Review

Next generation of antibody therapy for cancer

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Abstract

      Monoclonal antibodies (mAbs) have become a major class of therapeutic agents providing effective alternatives to treating various human diseases. To date, 15 mAbs have been approved by regulatory agencies in the world for clinical use in oncology indications. The selectivity and specificity, the unique pharmacokinetics, and the ability to engage and activate the host immune system differentiate these biologics from traditional small molecule anticancer drugs. mAb-based regimens have brought clinical benefits, including improvements in overall survival, to patients with a variety of cancers. Many challenges still remain, however, to fully realize the potential of these new medicines. With our further understanding of cancer biology, mechanism of antibody action, and advancement of antibody engineering technologies, many novel antibody formats or antibody-derived molecules are emerging as promising new generation therapeutics. Carefully designed and engineered, they retain the advantage of specificity and selectivity of original antibodies, but in the meantime acquire additional special features such as improved pharmacokinetics, increased selectivity, and enhanced anticancer efficacy. Promising clinical results are being generated with these newly improved antibody-based therapeutics.

Key words Cancer, antibodies, antibody engineering, cancer therapeutics

The first major milestone in the history of antibody research and development was the invention of hybridoma technology to create monoclonal antibodies (mAbs) in 1975 by Georges Kohler and Cesar Milstein[1], who were awarded the Nobel Prize in 1984. In 1986, OKT3, the first antibody derived from mouse hybridoma, was approved for use in organ transplant patients to prevent rejection. The mouse hybridoma-derived antibodies, however, can be recognized by the human immune system as foreign antibodies resulting in human anti-mouse antibody (HAMA) response, leading to shortened half-life, reduced efficacy, and increased toxicity in some patients due to immune responses. The immunogenicity of mouse-derived antibodies can be reduced by recombinant DNA engineering technologies, such as antibody chimerization[2] and humanization[3,4], by replacing portions of murine antibody with their human counterparts. Technologies were established to generate fully human antibodies, such as phage display libraries[5] and transgenic mice [6-8], to further reduce antibody immunogenicity.

Among over 30 therapeutic antibodies approved for clinical use, 15 of them are for oncology indications (Table 1). In combination with cytotoxics or radiation therapy, these mAbs have delivered significant clinical improvements in treating lymphoma [rituximab (Rituxan)], breast cancer [trastuzumab (Herceptin)], colorectal cancer [bevacizumab (Avastin), cetuximab (Erbitux), and panitumumab (Vectibix)], non–small cell lung cancer (NSCLC) [bevacizumab (Avastin)], and squamous cell cancer of the head and neck [cetuximab (Erbitux)], and are expanding into broader indications. However, clinical benefits are often limited to transient tumor responses seen only in a fraction of patients with incremental improvements in progression-free survival (PFS) and overall survival (OS) [9]. New approaches to further improve the efficacy of these mAb therapies include (a) selecting patients who may derive the most benefit based on the molecular characteristics of their tumors; (b) improving biodistribution to effectively deliver mAbs

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Table 1. Monoclonal antibodies approved for therapeutic use

