it is important to choose an antigen that is crucial for tumour survival, which would ascertain that the therapy kills only the desired target and the normal cells are unaffected. It has been observed that certain physical barriers restrict the access of monoclonal antibody to tumours by around 24–48 h after injection, in which case the bone marrow is exposed to emission coming from the circulating radioimmunoconjugate. A pretargeting approach, which takes the advantage of the dual specificity of an antibody, has been used to solve this problem. This was first shown by Reardan *et al.*[39], who demonstrated that it could be possible to prepare bispecific antibodies to a metal chelate as well as a tumour antigen. Essentially, pretargeting is a two-step process. First, a bispecific macromolecule, with affinity to a tumour antigen and a radioactive molecule is administered. This is done to maximize the concentration of the macromolecule in the tumour. Once this is achieved, the radioactive effector is injected. The success of this two-step process depends upon the efficient removal and clearance of the macromolecule from the blood and normal tissues, as the radioactive molecule will bind to the remaining traces of the macromolecule, leading to harmful side effects. This is often circumvented by a three-step approach in which a 'chaser' molecule is administered between the macromolecule and the radionuclide to remove the residual macromolecule[40]. The radioisotopes mainly used are alpha and beta emitters[41–43].

Antibody and enzyme conjugates

Antibody Directed Enzyme Prodrug Therapy (ADEPT) uses an antibody fused with an enzyme. The objective of this therapy is to localize therapeutic drugs to the site of the tumour without harming the adjoining tissue with any side effect. It is a two-step process in which the antibody enzyme conjugate is administered systemically near the tumour site, followed by administration of a drug in an inactive form. The enzyme component in the conjugate converts the drug to its active form, which becomes cytotoxic to the cancerous tissue[44]. An example of such a system is A33scFv::CDy, which is a recombinant fusion construct of anti-gpA33 single chain fragment, A33scFv, with cytosine deaminase from yeast (CDy), which converts 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU)[45]. This is essentially a deamination reaction which leads to the generation of 5-fluorouracil, a pyrimidine analogue which induces cell-cycle arrest and apoptosis in the targeted cells by inhibiting the ability of the cell to synthesize DNA[45]. In another example, a recombinant fusion protein composed of MFE-23, an anti-carcinoembryonic antigen (CEA) single-chain Fv fused to the amino terminus of the enzyme carboxypeptidase G2 (CPG2), has been constructed to achieve ADEPT in CEA-producing tumours[46]. The bacterial enzyme, carboxypeptidase G2 (CPG2), converts a prodrug, 4-[*N*,*N*-*bis*(2-iodoethyl) amino] phenoxycarbonyl L-glutamic acid (ZD2767P), into an active bifunctional alkylating drug (ZD2767). The drug can act by alkylating the DNA or by formation of cross bridges in the DNA[46]. The utility of ADEPT is documented in a number of *in vitro* models and clinical trials[47,48].

Engineering to modify serum persistence

Though it is possible to modify and introduce new effector function in antibodies by methodologies such as shuffling of domains and arming with toxic molecules respectively, such engineered molecules are often unstable and display short half-lives in the circulating serum. Therefore, it is important to modify antibodies to increase their persistence in the serum for improved clinical efficacy. Additionally, the use of antibodies with improved serum stability will reduce the frequency of administration to the patients, especially in a long-term therapeutic regimen.

