Advances in Experimental Medicine and Biology 909

Shuren Zhang *Editor*

Progress in Cancer Immunotherapy

Springer

Advances in Experimental Medicine and Biology

Volume 909

More information about this series at: http://www.springer.com/series/5584

Shuren Zhang
Editor

Progress in Cancer Immunotherapy

Springer

Editor
Shuren Zhang
Department of Immunology
Cancer Hospital & Institute
Chinese Academy of Medical Sciences and Peking Union Medical College
Beijing, China

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-94-017-7553-3 ISBN 978-94-017-7555-7 (eBook)
DOI 10.1007/978-94-017-7555-7

Library of Congress Control Number: 2016937714

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Science+Business Media B.V. Dordrecht

Preface

The great success achieved by immune checkpoint inhibitors and CAR-T cells dismissed the doubt of efficacy of immunotherapy in the treatment of cancer over the past century. The treatments targeting immune negative regulators have verified the immune editing theory, which have been established at the turn of the century. Previously, immune surveillance theory provided the notion of how the immune system can recognize and kill tumors, but did not fully consider reciprocity in the interaction between the immune system and the tumor. Immune editing theory makes us aware of regulatory controls that promote the development of tumors. Targeting negative immune checkpoints (such as the use of anti-CTLA4 and anti-PD-1 therapeutic antibodies), which are not against tumor targets, can elicit significant anti-tumor effects. This liberation of the once suppressed anti-tumor immune responses suggests that the immune system has adequate resources to destroy tumors.

Any kind of treatment need to analysis dialectically. There are advantages and disadvantages for each conventional therapy (surgery, radiotherapy and chemotherapy). Similarly, not all cancer patients respond well to immunotherapeutic interventions, and further precise medical research is required to determine these disparities in responses. Immunotherapy can also cause serious side effects, such as elicitation of cytokine storm, autoimmune, and even adverse event-related death. The combination of immunotherapy with conventional therapies can mutually complement and synergize with one another. Appropriate surgery, radiotherapy and chemotherapy may reduce tumor load; induce immunogenic cells death (ICD) of cancer cells; increase tumor sensitivity to immunotherapy and thus enhance immune therapeutic effect. This conforms to the traditional Chinese medicine theory of “strengthening bodily resistance and eliminating evil”. After the introduction of efficacious immunotherapy, tumor therapeutic principle will gradually change; their curative effects are mainly judged by overall survival rates, quality of life and tumor size, inclusive of survival without tumor clearance. However, if treatment seriously undermines the patient’s health and lowers their will to survive this is considered as a loss of therapeutic significance.

We are at the beginning of an era where potent immunotherapies are entering the market. Compared with conventional therapies, the developmental potential of immunotherapy is the largest and will be the key to the generation of precise medical treatments. Therapeutic antibodies, vaccines, immune cells and oncolytic immunotherapy for cancer are reviewed in this book. Discussed herein includes the progress of tumor immunotherapy, their advantages and existing problems, and hope can bring better enlightenment to the treatment of tumor.

Beijing, China Shuren Zhang

Contents

Contributors

Yiwei Chu Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Yuting Deng Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Hao Jin Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

Enyu Huang Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Mingming Jia CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Xuechao Jiang Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Huajun Jin Laboratory of Viral and Gene Therapy, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China

Zhong Li Laboratory of Viral and Gene Therapy, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China

Ronghua Liu Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Xiaoming Liu Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Department of Dermatology, Shenzhen Hospital, Peking University, Shenzhen, Guangdong, China

Zhou Lu Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Feifei Luo Biotherapy Research Center, Fudan University, Shanghai, China

Department of Digestive Diseases of Huashan Hospital, Fudan University, Shanghai, China

X.N. Miao Innovent Biologics, Inc., Suzhou Industrial Park, China

Jiawen Qian Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Qijun Qian Laboratory of Viral and Gene Therapy, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China

Xiubao Ren Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

Department of Biotherapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Qian Sun Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

A. Tsun Innovent Biologics, Inc., Suzhou Industrial Park, China

C.M. Wang Innovent Biologics, Inc., Suzhou Industrial Park, China

Jian Wang Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

Luman Wang Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Shengdian Wang CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Feng Wei Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

Cihui Yan Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

Fan Yang Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

Department of Biotherapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Jiao Yang Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Zhenlong Ye Laboratory of Viral and Gene Therapy, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China

D.C. Yu Innovent Biologics, Inc., Suzhou Industrial Park, China

Dan Zhang Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, Shanghai, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai, China

Chapter 1
Antibody Therapies in Cancer

Shengdian Wang and Mingming Jia

Abstract Antibody-based immunotherapy has become a standard treatment for a variety of cancers. Many well-developed antibodies disrupt signaling of various growth factor receptors for the treatment of a number of cancers by targeting surface antigens expressed on tumor cells. In recent years, a new family of antibodies is currently emerging in the clinic, which target immune cells rather than cancer cells. These immune-targeted therapies strive to augment antitumor immune responses by antagonizing immunosuppressive pathways or providing exogenous immune-activating stimuli, which have achieved dramatic results in several cancers. The future of cancer therapies is likely to combine these approaches with other treatments, including conventional therapies, to generate more effective treatments.