Generic name	Trade name	Antibody format	Antigen	Approved indication	FDA approval	EMEA approval	Sponsor
Rituximab	Rituxan	Chimeric IgG1κ	CD20	Non-Hodgkin's lymphoma, chronic lymphocytic leukemia and rheumatoid arthritis	Dec 26, 1997	Jul 2, 1998	Genentech and Biogen Idec
Trastuzumab	Herceptin	Humanized IgG1κ	Her2	Breast cancer	Sep 25, 1998	Aug 28, 2000	Genentech
Gemtuzumab Ozogamicin[a]	Mylotarg[a]	Calicheamicin-humanized IgG4κ	CD33	Acute myeloid leukemia	May 17, 2000	NA	Wyeth
Alemtuzumab	Campath	Humanized IgG1κ	CD52	B-cell chronic lymphocytic leukemia	May 7, 2001	Jul 6, 2001	Ilex Pharma
Ibritumomab Tiuxetan	Zevalin	^{90}Y-murine IgG1κ	CD20	B-cell non-Hodgkin's lymphoma	Feb 19, 2002	Jan 16, 2004	Spectrum Pharms
Tositumomab	Bexxar	^{131}I-murine IgG2aλ	CD20	Non-Hodgkin's lymphoma	Jun 27, 2003	NA	Smithkline Beecham
Cetuximab	Erbitux	Chimeric IgG1κ	EGFR	Head and neck cancer, colorectal cancer	Feb 12, 2004	Jun 29, 2004	ImClone/BMS/Merck kGa
Bevacizumab	Avastin	Humanized IgG1κ	VEGF-A	Various solid tumors	Feb 26, 2004	Jan 12, 2005	Genentech
Panitumumab	Vectibix	Human IgG2κ	EGFR	Metastatic colorectal carcinoma	Sep 27, 2006	Dec 19, 2007	Amgen
Ofatumumab	Arzerra	Human IgG1κ	CD20	Chronic lymphocytic leukemia	Oct 26, 2009	NA	Glaxo Grp Ltd
Denosumab	Xgeva	Human IgG2	RANKL	Prevention of cancer skeleton-related events	Nov 18, 2010	NA	Amgen
Catumaxomab	Removab	Murine/rat hybrid IgG	EpCAM and CD3	Intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas		Apr 20, 2009	TRION Pharma
Edrecolomab[b]	Panorex[b]	Murine IgG2a	EpCAM	Colon cancer			Centocor
I131-TNT[c]	Cotara[c]	^{131}I-chimeric IgG1	DNA	Lung cancer			Peregrine Pharma
Nimotuzumab[d]	Theracim[d]	Humanized IgG1	EGFR	Nasopharyngeal carcinomas, glioblastoma, and head and neck tumors			CIM/CIMAB/YM Biosciences

[a]Gemtuzumab Ozogamicin (Mylotarg) was withdrawn from the market in 2010. [b]Edrecolomab (Panorex) was approved in Germany in 1995. [c] ^{131}I-TNT (Cotara) was approved in China in 2003. [d]Nimotuzumab (Theracim) was approved in Cuba, Argentina, Colombia, India, and China in 2005 and 2006.

to susceptible tumor cells to achieve maximal target and pathway inhibition; (c) optimizing antibody immune effector mechanisms such as complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC); (d) molecular engineering of new antibody formats, for example, bispecific antibody, antibody-drug conjugate, and Fc modification for prolonged *in vivo* half-life[10].

Major Classes of Anti-Cancer Antibody Therapeutics

Anti-CD20 antibodies

CD20, human B-lymphocyte-restricted differentiation antigen Bp35, is a non-glycosylated phosphoprotein of 33–37 kDa expressed on cell surface of normal B lymphocytes and B-cell lymphomas.

Rituximab (Rituxan) Rituximab (IDEC-C2B8, Rituxan, MabThera) is a chimeric IgG1 anti-CD20 mAb. The mechanisms of action of rituximab include ADCC, CDC, induction of apoptosis, anti-proliferation, and chemosensitization [11-13]. Rituximab was genetically engineered by fusing the murine variable regions of the anti-CD20 mAb 2B8 with the human IgG1 constant regions[14]. In the pivotal trial of 166 patients with relapsed low grade or follicular lymphoma, the overall response rate (ORR) was 48%, with 6% complete response (CR) and 42% partial response (PR), and 76% of patients had at least a 20% reduction in tumor size[15]. The median duration of response was 11.2 months, with a time-to-progression (TTP) of 13.0 months. In 1997, only 4 years after initiation of the phase I study, rituximab was approved by the US FDA as the first mAb for treating relapsed or refractory, low grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma (NHL).

⁹⁰Y-ibritumomab tiuxetan (Zevalin) and ¹³¹I-tositumamab (Bexxar) Two radioisotope-conjugated anti-CD20 antibodies, ibritumomab tiuxetan (Zevalin), a ⁹⁰Y-labeled anti-CD20 antibody[16], and ¹³¹I-tositumamab (Bexxar), a ¹³¹I-labeled anti-CD20 antibody for NHL[17], have also been developed. Although these antibodies have demonstrated impressive clinical activity and efficacy, their use has been hindered by the requirements for specialized facility and professionals to administrate the radioactive treatments.