In order to modify serum persistence, antibodies can be genetically engineered to regulate their half-lives in the serum. This has been achieved by mutating crucial residues in the Fc region of the IgG to increase or decrease the serum persistence of an antibody. The neonatal Fc receptor, FcRn, is responsible for the transport of immunoglobulin from the mother to the offspring. Homologous to MHC class1-like molecule, the FcRn is also responsible for regulating IgG homeostasis by rescuing IgG from intracellular degradation[49,50]. However, all IgG molecules cannot be salvaged by the FcRn, primarily due to either the low binding affinity of an IgG for FcRn or the saturating concentration of IgG for binding to FcRn. Ghetie *et al.*[51] used a random mutagenesis strategy to alter certain residues in murine Fc present in the proximity of the Fc–FcRn interaction site, to increase the serum half-life of IgG. In another study, a single amino acid substitution in the Fc region of a human mouse chimeric TNT-3 monoclonal antibody showed accelerated clearance and improved imaging in tumour-bearing mice[52]. Hinton *et al.*[53] also carried out the mutagenesis of several human IgG1 molecules and demonstrated that these mutants have a long serum persistence. In another recent study, Vaccaro *et al.*[54] engineered the Fc region of an IgG to block FcRn-mediated recycling of endogenous IgG in mice. Referred to as Abdegs, antibodies that enhance IgG degradation, they have application in the treatment of antibody-mediated diseases where self-reactive antibodies are involved.

Humanization of murine antibodies

The earlier efforts to combat malignancy using murine mAbs were often hindered due to rapid clearance of the murine antibody by human anti mouse antibody (HAMA) response[55,56]. This problem has been overcome to a great extent using recombinant DNA techniques, which enabled the generation of less antigenic forms of the murine antibody. Initially, chimeric mouse–human antibodies were developed that contained the antigen-binding regions of murine antibody linked with the constant regions of the human antibody. The chimeric antibodies showed greater serum stability and less antigenicity compared to the murine antibodies[57,58]. However, since chimeric antibodies still contain the variable region of the mouse antibody, human anti-chimeric antibody responses have been observed in patients[59]. To further counter the HAMA response, the concept of antibody humanization using complementarity determining region (CDR) grafting was introduced. As the name implies, CDR grafting involves the grafting of CDRs from a mouse monoclonal antibody into human variable regions, which are then joined to human constant regions to create a reshaped human antibody[60,61]. CDR grafting has been used widely for antibody humanization over the years[62–65]. With bioinformatics tools being available, the designing has become knowledge-based now. For example, Hou *et al.*[66] have humanized a mouse anti-human CD34 monoclonal antibody by CDR grafting. A molecular model of the antibody was used to identify framework region residues of potential importance to the antigen binding, which together with the mouse CDR residues were then transferred onto the framework of a human antibody selected based on homology to the mouse antibody to generate the humanized antibody. The resultant humanized antibody possessed antigen-binding affinity and specificity similar to that of the original murine antibody, suggesting that it might be an alternative to mouse anti-CD34 antibodies routinely used clinically[66]. Though the murine content after CDR grafting remains only around 5–10%, this was enough to elicit a variable immune response in some cases, accompanied with a low affinity for the antigen. CDR grafting as a method of humanization, therefore, became questionable after the observation that patients administered with CDR-grafted mAbs elicited feeble to strong immune response in clinical trials[67–69]. A close examination and molecular analyses of the CDR reveal that only 20–30% of CDR residues are critical in antigen recognition. These residues, located in the hypervariable regions of the antibody, are called specificity determining residues (SDRs). SDR regions have been mapped by analysing three-dimensional structures of antigen–antibody complexes[69] and incorporated for antibody humanization[70–72].

Humanization of therapeutic proteins can also be carried out by the process of de-immunization, which involves identification and removal of sequences within the therapeutic protein that will become MHC class 2 binding peptides[73]. A number of *in silico*-based strategies have been employed for the prediction of MHC class 2 binding peptides. The commonly used methods are RANKPEP[74] and PepDist[75], which provide peptide-binding data for 50 and 24 MHC class 2 alleles respectively. In the next step, targeted amino acid substitution strategies are employed to replace the immunogenic residues with non-immunogenic ones without compromising on the biological activity[76].

Resurfacing is another technique used to humanize monoclonal antibodies. Resurfacing, first described by

Padlan[77], involves changing the murine surface framework residues which do not occur in human immunoglobulins (Igs), into the most resembling human counterpart[77,78]. This technique of resurfacing an antibody is based on the observation that the antigenicity of a protein is determined by the accessible and protruding residues[79–81]. Known antibody structures are used for determining the surface-exposed residues in the variable regions of the murine antibody which differ from those usually found in human antibodies. These residues can then be modified by mutagenesis to change the pattern of surface-accessible residues homologous to the host residues[78,82]. Since there is no change in the CDR-framework interactions in the interior and only subtle changes are made on the exterior, this usually has only minor effects on the conformation and the activity of the resurfaced antibody.