Keywords Immunotherapy • Therapeutic antibody • Cancer

Over the past 20 years, antibodies have been used as passive immunotherapy strategies as part of the standard treatment of many cancers. Many of these antibodies are specific for surface antigens expressed by tumor cells. A major class of them targeting growth factor receptors, such as epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2), are commonly used for the treatment of non-leukemic cancers. The antibodies targeting the lineage markers of hematopoietic cells, such as CD20, have shown the therapeutic efficacy in hematological malignancies. By directly binding to these membrane-bound receptors, these antibodies result in tumor cell death through dampening the downstream signaling cascades that promote cell cycle and function and Fc-mediated innate immunological effector mechanisms, such as antibody-dependent cell-mediated cytotoxicity (ADCC). In addition, therapies of these tumor-targeted antibodies can induce endogenous adaptive antitumor immune responses which were recently shown to play important roles in the therapeutic efficacy.

S. Wang (✉) • M. Jia
CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Datun Road #15, Chaoyang District, 100101 Beijing, China
e-mail: sdwang@moon.ibp.ac.cn

S. Zhang (ed.), *Progress in Cancer Immunotherapy*, Advances in Experimental Medicine and Biology 909, DOI 10.1007/978-94-017-7555-7_1

In recent years, it has been demonstrated that antibodies could be used to manipulate the host's immune responses by targeting the immune cells to generate active antitumor immunity in cancer patients. Such immunomodulatory antibodies may be either agonistic, targeting costimulatory molecules, or antagonistic, "blocking" inhibitory molecules expressed on the surface of immune cells. The aim of these approaches is to augment endogenous antitumor immune responses, either by providing direct immune stimulation or by releasing immunosuppressive mechanisms. They have resulted in a paradigm shift in cancer therapy, where instead of using drugs to target the tumor cells, molecules are designed to target the immune system in order to break the tumor tolerance and stimulate the antitumor immune response. The diversity of these targeted approaches reflects the versatility of antibodies as platforms for therapeutic development.

1 Historical Review of Antibody Therapeutics in Cancer

1.1 Characteristics of Antibody

Antibodies are composed of two identical light chains and two identical heavy chains and comprised of two distinct functional units: the fragment of antigen binding (Fab) and the constant fragment (Fc). Heavy and light chains each have variable and constant regions. The variable regions of a heavy chain and a light chain combine to form an antigen-binding site, so that an antibody molecule has two identical antigen-binding sites (Fig. 1.1). The Fc implements immune effector functions by binding to Fc receptors (FcRs) expressed on immune cells or initiating complement-dependent cytotoxicity.

Based on the sequence of the heavy-chain constant regions, antibodies are grouped into five classes: IgM, IgD, IgG, IgE, and IgA. IgG can be further subdivided into four subclasses (IgG1, IgG2, IgG3, and IgG4). Most of the approved antibodies in oncology are of the human IgG1 subclass, which is the most effective at engaging Fcγ receptors (FcγRs) on natural killer (NK) cells, macrophages, and neutrophils. Antibody engagement of these receptors leads to the killing of antibody-bound target cells by ADCC or antibody-dependent phagocytosis. In addition, IgG1 and IgG3 are potent activators of the classical complement pathway. The binding of two or more IgG molecules to the cell surface leads to high-affinity binding of complement component 1q (C1q) to the Fc domain, followed by activation of C1r enzymatic activity and subsequent activation of downstream complement proteins, resulting in cell lysis.

ADCC can be augmented through modification of the antibody Fc region to produce a more favorable binding profile for the FcRs expressed on monocytes and NK cells. These modifications include mutations in the amino acids and alterations in the glycosylation pattern of the Fc region. A triple alanine substitution mutant trastuzumab (S298A/E333A/K334A), an anti-HER2/neu antibody, has significantly