Ofatumumab (Arzerra) Ofatumumab (Arzerra), also known as HuMax-CD20, was developed by Genmab and GSK. Ofatumumab is a full human IgG1 anti-CD20 antibody targeting a distinct small-loop epitope on the CD20 molecule different than that of rituximab, with improved CDC and ADCC compared with rituximab. Therefore, ofatumumab is able to lyse rituximab-resistant cells that express low levels of CD20. In the pivotal trial, the primary efficacy population consisted of 59 patients with chronic lymphocytic leukemia (CLL) refractory to fludarabine and alemtuzumab. Objective RR was 42% [99% confidence interval (CI), 26% to 60%], with a median duration of response of 6.5 months (95% CI, 5.8 to 8.3 months). In 2009, US FDA granted accelerated approval of ofatumumab for treating CLL refractory to fludarabine and alemtuzumab[18-20].

Anti-HER2 antibodies

The HER2/neu (*erbB2*) proto-oncogene product HER2 is a member of the HER (erbB) family of receptor tyrosine kinases overexpressed in ~25% of breast cancer patients with or without HER2/neu gene amplification[21].

Trastuzumab (Herceptin) Trastuzumab (Herceptin) is a humanized IgG1 derived from 4D5, one of over 100 murine mAbs generated following immunization of mice with cells overexpressing human HER2. Trastuzumab binds the HER2 extracellular domain with high affinity (K_d = 0.10 nmol/L) to block HER2 homodimer formation and therefore HER2 signaling.

Two pivotal trials were conducted to investigate trastuzumab in patients with metastatic breast cancer, either as a single agent in previously treated patients[22] or in combination with chemotherapy drugs in the first-line setting[23]. Eight CR and 26 PR were observed in 222 patients enrolled, accounting for an objective RR of 15%, with 26% of patients deriving clinical benefits of stable disease (SD) $\geq$ 6 months. The median duration of response was 9.1 months; the median OS was 13 months. The most clinically significant adverse event, cardiac dysfunction, occurred in 4.7% of patients. In the combination trial, 469 patients with HER2-overexpressing breast cancer [2+ or 3+ immunohistochemistry (IHC) score] were randomized to undergo chemotherapy alone or in combination with trastuzumab. Patients who underwent combination treatment experienced significantly improved median TTP (7.4 vs. 4.6 months), RR (50% vs. 32%), and OS (25.1 vs. 20.3 months) despite that 65% of patients undergoing chemotherapy were allowed to cross-over at disease progression. The most important adverse event was cardiac dysfunction, which occurred more frequently in patients undergoing concurrent trastuzumab and anthracycline.

Herceptin was approved in 1998 for patients with tumors evaluated to overexpress HER2 by HercepTest (IHC test) or to have HER2 gene amplification by PathVysion (FISH assay). The inclusion of only HER2-overexpressing patients in the trial represents the first such approach to including a biomarker in order to prospectively select patients in the clinical development of an anti-cancer therapy.

Pertuzumab (Omnitarg) Pertuzumab (Omnitarg) is a humanized IgG1 anti-HER2 antibody that binds to different epitope(s) than that of trastuzumab, and prevents HER2 from both homodimerizing with HER2 and heterodimerizing with HER1 and HER3. When combined with trastuzumab, pertuzumab elicits complementary mechanisms of action and results in enhanced antitumor activity. In a phase II study in patients with HER2+ metastatic breast cancer who progressed during prior trastuzumab therapy, the objective RR was 24.2%, and the clinical benefit rate (CBR) was 50%. Five patients (7.6%) experienced a CR, 11 (16.7%) experienced a PR, and 17 (25.8%) experienced stable disease of $\geq$ 6 months with a median PFS of 5.5 months[24]. This combination is now being evaluated in a phase III trial[25]. Phase II studies conducted in patients with prostate cancer, ovarian cancer, or NSCLC only showed limited clinical activities.

Anti-VEGF antibody

Bevacizumab (Avastin) Bevacizumab is a humanized IgG1 derived from a murine anti-human vascular endothelial growth factor (VEGF) mAb A.4.6.1. Bevacizumab recognizes all isoforms of VEGF with high affinity (Kd, 0.8 nmol/L), and inhibits VEGF-induced proliferation of endothelial cells *in vitro* and tumor growth *in vivo* with potency and efficacy similar to the parent murine antibody.

