ANTIBODY-MEDIATED
DRUG DELIVERY SYSTEMS
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*Navdeep Kaur, Karthikeyan Subramani, and Yashwant Pathak*

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*Tatyana Levchenko, William Hartner, and Vladimir P. Torchilin*

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CONTRIBUTORS

Leonor Munoz Alcivar, Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, Florida

Simon Benita, The Institute for Drug Research of the School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Nikolai Borisjuk, Biotechnology Foundation Laboratories, Thomas Jefferson University, Philadelphia, Pennsylvania

Manuela Calin, Institute of Macromolecular Chemistry “Petru Poni,” Iasi, Romania; Institute of Cellular Biology and Pathology “Nicolae Simionescu,” Bucharest, Romania

Luca Campana, Melanoma and Sarcoma Unit, Istituto Oncologico Veneto, Department of Oncological and Surgical Sciences, University of Padova, Padova, Italy

Weiyuan Chang, Department of Environmental and Occupational Health, School of Public Health, University of Louisville, Louisville, Kentucky; currently at Division of Preclinical Science, Center For Drug Evaluation, Taipei, Taiwan

Dave Chen, ANP Technologies, Inc., Newark, Delaware

Hong Ding, Department of Pharmaceutical Sciences, The State University of New York at Buffalo, Buffalo, New York

Mohammad Fallahi-Sichani, Department of Chemical Engineering, College of Engineering, University of Michigan, Ann Arbor, Michigan

Oren Giladi, The Institute for Drug Research of the School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

William Hartner, The Center for Pharmaceutical Biotechnology and Nanomedicine, Department of Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts

Yoshitaka Isaka, Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan

Navdeep Kaur, Department of Pharmaceutics and Medicinal Chemistry, T.J.L School of Pharmacy and Health Sciences, University of the Pacific, Stockton, California

Denise E. Kirschner, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan
Slavko Komarnytsky, Plants for Human Health Institute, FBNS, North Carolina State University, Kannapolis, North Carolina

Girish J. Kotwal, Kotwal Bioconsulting, LLC and InFlaMed, Inc., Louisville, Kentucky; currently at University of Medicine and Health Sciences, St. Kitts, WI

Uyen Minh Le, Department of Pharmaceutical Sciences, Sullivan University College of Pharmacy, Louisville, Kentucky

Tatyana Levchenko, The Center for Pharmaceutical Biotechnology and Nanomedicine, Department of Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts

Junling Li, University of Louisville School of Medicine, Louisville, Kentucky

Jennifer J. Linderman, Department of Chemical Engineering, College of Engineering, University of Michigan, Ann Arbor, Michigan

Yijuan Liu, ANP Technologies, Inc., Newark, Delaware

Simeone Marino, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan

David Milunic, ANP Technologies, Inc., Newark, Delaware

Misty Muscarella, Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, Florida

Kutty Selva Nandakumar, Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

Arutselvan Natarajan, Molecular Imaging Program at Stanford, Department of Radiology, School of Medicine, Stanford University, Stanford, California

Chin K. Ng, University of Louisville School of Medicine, Louisville, Kentucky

Jing Pan, ANP Technologies, Inc., Newark, Delaware

Yashwant Pathak, Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, Florida

Chris Pohl, Thermo Fisher Scientific, Sunnyvale, California

Dujie Qin, ANP Technologies, Inc., Newark, Delaware

Hiromi Rakugi, Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Osaka Japan

Srinivasa Rao, Thermo Fisher Scientific, Sunnyvale, California

Helen Reidler, ANP Technologies, Inc., Newark, Delaware

Jeff Rohrer, Thermo Fisher Scientific, Sunnyvale, California
Rakesh Sharma, Center of Nanomagnetics and Biotechnology, Florida State University, Tallahassee, Florida; currently at Amity Institute of Nanotechnology, Amity University, Noida, India

Karthikeyan Subramani, Department of Oral Implantology and Prosthodontics, Academic Centre for Dentistry Amsterdam, Research Institute MOVE, University of Amsterdam and VU, Amsterdam, The Netherlands

Raji Sundararajan, Electrical and Computer Engineering Technology, Purdue University, West Lafayette, Indiana