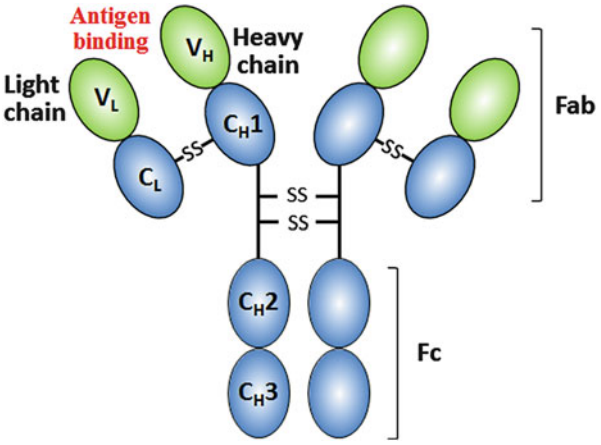

Fig. 1.1 The structure of antibody (IgG). Antibody is composed of two heavy (*H*) and two light (*L*) chains. These chains comprise constant (*C*) regions, which constitute the Fc domain, and variable (*V*) regions, which constitute the Fab domain and allow antigen specificity

improved binding to FcγRIIIA, the principal activating FcR on monocytes and NK cells. Consistent with the improved binding, this substituted trastuzumab has a superior ability to activate ADCC in vitro. Most of the currently used therapeutic antibodies are highly fucosylated owing to the nature of the cell lines used for manufacturing. However, antibodies with defucosylated oligosaccharides can promote FcγRIIIA binding and show a significant enhancement in ADCC in vitro and enhanced in vivo antitumor activity. Antibody-mediated killing can also be enhanced by decreasing binding to the inhibitory FcγRIIB. Conversely, ADCC can be eliminated by modifying specific residues in the Fc domain that bind to FcγR or by producing recombinant antibodies that lack the N-glycosylation of Fc regions. The IgG4 subclass has also been used for reducing ADCC.

The neonatal FcR (FcRn) is structurally distinct from FcγR. By binding to Fc, FcRn expressed on the vascular endothelium can protect antibody from transcytotic lysosomal catabolism after antibody internalization by endothelial cells and return it to the circulation (Roopenian and Akilesh 2007). FcRn is largely responsible for the serum half-life of antibodies. Thus, antibody half-life can be extended or reduced by introducing mutations into Fc region that enhance or diminish FcRn binding. These may prove to be important considerations in controlling the pharmacokinetic exposure levels of a given antibody, with a potential toxicity possibly mitigated by faster clearance (Yeung et al. 2009).

1.2 Development of Antibody Therapies for Cancer

Since the first description of monoclonal antibodies (mAbs) in 1975 (Kohler and Milstein 1975), mAbs were recognized as unique biological tools and quickly became invaluable in pathological diagnosis. Meanwhile there was equal excitement about their therapeutic potential based on the ability to manufacture mAbs of

defined specificity and class in essentially unlimited amounts. This would allow for highly specific targeting of cancer cells on the basis of their molecular phenotype.

However, early clinical results exploring mAb-based therapeutics were disappointing (Vaickus and Foon 1991). The first mAb evaluated in clinic as cancer treatments was a murine mAb. Although there were intriguing hints that antibody therapy could be successful, the treatments with murine mAbs were often associated with the development of an immune response against the therapeutic antibody itself and the rapid clearance of the antibody due to their immunogenicity for human, which limited their clinical applicability. To overcome these side effects, chimeric mouse-human antibodies were developed by grafting the entire antigen-specific domain of a mouse antibody onto the constant domains of a human antibody using genetic engineering techniques (Morrison et al. 1984) (Fig. 1.2). In 1997, rituximab (Rituxan), a mouse-human chimeric mAb against the B-cell lineage marker CD20, was approved by FDA for treatment of B-cell non-Hodgkin lymphoma. This is the first antibody approved for cancer therapy. Since then, no less than 15 distinct antibodies have been approved for the treatment of hematologic and solid tumors. In 2004, cetuximab (Erbitux), another chimeric mAb against Her-1, a member of epidermal growth factor receptor (EGFR) family, was approved for treatment of colorectal carcinoma (Galizia et al. 2007).

With the advent of in vitro phage display technology and the generation of transgenic rodents expressing human immunoglobulin genes, the humanized antibodies and fully human antibodies were generated (Fig. 1.2). In 1998, trastuzumab (Herceptin), a humanized antibody binding the extracellular domain of the HER2, was approved for the treatment of metastatic HER2-overexpressing breast cancer (Hudis 2007). In 2001, alemtuzumab (Campath), a humanized mAb against CD52, a cellular surface glycoprotein expressed on both normal and malignant B and T lymphocytes, was approved by FDA for treatment of drug-resistant chronic lymphocytic leukemia (Alinari et al. 2007). In 2004, the first anti-angiogenic agent, bevacizumab (Avastin), was approved by FDA (Fig. 1.3). Bevacizumab is a humanized version of a murine mAb against VEGF, which binds and neutralizes all human VEGF isoforms and bioactive proteolytic fragments. Bevacizumab has been used in combination with conventional chemotherapy and/or targeted