The first pivotal phase III trial[26] was conducted in over 800 patients with metastatic colorectal cancer (mCRC) randomized to bolus IFL [irinotecan, 5-fluorouracil (5-FU) and leucovorin] plus placebo or bevacizumab. The overall RR was 44.8% vs. 34.8% with duration of 10.4 and 7.1 months in the bevacizumab

group and the placebo group, respectively. The median PFS was 10.6 vs. 6.2 months, and median survival was 20.3 vs. 15.6 months. The main toxicities were grade 3 hypertension (11% vs. 2.3%), proteinuria, and arterial thromboembolic events (4.4% vs. 1.9%). These promising outcomes led to the FDA approval of the bevacizumab as first-line therapy in combination with IFL in mCRC patients in February 2004. Recent phase III study results[27] showed that bevacizumab plus FOLFOX4 also extended survival in second-line setting with median survival of 13.0 vs. 10.8 months. In 2006, bevacizumab was approved by the FDA for second-line mCRC treatment in combination with intravenous 5-FU based chemotherapy.

In ECOG4599 phase III study, 878 patients with NSCLC were randomized to receive paclitaxel and carboplatin with or without bevacizumab[28]. The RR was 27% vs. 10% with median OS of 12.3 vs. 10.3 months, and median PFS of 6.4 vs. 4.5 months, respectively. Bevacizumab was approved to be used with chemotherapy as first-line NSCLC treatment in 2006. In the AVAiL trial, bevacizumab was combined with cisplatin and gemcitabine at two dose levels, 7.5 mg/kg and 15 mg/kg, every three weeks. Although improvements in RR and PFS were observed in both dose level groups, no difference in PFS or OS was observed between the high and low doses[29]. Furthermore, no prolongation of OS was achieved when bevacizumab was added to chemotherapy in these lung cancer patients[30].

Two pivotal phase III trials investigated bevacizumab plus capecitabine in patients with metastatic breast cancer who received prior chemotherapy[31], and bevacizumab plus paclitaxel in patients with previously untreated metastatic breast cancer[32]. In the first study, no difference was observed in either PFS (4.9 vs. 4.2 months) or OS (15.1 vs. 14.5 months) although the bevacizumab combination yielded a higher RR (19.8% vs. 9.1%). In the second study, E2100, paclitaxel plus bevacizumab significantly increased median PFS (11.8 vs. 5.9 months) and objective RR (36.9% vs. 21.2%). However, median OS was similar in the two groups (26.7 vs. 25.2 months). On February 22, 2008, the FDA granted accelerated approval of bevacizumab in combination with paclitaxel as front-line treatment of metastatic HER2-negative breast cancer. However, results from additional phase III trials, including RIBBON-1, RIBBON-2, and AVADO (BO17708) studies in patients with locally recurrent or metastatic HER2-negative breast cancer, demonstrated only PFS but not OS improvements of bevacizumab in combination with various chemotherapies[33]. The US FDA in December 2010 recommended to withdraw the approval of bevacizumab for treating metastatic breast cancer.

Anti-EGFR antibodies

Dysregulated activation of the EGFR signaling pathway leads to aberrant stimulation of tumor proliferation, angiogenesis, invasion, metastasis, and inhibition of apoptosis. EGFR expression levels are highly predictive of clinical outcomes for patients with head and neck, ovarian, cervical, bladder, and esophageal cancers[34]. K-Ras mutations are negative predictor of clinical outcomes in colorectal cancer patients undergoing anti-EGFR therapies[35].

Cetuximab (Erbitux) Cetuximab is a chimeric monoclonal IgG1 antibody that binds to the EGFR with high affinity. The antibody blocks ligand binding and induces receptor internalization and degradation, resulting in down-regulation of surface EGFR expression.