Yoshitsugu Takabatake, Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan

Vladimir P. Torchilin, The Center for Pharmaceutical Biotechnology and Nanomedicine, Department of Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts

Hieu Tran, Department of Pharmaceutical Sciences, Sullivan University College of Pharmacy, Louisville, Kentucky

Yli Remo Vallejo, ANP Technologies, Inc., Newark, Delaware

Glenn J. Whelan, College of Pharmacy, University of South Florida, Tampa, Florida

William G. Whitford, Thermo Scientific Cell Culture and BioProcessing, Thermo Fisher Scientific, Logan, Utah

Fang Wu, Department of Pharmaceutical Sciences, The State University of New York at Buffalo, Buffalo, New York

Ray Yin, ANP Technologies, Inc., Newark, Delaware

Zhirong Zhang, Key Laboratory of Drug Targeting and Drug Delivery Systems, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu, China

Ting Zheng, Thermo Fisher Scientific, Sunnyvale, California
In 1988 the first comprehensive book on antibody-mediated delivery systems was published. Although the field has developed rapidly and immensely since then, until now no attempt had been made to compile an inclusive and detailed review of the current status of antibody-medicated drug delivery systems. The aim of our book is to provide medical and scientific researchers and students working in this field with an up-to-date, practical, all-encompassing reference source on the concept, analytical development, antibody processing, and applications of antibody-mediated drug delivery systems. Leading scientists working in the field contributed to this effort with chapters on their specific expertise.

Since 1975, when J. F. Köhler and César Milstein developed hybridoma technology to produce monoclonal antibodies (mAbs) efficiently, a number of therapeutic agents based on monoclonal antibodies have emerged for the treatment of various diseases. For their groundbreaking work, Köhler and Milstein won the Nobel Prize in Physiology or Medicine in 1984. Monoclonal antibodies (mAbs) were developed originally from mice as a tool for studying the immune system. The early applications of mAbs included grouping blood types, identifying viruses, purifying drugs, and testing for pregnancy, cancers, heart diseases, and blood clots. mAbs began to reveal their full potential in 1986 when Medical Research Council researcher Gregory Winter pioneered a technique to humanize mouse mAbs. This made them better suited for human medical use, as they were much less likely to elicit an inappropriate immune response in patients. Gregory’s techniques have been licensed to more than 50 companies worldwide. Subsequently, Humira became the first fully human mAb drug, launched in 2002 as a treatment for rheumatoid arthritis.

Briefly, the mAb time line is as follows:

- **1975** Method devised to isolate and reproduce mAbs
- **1986** Techniques pioneered to humanize mouse mAbs
- **1990** Test tube production of highly specific human mAbs
- **1997** First chimeric mAb, Rituxan (rituximab), approved by the U.S. Food and Drug Administration (FDA)
- **1998** First humanized mAb, Herceptin (trastuzumab), approved by FDA
- **2002** First fully human mAb Humira (adalimumab), approved by FDA
- **2003** First fully human mAb, Humira, launched in the UK
- **2005** Humira sales reach more than $200 million

It was quite interesting to note that despite the enormous effort concentrated in producing fully human mAbs, it appears that a significant number of immune
responses are related to the use of such fully human mAbs. Apparently, there are other parameters not yet fully identified that elicit at least some of these immune responses (some can be associated with the excipients used in the design of the formulation of these mAbs). Although today it is not conceivable from a marketing point of view to develop mAbs that are not fully human, the chimeric forms of antibodies that are currently on the market, such as Rituxan, still have their place and continue to expand. For example, annual sales of Rituxan increased continuously have reaching a peak of $5.7 billion in 2009. A total of 28 antibody-based therapeutics have been approved to date by the FDA for clinical applications, and numerous others are currently undergoing development. The market value of antibody-based therapeutics has already reached $40 billion and is expected to reach $68 billion by year 2015. It should be emphasized that of the 10 top-selling drugs today, six are therapeutic antibodies.