In immunotoxins, a limited 'humanization' may be achieved using a humanized antibody and modifying the toxin by genetic engineering to overcome the problem of immunogenicity[83]. Recently, Gao *et al.*[84] have developed a novel bioconjugate consisting of *Pseudomonas* exotoxin-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles conjugated with Fab′ fragments of a humanized anti-HER2 monoclonal antibody. This conjugate displayed significant cytotoxicity, inhibited tumour growth in a xenograft model and was found to be weakly immunogenic when injected in experimental animals[84]. In a recent study by Onda *et al.*[85], a mouse model has been used to identify B-cell epitopes present in *Pseudomonas* exotoxin, PE38 portion of an immunotoxin. Mice were immunized with PE38-containing immunotoxin, and the resulting monoclonal antibodies were used to determine the epitopes on PE38. The residues present in the epitopes were then substituted to generate an immunotoxin with significantly decreased immunogenicity[86].

Human monoclonal antibodies

Several recent technologies have been developed for generating human monoclonal antibodies[87]. One of these approaches includes the display of repertoires of scFvs, Fabs or domain antibodies (Dabs) on the surface of phage, yeast, bacteria, viruses, ribosomes or mammalian cells, from where they can be enriched and isolated through repeated cycles of panning[88–90]. The phage display technique involves library construction of human antibody fragments by PCR from human B cells of immune or non-immune sources and display onto the phage surface. The phage display library is used to select antibodies against the desired antigen by repeated rounds of panning. After several rounds of such selection, once the desired specificity is obtained, the genes of antibody-variable regions can be cloned into whole human IgG expression vectors and transfected into cell lines to produce fully human monoclonal antibodies. The display technology has also been successfully used to mimic affinity maturation *in vitro* to select high-affinity monoclonal antibodies[91–94].

Another technique to generate human monoclonal antibodies uses human immunoglobulin transgenic mice. In this technique, human monoclonal antibodies expressing B cells are isolated from immunized transgenic mice, in which mouse immunoglobulin genes have been genetically knocked-out and replaced with human counterparts. The transgenic mouse will make human antibodies after foreign antigen immunization. The B cells harvested from immunized mice are immortalized by fusion with a myeloma cell line, as in traditional hybridoma technology. The hybridomas are then screened for desired specificity. Using human immunoglobulin transgenic mice, human monoclonal antibodies against severe acute respiratory syndrome coronavirus (SARS-CoV) and rabies have been generated[95–97].

Another technique involves the use of human memory B cells, obtained from the blood of an individual as a source of human mAbs[98]. These cells are then transformed with Epstein-Barr virus (EBV) in the presence of polyclonal memory B-cell activating agents[99]. Unlike the hybridoma technique, this technique does not need a fusion step to generate hybrid cells. The culture supernatants are then screened directly for specific antibodies, and positive cultures are further cloned by limiting dilution and fully human monoclonal antibodies can then be produced from the cloned B cells. Memory B cells isolated from the blood of individuals who have recovered from SARS-CoV and H5N1 avian influenza infections have been immortalized using this method to produce neutralizing antibodies against these viruses[99,100].

Altering antibody size

The basic structure of an antibody consists of a bivalent antigen-binding site, present on the variable regions of the light and the heavy chains, and the Fc domain which mediates effector and recycling functions through its interaction with the Fc gamma and FcRn receptor respectively. Using papain cleavage, antibodies can be resolved into their constituent domains and smaller fragments. These smaller antibody fragments, scFvs[101,102], diabodies[103] and triabodies[104], capable of binding antigen can be generated using recombinant DNA technology in *Escherichia coli* as recombinant proteins. ScFv is a fusion of the variable regions of the heavy and light chain of an immunoglobulin using a short amino acid linker. This chimeric molecule retains the specific, monovalent, antigen-binding affinity of the parent immunoglobulin, despite removal of the constant regions and is of immense application where Fc-mediated effector functions are not required. Diabodies are bispecific or bivalent antibodies which are generated by combining the heavy chain vari-