Cetuximab is approved as monotherapy or in combination with irinotecan for treating irinotecan-refractory mCRC, as monotherapy for metastatic squamous cell cancer of the head and neck (SCCHN), or in combination with radiation therapy for unresectable SCCHN. In a pivotal trial, 329 mCRC patients whose disease had progressed during or within 3 months after treatment with an irinotecan-based regimen were randomized 2:1 to undergo either cetuximab and irinotecan or cetuximab monotherapy. The RR was significantly higher in the combination therapy group than in the monotherapy group [22.9% (95% CI, 17.5% to 29.1%) vs. 10.8% (95% CI, 5.7% to 18.1%), *P* = 0.007]. The median TTP was significantly greater too (4.1 vs. 1.5 months, *P* < 0.001). The median survival time was 8.6 vs. 6.9 months (*P* = 0.48)[36]. In another randomized, multicenter, phase III trial, cetuximab plus best supportive care (BSC) was compared to BSC alone in 572 patients with chemotherapy-refractory mCRC. A 23% increase in OS (6.1 vs. 4.6 months, *P* = 0.005) and an increase in PR (8% vs. 0, *P* < 0.001) were observed[37].

Likewise, in a phase III trial involving 424 patients with locoregionally advanced SCCHN, the addition of cetuximab to high-dose radiation resulted in a median survival of 49.0 months compared with 29.3 months with radiation alone and a 26% reduction in the risk of mortality (*P* = 0.03)[38]. In the first-line phase III study (EXTREME study), 442 patients with stage III/IV recurrent and/or metastatic SCCHN were treated with 5-fluorouracil plus either cisplatin or carboplatin with or without cetuximab. The addition of cetuximab significantly improved median OS (10.1 vs. 7.4 months) and median PFS (5.6 vs. 3.3 months), and increased RR (36% vs. 20%) compared with chemotherapy alone[39].

In addition to mCRC and SCCHN, cetuximab has also been tested in combination with chemotherapeutic

agents in patients with advanced NSCLC. In a large, randomized multi-national, phase III study (FLEX study), cetuximab in combination with platinum-based chemotherapy (vinorelbine plus cisplatin) significantly increased the patient's overall survival compared with chemotherapy alone. Cetuximab thus may provide a new option for NSCLC patients, particularly those who are not eligible for or can not tolerate bevacizumab treatment.

Panitumumab (Vectibix)   Panitumumab is a fully human IgG2 anti-EGFR antibody originally derived from transgenic human mouse. In an open-labeled phase III trial[40], 463 patients with mCRC who had failed standard chemotherapy were randomized to undergo 6 mg/kg panitumumab plus BSC or BSC alone. The objective RR was 10% vs. 0, with the median PFS of 8 vs. 7.3 weeks. No difference was observed in OS likely due to a large number of patients cross-over from BSC to panitumumab arm. In a recent retrospective analysis of the trial, responses to panitumumab were seen only in those patients with wild-type *K-Ras*, and these patients also had a longer median TTP (12.3 vs. 7.4 weeks). Further, a longer OS was seen in patients with wild-type *K-Ras* than those with *K-Ras* mutations[35]. Panitumumab is approved as monotherapy for refractory mCRC in the US, and recently, in European Union as monotherapy for mCRC patients with wild-type *K-Ras* selected using TheraScreen K-RAS kit[41].

Anti-RANKL antibody

Denosumab (Xgeva)   Denosumab is a full human IgG2 mAb that binds to receptor activator of nuclear kappa-B ligand (RANKL) with high affinity and specificity, preventing RANKL binding to its receptor RANK on esteoclasts and their precursor cells. Denosumab therefore decrease bone resorption and increase bone mass and strength.

The efficacy and safety of denosumab were investigated in 1468 men with non-metastatic prostate cancer undergoing androgen-deprivation therapy at increased risk of fracture [42]. The primary efficacy end point was percent change in bone mineral density (BMD) at the lumbar spine after 24 months; key secondary end points included percent change in BMD at the femoral neck and total hip after 24 months and at all three sites at 36 months, as well as the incidence of new vertebral fractures. At 24 months, BMD of the lumbar spine had increased by 5.6% in the denosumab group as compared with a loss of 1.0% in the placebo group ($P < 0.001$); significant differences between the two groups were seen at as early as 1 month and sustained 36 months. Denosumab therapy was also associated with significant increases in BMD at the total hip, femoral neck, and distal third of the radius at all time points.

Patients who underwent denosumab therapy had a decreased incidence of new vertebral fractures at 36 months (1.5%, vs. 3.9% with placebo; relative risk, 0.38; 95% CI, 19% to 78%; $P = 0.006$).