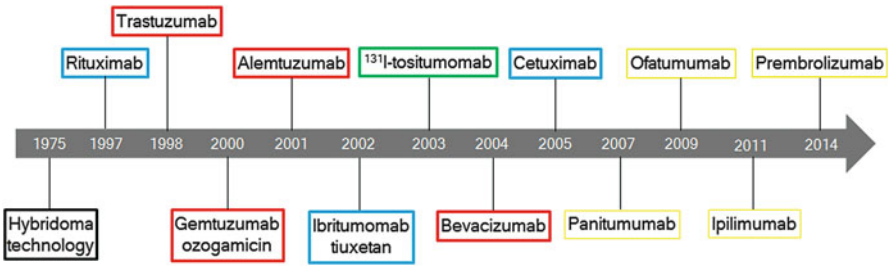

Fig. 1.2 Timeline of antibody development for cancer therapy. *Box outline*: *blue*, chimeric antibody; *red*, humanized antibody; *yellow*, human antibody

anticancer agents for colorectal cancer, acute myeloid leukemia, multiple myeloma, head and neck squamous cell carcinoma, etc. (Hurwitz et al. 2004).

In 2013, the use of antibodies to harness the power of the immune system to fight cancers was heralded in science as the "breakthrough of the year." This was mainly due to the great early successes of antibodies to two co-inhibitory receptors, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1), expressed on activated T cells (Couzin-Frankel 2013). Ipilimumab, a fully human IgG1 monoclonal antibody targeting CTLA-4, is the first immune checkpoint inhibitor approved by FDA for treatment of cancer in 2011. Ipilimumab blocks CTLA-4 signaling pathway in activated T cells and can induce sustained antitumor responses (Hodi et al. 2010). The next generation of immune checkpoint inhibitors blocks the interaction of co-inhibitory receptor PD-1 on T cells and its ligand PD-L1 on tumor cells and antigen-presenting cells (APCs). Multiple breakthrough designations for PD-1- and PD-L1-blocking antibodies have been granted by the FDA in melanoma, non-small cell lung cancer (NSCLC), Hodgkin

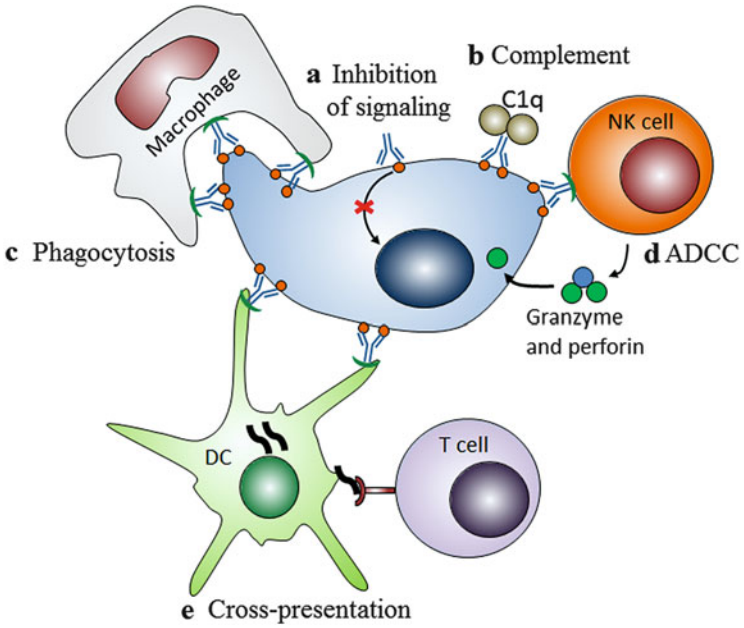

Fig. 1.3 Mechanisms of tumor-targeted antibody therapy in cancers. (**a**) Antibodies directed against TAA (such as EGFR and HER2) inhibit oncogene signaling. (**b**) The complex of antibody and tumor antigen initiates soluble complement-mediated cytotoxicity. (**c**) Antibody-coated apoptotic tumor cells can bind Fc receptors on phagocytes and initiate Fc-dependent phagocytosis. (**d**) Recognition of antibody-coated tumors by Fcγ receptors (*FcγRs*) on effector immune cells such as natural killer (*NK*) cells, macrophages, and neutrophils leads to ADCC and tumor cell apoptosis, which is mediated by the delivery of perforin and granzymes to the tumor cell. (**e**) Antibody-coated tumor antigens released by dying cells are taken up by DCs, processed, and cross-presented to T cells

lymphoma, bladder cancer, renal cell carcinoma, etc. Such designations have led to the accelerated FDA approval of fully human anti-PD-1 mAb prembrolizumab for patients with melanoma in 2014 (Hamid et al. 2013) and nivolumab for patients with melanoma or squamous cell NSCLC in 2014 and 2015, respectively. This class of immunomodulatory antibodies blocking PD-1 co-inhibitory pathway are arguably the most exciting development in current cancer drug development.