Table 2. Recently carried out clinical trials using engineered antibodies and immunotoxins

Antibody/immunotoxin	Cancer type	Trial phase	Reference
Trastuzumab	Breast	3	109
Bevacizumab	Colorectal	3	110
Rituximab	Non-Hodgkin lymphoma	3	111
Cetuximab	Colorectal	3	112
Lintuzumab	Acute myeloid leukaemia	3	113
Anti-mesothelin immunotoxin	Ovarian, pancreatic	1	114
Anti-CD22Fv	B cell	1	115
IL-2R targeted Diptheria toxin	T-Cell lymphoma	3	116
Ricin A chain	Hodgkin's lymphoma	1/2	117
Panitumumab	Colorectal	3	118
Edrecolomab	Colon	3	119

able and light chain variable regions of two antibodies using a linker. In practice, the variable domains of antibody 1 and antibody 2 are fused to create two chains, V_{H1}–V_{L2} and V_{H2}–V_{L1}. The linker used in this case is too small, usually 3–12 residues, which prevents pairing between two domains on the same chain. The domains as a result, are forced to pair with complementary domains of another chain to create two antigen-binding sites. Triabodies are molecules with three active antigen combining sites. The reduction of linker length to less than three residues between V_H and V_L domains of scFvs forces scFv association to form triabodies. This usually leads to high avidity and fast clearance rate compared to the parent IgG.

Because of their small size compared to the parent IgG molecules, these fragments show improved pharmacokinetics and tissue penetration. Additionally, they show better clearance from blood circulation. Hence, they are better suited in certain radio-diagnosis and therapy protocols, where Fc-related effector mechanisms are not required. This is evident from their application in the therapy of certain tumours[105–107].

Clinical trials using engineered antibodies

Engineered antibodies have come a long way from their production and modification in the laboratory to their translation into therapeutic regimens for early as well as advanced stages of cancer. This is evident from a number of clinical trials being carried out to assess their efficacy, either alone or in combination with drugs for the treatment of solid as well as haematological malignancies. Based on the results of these clinical trials, several monoclonal antibodies have been approved by the FDA for cancer treatment[108]. Results of most of the clinical trials have been able to establish a maximum tolerated dose at which there is progression-free survival and regression of tumour. However, in a few cases minimal to moderate side effects have also been observed. Though these side effects and toxicities are not as adverse as those observed in chemotherapy or radiotherapy, their occurrence warrants a systematic and scientific reevaluation of these studies. Table 2 presents a brief summary of some of the recently carried out clinical trials[109–119].

Conclusion

Antibody engineering has come of age. The use of recombinant DNA technology has aided the molecular design and modification of antibodies to suit specific treatment regimens. Engineered antibodies are being explored in clinical trials, alone or as part of conjugates, for the treatment of a wide variety of carcinomas. A key factor in the use of engineered antibodies is the identification of tumour antigens which are expressed exclusively on the cancerous tissue. An ideal tumour antigen candidate therefore would be one that is expressed during all stages of tumour development, is crucial for tumour growth and survival, and is expressed in a broad range of carcinomas. Identification and characterization of such target antigens would aid in the design of broad-spectrum therapeutics. The latest concept of antibody humanization based on SDR grafting has resulted in generating weakly immunogenic chimeric antibody molecules. Furthermore, engineering effector mechanisms, serum persistence, generation of single-domain molecules and their arming with toxins and radionuclides have resulted in the development of highly effective molecules. Treatment of haematologic malignancies using mAb therapy has yielded better results compared to that of solid tumours. This is mainly due to certain physical barriers which the antibody has to face while traversing the tumour mass[120]. This, aided with complexities such as clearance, endocytosis and poor microdistribution render certain immunotherapy regimens weakly effective[121]. Further systematic studies employing the use of knowledge generated will immensely benefit the development of the next generation of immunotherapy regimens.

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