This book covers important therapeutic and diagnostic aspects of mAbs. Indeed, Chapter 2 deals with applications of immunoliposomes for cardiovascular targeting. mAbs are well known for their ability to bind to a wide variety of cell-surface proteins, including tumor cell–specific proteins. mAbs can be produced that are directed against virtually any molecule, and unlike polyclonal antisera, they are highly specific. This unique feature of mAbs has opened an important arena of cancer treatment, including immunotherapy, radioimmunotherapy, and pre-targeted therapy (Chapter 3). All these treatment modalities have been developed either with mAbs alone or as conjugates of radionuclides, drugs, and toxins (effector moiety), to seek out and destroy tumor cells selectively. Although many obstacles still have to be overcome, immunoconjugates (Chapter 4) have become a valuable arsenal in the treatment of human diseases, including cancer imaging and therapy in specific targeted drug delivery therapy. Thus, mAb-based immunoconjugates are unique targeting agents for cancer diagnosis, imaging, and therapy. In addition, engineered mAb fragments and nontraditional antibody-like scaffolds (e.g., fibronectin, affibodies) directed toward many novel protein markers are under development and will provide novel options to improve drug delivery. Furthermore, as the authors of Chapter 5, Chapter 9, Chapter 12, and Chapter 18 clearly point out, antibody-mediated drug delivery systems offer promise as carriers of drugs with targeting to specific sites by the binding of mAbs and antigens on malignant or other target cells. Antibody-based therapies using antibody-mediated drug delivery systems target tumor cells while potentially sparing normal cells. Such targeted therapy approaches are employed to reduce the nonspecific toxicity of cytotoxic chemotherapy and to improve the efficacy of treatment. Some antibody-drug conjugates, such as SGN-35 and CMC-544, have demonstrated promising results in clinical trials for the treatment of Hodgkin and non-Hodgkin lymphomas. Most polymer and liposome antibody conjugates are in the preclinical stages, and further clinical studies need to be carried out to confirm the observations from in vitro cell culture experiments and in vivo animal tumor models. The concept of targeted drug delivery using immunoliposomes (liposomes bearing on their surface covalently coupled antibodies) is an appealing therapeutic strategy because of advantages such as the ability to target specific and restricted locations in the body,
to deliver effective concentration of drugs to the diseased sites, and to reduce the drug concentrations at nontarget sites, resulting in fewer side effects.

In addition, the potential of renal gene therapy, which offers new strategies to treat kidney diseases, is reviewed in Chapter 13. Several experimental techniques have been developed and employed using nonviral and viral vectors. Gene transfer consists of carrying a therapeutic gene to the surface of target cells, introducing it into cells, and recruiting it into the nucleus. The development of a gene transfer method is developed to enhance the second step. In addition to the choice of delivery vehicle, the administration route and intrinsic pressure determine the site of transduction.

In Chapter 4, Chapter 6, Chapter 15, and Chapter 18, the diagnostic applications of mAbs are covered. Poly(ethylene glycol) (PEG) polymers attached to biotherapeutic molecules enhance the in vivo delivery and stability of these high-molecular-weight drugs. However, these polymers may, by themselves, be immunogenic and elicit antibodies that can reduce the efficacy of the drug and contribute to potential patient morbidity. A double-antigen-bridging ELISA immunogenicity assay for the detection of specific antidrug antibodies to PEG polymers of various sizes has now been developed.

The authors of Chapter 6, Chapter 10, and Chapter 15 emphasize the contribution of nanotechnology to the expansion of mAbs. With the emergence of nanotechnology, antibody-coated magnetic nanoparticles, portable magnetic immunoassays, nanoparticle-based antigen–nanometal conjugates, and several biomarker bioapplications are in the developmental stages to achieve microimaging at microscale, point-of-care detection devices, nano-drug delivery systems, and nanorobots, respectively.