1.3 Classes of Antibody Therapeutics in Cancer

Anticancer immunotherapies are generally classified as "passive" or "active" based on their ability to activate the host immune system against malignant cells. From this standpoint, tumor-targeting antibody therapeutics are considered passive immunotherapy, as they are endowed with intrinsic antineoplastic activity. Conversely, immunostimulatory antibodies and checkpoint inhibitors constitute clear examples of active immunotherapy as they exert anticancer effects by modulating antitumor immune responses only upon the engagement of the host immune system.

Tumor-targeting antibodies exist in at least four functionally distinct variants. First, the antibodies inhibit signaling pathways required for the survival or progression of neoplastic cells, such as the EGFR-specific antibody (cetuximab) for the treatment of head and neck cancer and colorectal carcinoma (CRC) (Weiner et al. 2008). Second, the TAA-specific antibodies opsonize cancer cells and hence activate ADCC, CDC, and antibody-dependent cellular phagocytosis, such as the CD20-specific antibody rituximab for the treatment of chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma (Scott 1998; Jones 2013). Third, immunoconjugates, i.e., TAA-specific antibodies, coupled with toxins or radionuclides, such as gemtuzumab ozogamicin, an anti-CD33 calicheamicin conjugate for the treatment of acute myeloid leukemia (Hughes 2010). Fourth, the "bispecific T-cell engagers" (BiTEs) consist of two single-chain variable fragments from distinct mAbs, one targeting a TAA and one specific for a T-cell surface antigen, such as blinatumomab, a CD19 and CD3 BiTE recently approved for the therapy of Philadelphia chromosome-negative precursor B-cell acute lymphoblastic leukemia (Walter 2014).

The immunomodulating antibodies operate by interacting with the immune system to elicit a novel or reinstate an existing anticancer immune response. So far, this has been achieved through four kinds of antibodies: (1) Antagonistic antibodies block immunosuppressive receptors expressed by activated T lymphocytes, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1) or NK cells, like various members of the killer cell immunoglobulin-like receptor (KIR) family (Long 2008); (2) the antibodies against the ligands of these immunosuppressive receptors block the interactions of these receptors and ligands (Zou and Chen 2008); (3) agonistic antibodies activate the costimulatory receptors expressed on the surface of immune effector cells, such as tumor necrosis factor receptor superfamily, member 4 (TNFRSF4, best known as

OX40), TNFRSF9 (best known as CD137 or 4-1BB), and TNFRSF18 (best known as GITR) (Croft 2009); and (4) neutralizing antibodies neutralize the activities of immunosuppressive factors released in the tumor microenvironment, such as transforming growth factor β1 (TGFβ1) (Pickup et al. 2013).

2 Tumor-Targeted Antibody Therapies

Tumor-targeted antibody therapy has shown efficacy in the past 30 years and is now one of the most successful and leading strategies for targeted treatment of patients harboring hematological malignancies and solid tumors. Tumor-targeting antibodies are the best-characterized form of anticancer immunotherapy. These therapeutics include unconjugated antibodies or antibody fragments targeting TAA, as well as antibody-drug conjugates, radioimmunoconjugates, and bispecific/trispecific molecules targeting TAA. Currently several FDA-approved monoclonal antibodies are used in the clinic, either alone or in combination with chemotherapy or radiation.

2.1 An Outline of Tumor-Targeted Antibody Therapeutics

Many of the tumor-expressed targets for therapeutic antibodies are growth factor receptors and differentiation antigens that are involved in growth and differentiation signaling, such as EGFR, HER2, CD20, CD30, etc. By blocking ligand binding and/or signaling pathways through these receptors, monoclonal antibodies may serve to normalize growth rates, induce apoptosis, and/or help sensitize tumors to chemotherapeutic agents. These include antibodies that target receptors expressed on the tumor cells. In addition, antibodies that target tumor microenvironment and inhibit processes such as angiogenesis have shown therapeutic promise.

2.1.1 Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor overexpressed in many different malignancies, including those originating in the colon, head and neck, ovary, lung, and brain. Ligand binding causes EGFR dimerization, leading to activation of the tyrosine kinase domain which promotes cell proliferation, migration, and invasion via activation of the MAPK and AKT pathways (Li et al. 2005). Some EGFR-expressing tumors have rearrangements of the EGFR gene that lead to the expression of constitutively activated mutant receptors. The most common EGFR mutation in the extracellular domain is EGFRvIII, which has an in-frame deletion of exons II–IV. EGFRvIII is found in glioblastoma, head and neck cancers, and NSCLC (Li et al. 2007). This mutated receptor has

constitutive tyrosine kinase activity and has important pro-oncogenic effects including proliferation and chemotherapeutic resistance (Fan et al. 2013).