In 2010, denosumab was granted marketing authorization by the European Commission for treating bone loss associated with hormone ablation therapy in men with prostate cancer at increased risk of fracture.

Denosumab also demonstrated clinical efficacy in delaying or preventing skeletal-related events (SREs) in breast cancer patients with bone metastases. In the phase III study of 1026 breast cancer patients, denosumab was superior to zoledronic acid in delaying time to first on-study SRE (hazard ratio, 0.82; 95% CI, 0.71 to 0.95; $P = 0.01$) and time to first and subsequent (multiple) on-study SREs (rate ratio, 0.77; 95% CI, 0.66 to 0.89; $P = 0.001$). With the convenience of a subcutaneous injection and no requirement for renal monitoring, denosumab represents an attractive treatment option for patients with bone metastases[43].

Engineering of Next Generation Antibody Therapeutics

The major focus of antibody engineering in the past three decades has been to reduce immunogenicity of murine antibodies and to improve manufacturability. With recombinant engineering technology and our further understanding of disease biology and mechanisms of action of antibodies, an array of novel classes of antibody formats or antibody-derived molecules are emerging as promising new generation therapeutics. These new biologics are carefully designed and engineered to acquire special features, such as improved pharmacokinetics, increased selectivity, and enhanced efficacy. For oncology applications, antibody drug conjugate (ADC) has recently shown clinical promises.

Antibody drug conjugates

ADC technology was developed to enhance the clinical efficacy of therapeutic antibodies by leveraging the target specificity of mAbs to deliver therapeutic payloads, typically radioactive isotopes, chemotherapeutic drugs, or toxins, to target cancer cells. The non-specific toxic side effects of chemotherapy on normal tissues can therefore be potentially minimized by reducing systemic exposure.

Three such ADCs have been approved by the US FDA: gemtuzumab ozogamicin (Mylotarg), a humanized anti-CD33 antibody conjugated to calicheamicin for acute myeloid leukemia [44], ibritumomab tiuxetan (Zevalin), an ^{90}Y-labeled anti-CD20 antibody, and tositumomab

(Bexxar), an ^{131}I-labeled murine anti-CD20 antibody for NHL[16,17,45].

Trastuzumab-DM1 (T-DM1) Recently, Genentech has developed trastuzumab-DM1 (T-DM1), a HER2 ADC, which shows greater activity compared with nonconjugated trastuzumab (Herceptin) while maintaining selectivity for HER2-overexpressing tumor cells [46,47]. T-DM1 consists of the potent anti-microtubule cell-killing agent DM1, attached to the HER2-binding antibody, trastuzumab, via a stable linker. A single-arm, multi-center trial assessed T-DM1 as a single agent in 110 women with HER2-positive advanced breast cancer whose disease had progressed after prior HER2-targeted therapy and chemotherapy. The ORR by independent assessment was 25.9% (95% CI, 18.4% to 34.4%). Median duration of response was not reached as a result of insufficient events (lower limit of 95% CI, 6.2 months), and median PFS time was 4.6 months (95% CI, 3.9 to 8.6 months). The RR was higher among patients with confirmed HER2-positive tumors (immunohistochemistry 3+ or fluorescent *in situ* hybridization positive). Genentech submitted a Biologics License Application (BLA) on July 7, 2010, but the filing was refused by the FDA (refuse-to-file) on August 27, 2010. Genentech plans to resubmit the BLA around mid 2012.

Brentuximab Vedotin (SGN-35) Another promising ADC is brentuximab vedotin (SGN-35, Seattle Genetics), consisting of an anti-CD30 mAb linked to a potent, synthetic drug payload monomethyl auristatin E (MMAE) via an enzyme cleavable linker[48]. In a pivotal trial, 102 patients with relapsed or refractory Hodgkin lymphoma (HL) were treated with 1.8 mg/kg of brentuximab vedotin q3w for up to 16 total doses, and 75% of them achieved an objective response. In a phase II trial, 58 patients with relapsed or refractory systemic anaplastic large cell lymphoma (ALCL) were treated with brentuximab vedotin, and 86% of them achieved an objective response. On February 28, 2011, Seattle Genetics submitted a BLA to the FAD for the use of SGN-35 in relapsed or refractory HL and relapsed or refractory systemic ALCL.