Plant-derived antibodies offer a wide range of applications in biomedical research and metabolic engineering, and as clinical diagnostic or therapeutic agents, as proposed in Chapter 17. Even though numerous breakthroughs have been achieved in the use of plants as hosts for the production of recombinant proteins, manufacturing complex immunoglobulins is not a simple procedure with an assured favorable outcome. One of the major problems is the low yield of the recombinant antibodies in plants. Careful selection of the host species, codon optimization, engineering of genetic elements capable of stabilizing and enhancing levels of the recombinant transcript, development of novel harvesting and purifying strategies, and use of various cell compartments are but a few potential avenues that may help increase the yield of the final product. The increasing number of plant antibody–based products entering clinical trials and the market indicates an exponential growth of activities in this field. This technology is just beginning to mature, and considerable evolution may be expected in the next few decades.

Additional applications for mAb modifications which have made a huge impact in biopharmaceuticals are reviewed in Chapter 18. The simple concept of fusing antibody-producing B cells from the spleen with myeloma cells followed by isolating clones secreting monospecific antibodies for which Köhler and Milstein received a Nobel prize translated into a lifesaving treatment that specifically targets tumor cells or proinflammatory cytokines with minimal collateral damage. mAbs are heterodimeric protein molecules with an antigen-binding region.
that can target receptors on cancer cells and a conserved or constant region that can
bind to complement components and recruit the destructive force of the immune
system to target and eliminate tumor cells. Using recombinant DNA technology,
the conserved parts of the mAbs can be humanized to prevent rapid clearance of
antibody molecules. Several mAbs have made it to the top 12 biotech drugs list,
and the application of mAbs has yet to be fully explored. The prohibitive cost of
these mAbs has raised questions about their widespread use to prolong life, and
questions have been raised as to whether the final 2% of life deserves to incur 98%
of the lifelong medical expenses.

Many different strategies have been discussed for application of antibodies
in the treatment of asthma using allergen-specific T cells and their cytokines, IgE
levels and IgE inhibitors, and TNFα therapies. Nevertheless, the continued interest
of academics, clinicians, and the pharmaceutical industry will help keep mAbs
central to the efforts of the biotech industry. Each chapter of the book deals with
the concepts, technology, and applications of mAb systems.

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Yashwant Pathak
Simon Benita
CHAPTER 1

ANTIBODY-MEDIATED DRUG DELIVERY SYSTEMS: GENERAL REVIEW AND APPLICATIONS

NAVDEEP KAUR
Department of Pharmaceutics and Medicinal Chemistry, T.J.L School of Pharmacy and Health Sciences, University of the Pacific, Stockton, California

KARTHIKEYAN SUBRAMANI
Department of Oral Implantology and Prosthodontics, Academic Centre for Dentistry Amsterdam, Research Institute MOVE, University of Amsterdam and VU, Amsterdam, The Netherlands

YASHWANT PATHAK
Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, Florida

1 HISTORICAL PERSPECTIVE

The term antibody was first used by Paul Ehrlich in year 1891 in his article “Experimental Studies on Immunity.” In 1890, Emil Von Behring and Shibasaburo Kitasato established the basis for serum therapy: that serum taken from animals treated with nonlethal doses of diphtheria and tetanus can be used for the treatment of diphtheria and tetanus. They followed this discovery with the theory of humoral immunity, which prompted Paul Ehrlich to propose side chain theory, which describes the interaction between antibodies and antigens. Later, in the 1920s and the 1930s, it was shown by Michael Heidelberger and Oswald Avery that antibodies are made of protein, and the biochemical aspect of antigen–antibody interactions was explained by John Marrack. In the following years, the structure of antibodies was characterized by a number of scientists independently [1].

In 1975, Köhler and Milstein successfully produced antibodies in vitro using “hybridoma technology.” This discovery allowed the production and use of antibodies on a large scale for diagnostic and therapeutic purposes. The first
antibody, OKT3, was approved by the U.S. Food and Drug Administration (FDA) in 1986 for use in patients to prevent transplant rejections [2]. Since then, numerous technologies have been developed to decrease the immunogenicity of mouse antibodies by generating partial or fully human antibodies. A total of 28 therapeutic antibodies approved by the FDA are currently available in the U.S. market. It is the fastest-growing market, and its revenue is expected to increase to $62.7 billion in 2015, according to DatamonitorPlc, a London-based health information firm [3].

2 ANTIBODIES

Antibodies (also known as immunoglobulins) are proteinacious in nature and are produced in response to an invasion of foreign substances in the body called antigens.