Cetuximab (Erbitux, ImClone Systems/Bristol-Myers Squibb) and panitumumab (Vectibix, Amgen, Inc.) are both EGFR-specific antibodies. The former is a chimeric IgG1 monoclonal antibody and the latter is a fully humanized IgG2 isotype. Both inhibit EGFR-mediated signal transduction by preventing ligand binding and receptor dimerization, which induce cell cycle arrest and apoptosis in tumor cells (Li et al. 2005; Kim 2009). Cetuximab and panitumumab have been used as second- or third-line therapy for the treatment of metastatic colorectal cancer. Cetuximab is often used in combination with other chemotherapeutic regimens. The combination of cetuximab with folinic acid, 5-fluorouracil, and irinotecan (FOLFIRI chemotherapy) has been shown to prolong progression-free survival of patients with metastatic CRC harboring wild-type KRAS alleles (Van Cutsem et al. 2009). Necitumumab and nimotuzumab are another two anti-EGFR antibodies, which are competitive inhibitors of EGFR's ligand. Necitumumab combined with pemetrexed and cisplatin recently failed to show a benefit in overall survival of patients with NSCLC compared to pemetrexed and cisplatin alone (Paz-Ares et al. 2015). Nimotuzumab is approved for the treatment of various epithelial malignancies in Europe. For example, it is approved for pancreatic cancer treatment in Germany. It is also approved for use in some countries in Asia, South America, and Africa for the treatment of head and neck cancer, glioma, and nasopharyngeal cancer. Efforts are underway to target a truncated form of EGFR, EGFRvIII. A phase I study using the monoclonal antibody 806 (Zymed) targeting EGFRvIII showed good antibody penetration of tumor tissue and no significant toxicities in patients with metastatic disease (Scott et al. 2007).

2.1.2 Human Epidermal Growth Factor Receptor

Human epidermal growth factor receptor 2 (HER2) is a member of the ErbB/HER growth factor superfamily, which is composed of HER1 EGFR (HER1, ErbB1), HER2 (ErbB2), HER3, and HER4. HER2 has no known ligand and constitutively adopts an open configuration priming it for heterodimerization and increased mitogenic signaling. It is gene amplified and overexpressed in approximately 30 % of breast cancers and is overexpressed, although rarely gene amplified, by some gastrointestinal, lung, prostate, and ovarian adenocarcinomas (Chen et al. 2003). The expression of HER2 in breast cancer is associated with aggressive disease, a high recurrence rate, and reduced patient survival. Overexpression of HER2 leads to increased signal transduction and activation of the MAPK and P13K/AKT pathways (Yarden and Sliwkowski 2001). Trastuzumab (Herceptin, Genentech/Roche) was the first FDA-approved anti-HER2 antibody for $HER2^{+}$ breast cancer, and pertuzumab (Omnitarg, Genentech/Roche) is a newer one. Both are humanized IgG1 anti-HER2 antibodies. Trastuzumab binds the juxtamembrane domain IV region of HER2 and inhibits homo- and heterodimerization and internalization of HER2, whereas pertuzumab binds HER2 at the extracellular

dimerization subdomain II which is critical for heterodimerization of HER2 with other HER-family receptors, most notably HER3 (Hudis 2007; Franklin et al. 2004). A new anti-HER3 antibody MM-121 (Merrimack Pharmaceuticals), which is currently being developed, has been shown to inhibit growth of human tumor xenograft in mice (Schoeberl et al. 2009).

2.1.3 VEGF

Vascular endothelial growth factor (VEGF) is a glycoprotein produced by normal and malignant cells. VEGF and its isoforms are mitogens which bind and activate three different tyrosine kinase receptors, VEGFR1, VEGFR2, and VEGFR3, and play a very important role in the regulation of angiogenesis for both normal and malignant tissues. VEGFR2 is mainly expressed on the surface of vascular endothelial cells and highly expressed in many tumor types, including cancers of the gastrointestinal tract. VEGFA binding to VEGFR2 leads to autophosphorylation of tyrosine residues at the carboxy-terminal of the receptor, initiating cell signaling and angiogenesis (Sia et al. 2014). VEGF binds to its receptor on the vascular endothelium to stimulate the growth of new blood vessels to allow for tumor growth, and it also maintains the immature blood vessels.