In addition to T-DM1 and SGN-35, a dozen of other ADCs, for example inotuzumab-ozogamicin, HuC242-DM4, CR011-MMAE, MDX1203-MGBA, and MEDI-547, are also in clinical trials[49].

Bispecific antibody

Cancer is an extremely complex disease process driven by multiple mechanisms involving interlinked signaling pathways. Individual therapeutic entities targeting a specific node of biology network in cancer cells cannot eradicate the disease or prevent the disease from recurrence. Simultaneously attacking pathways by combining different drugs that act on different targets and/or mechanisms presents an attractive means to overcome treatment resistance. Such approach has demonstrated efficacy exemplified by chemotherapy regimens consisting of several cytotoxic agents. The combinational strategy has also shown efficacy when combining therapeutic antibodies with cytotoxic agents, as well as simultaneously administrating two or more therapeutic antibodies.

A promising alternative approach to combining individual antibodies is to create a dual targeting bispecific antibody (BsAb), a single antibody molecule capable of strong and specific binding simultaneously with, and also modifying, two different disease-relevant targets[50].

Based on the mechanisms of action, dual-targeting BsAb can be constructed to bind to different targets of the signaling pathways within the same diseased cells. Simultaneously modifying (e.g., blocking or neutralizing) two disease-associated targets should provide the benefits of both enhancing the therapeutic efficacy of and also blocking the compensatory mechanisms associated with the individual antibody therapy, thus circumventing resistance to monotherapy[51-53]. By simultaneously binding to different cell surface targets, BsAb may result in enhanced binding avidity, leading to preferential (strong) binding to only cells that express both targets but not cells that only express a single target, thus fine-tuning the antibody selectivity[54]. BsAb can also be designed to bind to different targets expressed on different cell populations within the diseased tissues to achieve synergistic therapeutic effects and/or to enhance specific tissue distribution[55]. Further, BsAb can be created from two antibodies that bind to different epitopes on the same target (i.e., bi-paratopic binding) to enhance binding avidity[56] and to increase antibody load on tumor cells for enhanced effector functions, such as ADCC and/or CDC[53]. Furthermore, bi-paratopic BsAb, by simultaneously binding to two different epitopes on the same target molecule, could even potentially acquire new function that could not be achieved with the parent antibodies when used alone or in combination[56].

A major obstacle in the successful development of BsAb has been the difficulty of producing the materials in sufficient quality and quantity for both preclinical and clinical studies. The technological challenge is to construct a recombinant molecule with good pharmaceutical properties comparable to those of the conventional mAb, such as good molecule characteristics (e.g., homogeneity, stability, low aggregation propensity), and ease of manufacturing and downstream processing including high level productivity, simple purification process and no special need for

formulation. On the biology front, it has long been known that not every single combination of cytotoxics or other disease-modifying agents would necessarily lead to additive or synergistic therapeutic effect. Developing highly effective BsAbs will require clear elucidation and understanding of the molecular details in the aberrant signaling pathways that lead to various diseases to guide the selection of the target pairs for co-targeting.

Antibody with modified Fc functions

In addition to the direct effect of binding to and neutralizing an antigen, antibodies can mediate a variety of "effector" functions such as ADCC and CDC via their Fc regions. By fixing complement or activating immune cells such as natural killer cells, macrophages, and T cells, antibodies can mediate additional cell killing against target cells. These effector mechanisms are particularly relevant when antibodies are used to treat cancer. Better clinical responses to rituximab were observed in NHL patients carrying an IgG FcRIIIa of V158 allotype, an allotype with higher affinity binding to the Fc region of an IgG compared to the F158 allotype [57]. Similarly, patients carrying the 158 VV genotype of FcRIIIa were also associated with a better clinical response to trastuzumab [58] and cetuximab [59]. Based on these clinical observations, it is plausible to further enhance the therapeutic efficacy of a mAb by optimizing (increasing) its Fc interaction with the Fc regions on effector cells, via molecular engineering and/or manufacturing process modification[60-62].