2.1 Structure of Antibodies

Antibodies are heavy (~150 kDa), Y-shaped glycoproteins composed of four polypeptide chains: two long heavy or H chains and two short light or L chains. The end of light and heavy chains together constitutes a variable region (also known as antigen-binding site) consisting of 110 to 130 amino acids. The amino acid sequence in the variable region gives antibody its specificity for binding to a variety of antigens.

2.2 Types of Antibodies

There are five major types of antibodies, each having a specific role in the immune response:

1. IgG: comprises 75 to 80% of total antibodies circulating in the blood and body fluids. This is the principal antibody found in the body and provides the majority of antibody-mediated protection against bacterial and viral infections. It is produced one month following initial B-cell activation.
2. IgA: comprises 10 to 15% of total antibodies present in the body. These are involved predominantly in the protection of mucosal surfaces exposed to various pathogens and are thus found in mucosal areas such as the digestive tract, the respiratory tract, the urogenital tract, and the eyes.
3. IgM: makes up about 5 to 10% of total circulating antibodies in the body. IgM antibodies are the first to appear in the body post-infection. They are expressed on the surface of B cells and are also secreted by them.
4. IgD: comprises about 1% of total antibodies present in the body. The exact function of IgD antibodies is not very clear.
5. IgE: makes up about 0.05% of all immunoglobulins in the body. IgE binds to Fc receptors on the surface of mast cells and basophils to produce an immune response. These are particularly involved in allergic reactions and immune responses to parasitic worms [4–7].
2.3 Antibody Development

Over a period of time, numerous methods have been devised for the production of antibodies, the first being the hybridoma method proposed by Köhler and Milstein. This method involves immunization of mice with a mixture of antigens followed by fusion of their spleen cells with immortalized myeloma cells. These cells are then cloned and screened for production of the desired antibodies. Certain limitations associated with the method involve specificity issues, as the antibodies are derived from murine cells and thus resemble a rodent immune system and also because these antibodies are recognized as allogenic proteins in human patients, which leads to human antimouse antibody response.

Another method, the Epstein–Barr virus method, involves immortalization of human cells by the Epstein–Barr virus. The disadvantage of this method is its nonspecificity in terms of immortalizing antigen-specific B cells among a pool of peripheral blood lymphocytes.

To humanize murine antibodies further, chemical and molecular methods were devised, such as replacement of the Fc portion of murine antibodies by that of human antibodies to yield chimeric monoclonal antibodies. Also, immortalization of genes corresponding to specific antibodies, and grafting of DNA fragments determining the binding specificity of the antibody into the framework of human immunoglobulin genes, leads to the production of humanized antibodies.

The phage display method is an efficient method for the production of high-affinity antibodies. It involves ligation of a DNA library derived from B cells onto a surface protein gene of a bacteriophage. Further, phages expressing the required specificities are isolated, enriched, and used to infect Escherichia coli for the production of monoclonal antibody construct [8].

3 ANTIBODY MEDIATION

Antibody-mediated immunity is also called humoral immunity or humoral immune response. Lymphocytes (white blood cells) are divided into two types: B lymphocytes or B cells (which secrete antibodies and are involved in humoral immunity) and T lymphocytes or T cells (which are involved in cell-mediated immunity). Both types of cells originate from the bone marrow; they become B or T cells depending on their point of maturation. T cells develop in the thymus gland; B cells develop in the bone marrow. Antibodies are produced in the body by B lymphocytes or B cells. B cells develop in the bone marrow and travel from bone marrow to the spleen. Once in the spleen, the B cells undergo a maturation process during which the genes responsible for generating antibody recombine several times. This process renders the cells highly specific for a single antigenic sequence. During maturation, each B cell undergoes selection mechanisms which ensure that it is not only specific for one antigen, but also that it does not recognize self-antigen. During this process, any B cells that recognize self-antigen either die or their activity is permanently suppressed. When a B cell has gone through the entire recombination process, it becomes fully mature. Once fully matured, the cell is at a stage where it
will activate only when it recognizes a particular amino acid sequence during the course of a pathogenic infection. Mature B cells circulate throughout the body, via the bloodstream and lymphatic system, until they come into contact with the specific antigen that they recognize. When there is an infection, the invading pathogen produces antigen. Resting or naive B cells get activated when the antigen binds to its membrane, and this results in the production of numerous antibodies that bind specifically to that antigen. B cells can be activated in a T-cell-dependent or T-cell-independent manner.