Bevacizumab (Avastin, Genentech) is the first VEGFA-specific antibody that effectively blocks the activation of key pathways required in tumor angiogenesis by blocking the binding of VEGF to its receptor (Sullivan and Brekken 2010). It exerts its antitumor effect by functionally altering or slowing the formation of the tumor vasculature. Bevacizumab is approved for the treatment of breast, colorectal, and non-small cell lung cancer in combination with cytotoxic chemotherapy (Ellis and Hicklin 2008). The treatment of bevacizumab has led to production of bevacizumab-resistant tumors owing to upregulation of other pro-angiogenic mediators, such as platelet-derived growth factor (PDGF). PDGF receptor (PDGFR) signaling plays an important role in maintaining the endothelial support system, which stabilizes and promotes the growth of new blood vessels (Hirschi et al. 1998). Blockade of PDGFR signaling by a PDGFRβ-specific human antibody has been shown to synergize with anti-VEGFR2 therapy in preclinical models and suggests the utility of anti-PDGFRβ therapy in the setting of bevacizumab resistance (Shen et al. 2009). Ramucirumab (IMC-1121B, ImClone Systems) is a humanized anti-VEGFR2 antibody that blocks the VEGFR2-related signaling and activating pathways (Spratlin 2011). Ramucirumab was approved for use in advanced cases of gastric and gastroesophageal adenocarcinomas that have been refractory to first-line treatments. The therapeutic antibodies targeting VEGFR1 (IMC-18F1) are currently underway and have shown preclinical promise (Wu et al. 2006).

2.1.4 Hematopoietic Differentiation Antigens

Hematopoietic differentiation antigens are glycoproteins that are usually associated with cluster of differentiation (CD) grouping selectively expressed on hematopoietic cells. Some of them, such as CD20, CD52, CD19, etc., can be targeted by therapeutic antibodies for treatment of hematopoietic malignancies.

2.1.4.1 CD20

CD20 is a B-cell lineage marker expressed on the surface of normal B cells, but not mature plasma cells. It is also expressed on more than 90 % of B-cell neoplasms. Rituximab (Rituxan), a mouse-human chimeric monoclonal antibody against CD20, was initially developed in the early 1990s by FDA approved in 1997 for treatment of non-Hodgkin B-cell lymphoma and approved. Rituximab is the first antibody approved for malignancy therapy and perhaps the most studied (Grillo-Lopez et al. 2002). Ofatumumab (Arzerra, Genmab/GlaxoSmithKline), the first humanized anti-CD20 antibody, received accelerated approval in 2009 for the treatment of relapsed or refractory CLL which has failed to fludarabine and alemtuzumab (Gupta and Jewell 2012). Rituximab binds to the large loop of CD20 antigen alone, whereas ofatumumab binds to a novel epitope that includes both small and large loops. Binding kinetics of ofatumumab is superior, resulting in a lower off-rate when bounding to CD20 (Teeling et al. 2004). Accordingly, in vitro studies showed that ofatumumab activates complement more efficiently than rituximab (Pawluczkowycz et al. 2009). Ofatumumab has been shown to be more potent than rituximab against both rituximab-sensitive and rituximab-resistant cells (Barth et al. 2012). Its activity against rituximab-resistant cells and the potent cytotoxic effect are believed to be due to the proximal epitope of the small loop of CD20 molecule and the high capacity for C1q activation. The newer-generation humanized anti-CD20 antibodies have been developed to increase their binding affinity for the FcγRIIIA expressed on NK cells by engineering Fc region. These antibodies include obinutuzumab (GA-101), ocrelizumab (2H7, Genentech/Roche/Biogen Idec), and AME-133 (Applied Molecular Evolution/Eli Lilly), which are undergoing active clinical development (Cang et al. 2012). Obinutuzumab is a glycol-engineered anti-CD20 Ab in which Fc region was engineered to contain less fucose (Peipp et al. 2008). Obinutuzumab was approved by FDA for the treatment of CLL, and its activity in various B-cell malignancies is under clinical investigation.

2.1.4.2 CD52

CD52 is a cellular surface glycoprotein expressed on both normal and malignant B and T lymphocytes, but not on hematopoietic stem cells. It is also highly expressed

on B cell. Alemtuzumab (Campath), a humanized IgG1 antibody against CD52, was initially developed for the prevention of graft-versus-host disease (GVHD) in allogeneic bone marrow transplant. By binding to CD52, it induces ADCC of CLL cells (Hallek 2013). In 1997, a phase II trial of alemtuzumab was undertaken to evaluate the safety and efficacy in CLL patients who relapsed after standard chemotherapy. Alemtuzumab was approved in 2001 by FDA for the treatment of drug-resistant chronic lymphocytic leukemia (Ferrajoli et al. 2001). But it was withdrawn from the market in 2012. However, it is still available to patients with refractory CLL who have failed therapy with alkylating agents and second-line therapy with fludarabine.