By combining various molecular engineering methods, including alanine scanning, site-directed mutagenesis, computational structure-based design/algorithm and experimental selections [60,61,63,64], a large set of Fc variants has been generated to provide a broad spectrum of FcγR-binding profiles. Several variants provide up to 100-fold greater affinity for FcγRIIIa, resulting in an enhanced ADCC, for up to 2 to 3 logs higher than what can be achieved by the wild-type antibody [62,65]. For example, an Fc-engineered anti-CD19 antibody, with 100- to 1000-fold increased ADCC, delivered substantially more potent antitumor activity than its IgG1 analogue in mouse tumor models[66]. In addition to ADCC, an IgG1/IgG3 chimeric Fc, constructed via alternative domain shuffling, has shown markedly increased CDC activity both *in vitro* and *in vivo* in a cynomolgus monkey model[67].

The glycosylation of the antibody Fc domain has a major influence on its binding affinity for FcγRs. The carbohydrates at the position Asn297 are critical for Fcγ Rs binding in the presence of fucose and its glycosylation content at this position can negatively influence ADCC activity of mAbs. New cell lines capable of producing defucosylated mAbs have been established,

such as Chinese hamster ovary (CHO) cell lines that are genetically engineered (knock-out) to delete the FUT8 gene coding for the enzyme α-1,6-fucosyltransferase [68]. Alternatively, CHO cell lines that over-express a recombinant β-1,4-N-acetylglucosaminyltransferase III (GnTIII) have also been established. Antibodies produced in these cell lines are enriched in bisected and non-fucosylated oligosaccharides and are more potent than their counterparts produced in the wild-type CHO cell lines in mediating ADCC towards target cells[69].

Another major function of the antibody Fc is to bind to FcRn (the neonatal Fc receptor), the major mechanism responsible for the long circulation half-life of an antibody. A long serum half-life is generally desirable as it would enable less frequent dosing, thus potentially reduce both the inconvenience and the total cost of the treatments. Various mutations within the Fc domain of an IgG1 can result in significantly improved binding properties to FcRn. The enhanced FcRn-binding resulted in increased circulation half-life and improved *in vivo* dosing regimen[70-75]. The kinetics of Fc/FcRn interaction may also have an effect on the extent to which improved binding translates into extended serum half-life[76].

Perspectives

Compared to low molecular weight chemical drugs, therapeutic antibodies have higher target specificity, lower systemic toxicity, longer half-life, and potentially higher barrier for generic (or biosimilar) competition. The future growth of mAb-based therapeutics is still strong. Among all biologics that are being studied in clinical trials, 85% are mAbs or antibody-based molecules. Antibodies and antibody-based therapeutics consist of more than one third of all new agents currently under both preclinical and clinical development by biotechnology or pharmaceutical companies. Consensus sales forecasts predict that, by 2014, 6 out of the world's top 10 best-selling drugs will be therapeutic antibodies or antibody fusion proteins, and the total sales of mAb-based therapeutics will approach $58 billion dollars[77].

There are many challenges remaining in the continuous successful development of mAb therapeutics. New therapeutic targets are limited and therefore competition on validated targets is fierce. With the advancement of ADC technology, certain previously "undrugable" targets, such as antigens overexpressed on diseased cells with no known major pathophysiologic functions for intervention (e.g., CD33), may prove to be good candidates for ADC development. Targeting different epitopes of validated target antigens represents another opportunity to generate antibody therapeutics with differential clinical performances. Such examples include ofatumumab (anti-CD20), nimutuzumab (anti-EGFR), and others. Furthermore, there are

individual targets (pathways) that may not provide sufficient therapeutic benefit when being modified alone, but if modulated simultaneously with other targets (pathways), could produce additive and/or synergistic activity. These targets thus represent excellent candidates for the development of BsAb therapeutics.

Development of new technology platforms in antibody discovery, engineering, and production to increase the efficacy of therapeutic mAb and to reduce the cost of the therapy represent the other critical components. We expect to witness more novel mAbs and mAb-like molecules with precisely engineered features to enter

clinical development in the near future. New manufacturing and downstream process development (purification and formulation) are needed urgently to transform these novel therapeutics to affordable treatment options. Many other issues, for example, analytical and quality control, preclinical toxicology and pharmacology studies, regulatory and clinical development pathways, remain to be carefully researched and addressed before we see these new antibody formats become mainstream therapeutics.

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