1. **T-cell-dependent activation.** In this process, the B cells get help from T cells in the antibody response by acting as antigen-specific antigen-presenting cells. Ig receptors on the membrane of B cells bind antigens and internalize them by means of receptor-mediated endocytosis (a process by which cells absorb molecules such as proteins by engulfing them in vesicles). The pathogen is then digested in endosomal vesicles to yield peptide fragments, which are then attached to class II (major histocompatibility complex (MHC)) proteins and migrated to the plasma membrane of the B cells. Helper T cells recognize MHC–peptide complex on the surface of B cells and get stimulated to produce cytokines, which leads to activation and proliferation of B cells. Activated B cells subsequently mature into antibody-producing plasma cells which produce antibodies specific for the antigen presented to fight the infection. Once these antibodies are released into the bloodstream, they lock onto specific antigen. These antibody–antigen complexes are removed through the complement system or by the liver and spleen [9].

2. **T-cell-independent activation.** This process involves stimulation of antibody production in the absence of helper T cells. Many antigens are T-cell-independent and can deliver the signals directly to the B cell. T-cell-independent activation is brought about by T-cell-independent antigens such as polysaccharides, glycolipids, and nucleic acids. These antigens are not processed and presented along with MHC proteins and hence cannot be recognized by helper T cells. Many bacteria have repeating carbohydrate epitopes. Most of these antigens have multiple identical epitopes, which induces cross-linking of Ig receptors on B-cell surfaces and further stimulation of B cells, and there is no requirement for participation by antigen-specific helper T cells. These T-cell-independent (TI) antigens are of two types: **TI-1 antigen** is made up of lipopolysaccharide (LPS), and **TI-2 antigens** are polysaccharides, glycolipids, and nucleic acids. TI-1 antigens stimulate the B cells directly without the requirement of any other cell. At lower concentrations, gram-negative bacterial LPS stimulates specific antibody production, but at higher levels it acts as a polyclonal B-cell activator, stimulating growth and differentiation of most of the B cells without binding to the membrane receptors [10–12].

### 4 ANTIBODY-MEDIATED DRUG DELIVERY SYSTEMS

1. **Radioimmunotherapy:** a treatment method that employs radionuclide-labeled antibody to deliver cytotoxic radiation to target cells. Owing to the specificity of antibodies for the cancer antigens, radiolabeled antibodies have the
ability to localize in cancer cells and to kill the cells because of the cytotoxic radiations of radionuclide. Radioimmunotherapy has advantages over traditional chemotherapy, which distributes drug throughout the body (lack of selectivity) and is often associated with dose-limiting toxicities to various organs, and also over conventional radiation therapy, which has the disadvantage of killing normal healthy cells in addition to cancer cells. In addition to these advantages, radioimmunotherapy is better than conventional immunotherapy, as radiolabeled antibodies not only kill the cells to which they are bound but also the adjoining cancer cells [13].

Immunomedics, Inc. and IBC Pharmaceuticals, Inc. have designed a bispecific antibody, TF2, using patented dock-and-lock (DNL) protein engineering platform technology for pretargeted radiation therapy. Radiolabeled TF2 binds to carcinoembryonic antigen (CEA) and accumulates in CEA-expressing tumors, resulting in increased signal at tumor relative to nontumor tissues. Radiation can be targeted specifically to tissues bearing tumors. Results from the preclinical study of TF2 for pretargeted therapy suggests a fivefold increase in survival in one model and a twofold increase in another model. Temporary and mild side effects were found to be bone marrow and kidney toxicity. It is currently in early phase I studies with colorectal cancer [14].