2.1.4.3 CD19

CD19, a transmembrane protein, is a specific B-cell marker expressed on B cells along all differentiation stages of the lineage. In parallel, all cells derived from mature B-cell malignancies express CD19, except for plasma cell disorders, although the levels of CD19 expression are lower in CLL, mantle cell lymphoma, B-prolymphocytic leukemia, follicular lymphoma, and diffuse large B-cell lymphoma samples, compared with normal B cells. CD19 staining is considered mandatory in the immunophenotyping schemes of the acute lymphoblastic leukemias (ALLs). The fact that CD19 is expressed by a wide range of B-lymphoid malignancies, but not by hematopoietic stem cells and pro-B cells (van Zelm et al. 2005), makes it an attractive target for antibody-mediated therapy. Humanized anti-CD19 antibodies have been designed to attract components of the immune system, predominantly T cells, to eliminate $CD19^+$ target cells, such as modified anti-CD19 antibodies (Awan et al. 2010) and bispecific anti-CD19/anti-CD3 antibodies (Topp et al. 2011). One of the most attractive approaches to target malignant B cells is the introduction of chimeric antigen receptors (CARs), composed of single-chain anti-CD19 antibody and intracellular signaling components for T-cell activation, into patient-derived T cells (Porter et al. 2011). The novel anti-B-cell therapeutics have shown promising clinical effects against various B-cell malignancies, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin lymphoma (NHL).

2.1.4.4 CD30

CD30, a membrane glycoprotein, is a member of TNF receptor family which is expressed on activated, but not resting, T and B cells. CD30 expression is very low in normal tissues. However, CD30 shows highly selective expression on tumor cells. In particular, CD30 is broadly expressed in a variety of lymphoid malignancies. CD30 expression is also observed on nonlymphoid embryonal carcinomas and occasionally in nasopharyngeal cancers. Brentuximab vedotin (Adcetris, Seattle Genetics) is a chimeric anti-CD30 antibody conjugated to the highly potent

auristatin derivative MMAE through the cleavable linker (Sievers and Senter 2013). Brentuximab vedotin treatment causes complete regression of established tumors in xenograft models of Hodgkin lymphoma and anaplastic large-cell lymphoma. Brentuximab vedotin was approved in 2011 for the treatment of patients with Hodgkin lymphoma and systemic anaplastic large-cell lymphoma (Katz et al. 2011).

2.2 *Clinical Efficacy of Tumor-Targeted Antibody Therapeutics*

Over 13 tumor-targeted antibodies have been approved by the FDA for the treatment of a variety of solid tumors and hematological malignancies (Table 1.1). Meanwhile a large number of therapeutic antibodies are currently being tested in early and late-stage clinical trials. The most successful therapeutic antibodies in patients with solid tumors are the classes of antibodies targeting the members of EGFR family (such as EGFR and HER2) and VEGF. More importantly, there are some predictive biomarkers that are pivotal in optimal selection of patients for these therapeutics. For example, colorectal cancers bearing wild-type KRAS (Kirsten rat sarcoma viral oncogene) tumor treated with anti-EGFR antibodies have improved responses and survival (Van Cutsem et al. 2009; Amado et al. 2008). The use of trastuzumab has also been restricted to patients with either 3+ immunohistochemical staining or fluorescence in situ hybridization positive for ErbB2 (HER2) expression. In the hematologic realm, the antibody against CD20 has enjoyed considerable success in patients with non-Hodgkin B-cell lymphoma and chronic lymphocytic leukemia.

2.2.1 Hematological Malignancies

There are currently two chimeric antibodies (rituximab and brentuximab vedotin) and three fully humanized antibodies (alemtuzumab, eculizumab, and ofatumumab) that are FDA approved for treatment of hematologic diseases. The first approved antibody was rituximab, which was initially approved for the treatment of non-Hodgkin B-cell lymphoma. Since then, the use of rituximab has grown widely to encompass not only a variety of B-cell malignancies but also immune-mediated disorders (i.e., rheumatoid arthritis, systemic lupus erythematosus, immune-mediated thrombocytopenia, autoimmune hemolytic anemia, cryoglobulinemia, etc.). Rituximab has been studied in a number of clinical trials, which have successfully demonstrated improvement in progression-free and overall survival in non-Hodgkin lymphoma (including follicular lymphoma and diffuse large B-cell lymphoma) as well as improvement in progression-free survival in chronic lymphocytic leukemia. Rituximab in combination with cyclophosphamide,