2. Immunoliposomes: liposomal formulations with an encapsulated active agent and conjugated antibodies and antibody fragments on their surfaces. Antibodies and antibody fragments specific for certain tumor markers can be used for the targeted delivery of liposomes and can also help in internalization, owing to their ability to endocytose, resulting in overall improved bioavailability of chemotherapeutic agents. Various internalizing single-chain variable fragment (scFv) antibody fragments have been identified and are being used to deliver drugs to cancer cells, such as anti-CD166 scFv and a novel UA20 scFv which targets prostate cancer cells; anti-ErbB2 F5 scFv, which binds specifically to ErbB2 expressed on certain tumors; and anti-epidermal growth factor receptor (EGFR) scFv antibodies, which target EGFR overexpressed in a number of cancer cells [15,16]. Immunoliposomes have enhanced performance compared to liposomes, as these can be specifically targeted and internalized in cancer cells [17].

3. Immunotoxins: conjugates of antibody fragments linked chemically or genetically to toxins derived from bacterial, plant, or animal sources. Various toxins, such as *Pseudomonas*, anthrax and diphtheria (bacterial toxins), ricin, saporin, abrin, gelonin and pokeweed (plant toxins), restrictocin (fungal toxin), and hemolytic toxin from sea anemone (animal toxin), are being used for the treatment of cancer.

Denileukindifitox (Ontak) is an FDA-approved immunotoxin used for the treatment of cutaneous T-cell lymphoma. It is composed of interleukin-2 (IL-2) protein sequences conjugated to diphtheria toxin. IL-2 moiety of Ontak targets tumor cells expressing IL-2 receptors and delivers the immunotoxin inside the cells via receptor-mediated endocytosis, where diphtheria toxin fragment A is released into the cytosol, inhibiting the protein synthesis through the ADP ribosylation
of elongation factor 2 and leading to cell death [18]. Several immunotoxins are currently under development and in clinical trials.

A new anti-fAChR (fetal acetylcholine receptor) immunotoxin (scFv35-ETA) is currently being developed for the treatment of rhabdomyosarcoma (RMS). It is composed of fully human anti-fAChR Fab fragment fused to *Pseudomonas* exotoxin A. It showed promising results in vitro (killed RMS cell lines TE-671, FL-OH-1, and RD in a dose-dependent manner) and delayed RMS development in a murine transplantation model [19].

4. **Antibody–drug conjugates**: monoclonal antibodies linked or conjugated to cytotoxic drugs by means of a chemical linker. Antibody–drug conjugates exert their therapeutic efficacy by targeting the cytotoxic agents to tumors as a result of the ability of antibodies to recognize and bind specifically to tumor-specific and/or overexpressed antigens on cancer cells. Antibody–drug conjugates are superior to treatment with either monoclonal antibodies alone or cytotoxic drugs. Monoclonal antibodies can be used as single agents for the treatment of cancer; however, their efficacy is limited. Also, the efficacy of chemotherapy is limited because of lack of selectivity of cytotoxic agents, which leads to nonspecific toxicity of healthy tissues. In antibody–drug conjugates, antibody is attached to a cytotoxic drug by means of a linker (Fig. 1).

The challenges associated with antibody–drug conjugates are that the linker in these conjugates must be stable while circulating in the bloodstream and must release the drug while inside the tumor cells. Also, the conjugation must not affect the binding specificity of the antibody toward antigen and must be internalized effectively inside the cancer cells to attain sufficient intracellular drug concentration so as to kill the tumor cells [20,21]. Numerous antibody–drug conjugates currently on the market and under development are listed in Table 1.

5 **APPLICATIONS**

1. **Diabodies.** Diabodies are medium-sized bivalent and bispecific antibody fragments with a molecular weight of about 60 kDa. Diabodies consist of variable domains of heavy and light chains connected by a peptide linker. The short linker between the heavy and light domains hinders pairing between them while promoting pairing with the complementary domains of another chain, resulting in the formation of dimers called diabodies. Diabodies bind to multimeric antigens with great avidity because of their bivalency, and this leads to high tumor retention. Because of such advantages as rapid tissue penetration, high target retention, and rapid blood clearance, diabodies are particularly suitable for such applications as radioimmunotherapy and imaging.