The biopharmaceutical industry's pipeline of anticancer antibodies includes 165 candidates with substantial diversity in composition, targets and mechanisms of action that hold promise to be the cancer drugs of the future.

Foundation review: The future of antibodies as cancer drugs

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Targeted therapeutics such as monoclonal antibodies (mAbs) have proven successful as cancer drugs. To profile products that could be marketed in the future, we examined the current commercial clinical pipeline of mAb candidates for cancer. Our analysis revealed trends toward development of a variety of noncanonical mAbs, including antibody–drug conjugates (ADCs), bispecific antibodies, engineered antibodies and antibody fragments and/or domains. We found substantial diversity in the antibody sequence source, isotype, carbohydrate residues, targets and mechanisms of action (MOA). Although well-validated targets, such as epidermal growth factor receptor (EGFR) and CD20, continue to provide opportunities for companies, we found notable trends toward targeting less-well-validated antigens and exploration of innovative MOA such as the generation of anticancer immune responses or recruitment of cytotoxic T cells.

Introduction

The biopharmaceutical industry dedicated substantial resources to the research and development of cancer therapeutics during the 2000s, and this investment, coupled with increased knowledge about the biology of cancer and the mechanisms by which cancer therapeutics function, has led to record numbers of novel anticancer agents entering clinical study. Commercial development of cancer drugs has focused increasingly on personalized medicine and targeted therapeutics. As a consequence, the average number of novel monoclonal antibodies (mAbs) that entered clinical study per year as cancer treatments rose from approximately ten in the early 2000s to over 30 in 2011 (Fig. 1).

To profile the mAbs that might emerge from the cancer drugs pipeline over the next decade, we collected data from the public domain (e.g. company websites, clinicaltrials.gov, meeting abstracts, medical literature) for the mAbs currently in clinical study sponsored by commercial firms located worldwide. The data supplemented and updated a dataset of over 700 commercially sponsored mAbs studied in humans for an indication that has been maintained since the

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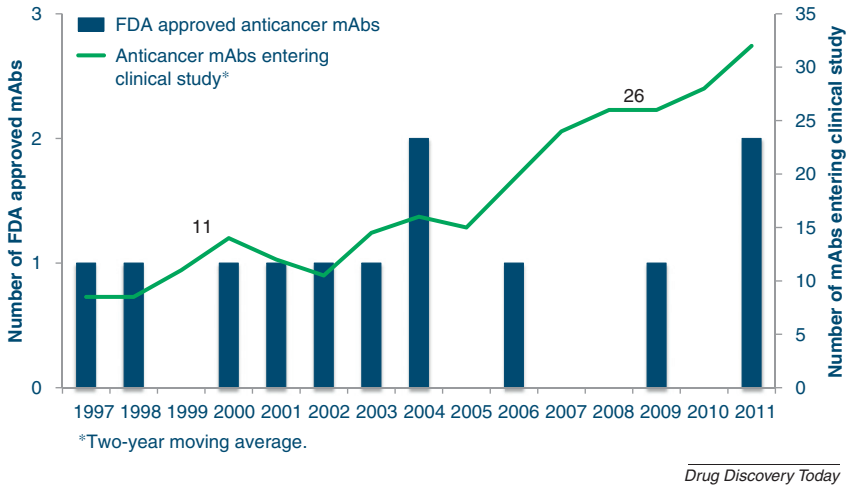

FIGURE 1

Number of novel anticancer mAbs entering clinical study and FDA-approved during 1997–2011.

1990s by the Center for the Study of Drug Development (CSDD) at Tufts University. Specific data collected included, but was not limited to, description (e.g. type, isotype, valency, specificity, modifications), target, mechanism of action (MOA) and clinical status (i.e. phase of clinical study, regulatory review, marketed). The diversity of the composition of matter, targets and MOA were evaluated and compared with those of marketed mAb products. Here, we discuss the trends in the development of antibody–drug conjugates (ADCs), bispecific antibodies, engineered antibodies and antibody fragments and/or domains that we observed. Details of approaches that apply novel formats to the validated targets epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER)2 and CD20, as well as approaches that explore antigens that are in relevant pathways, for example vascular endothelial growth factor (VEGF) and VEGF receptors, are provided. The use of antibodies with indirect MOA, for example agonism of immune activation receptors or antagonism of immune inhibitory receptors, and those that recruit T cells is also examined. Owing to the large volume of literature for the mAbs described here, only selected references are provided.

Current pipeline of mAbs

The pipeline of anticancer mAbs currently in clinical study includes a total of 165 candidates, with 89 (54%) at Phase I, and 64 (39%) and 12 (7%) that have advanced to Phase II and Phase III studies, respectively (Fig. 2). The canonical bivalent, monospecific, full-length IgG molecule is representative of only about half the anticancer mAbs in the pipeline. The rest are noncanonical candidates that can be conjugated to drugs or radiolabels; they can be multispecific or otherwise engineered for increased functionality; or they can be antibody fragments or domains. These candidates comprise 53% of all anticancer mAbs at Phase I, 38% at Phase II and 33% at Phase III.

Noncanonical antibodies in the pipeline

Although using antibodies as a means to guide drugs to a specific target has been explored for over 30 years, the development of ADCs has been challenging [1,2]. One ADC, gemtuzumab ozogamicin (Mylotarg®), was approved in the USA in 2000 through the FDA's accelerated approval mechanism as a treatment for acute myeloid leukemia, but was withdrawn in 2010 when the drug failed to demonstrate an improvement in clinical benefit in a confirmatory trial and new safety concerns were raised [3].

Advances in the knowledge of linker and drug properties, and of antibody engineering, design and selection, have enabled the development of a new generation of ADCs that are demonstrating promising clinical results [4]. Brentuximab vedotin, an anti-CD30 chimeric mAb conjugated to monomethyl auristatin E, was approved by the FDA as a treatment for Hodgkin's and systemic anaplastic large cell lymphomas in August 2011. Other ADCs that might reach the market within the next one to three years are trastuzumab emtansine, a humanized anti-HER2 antibody conjugated to DM1 that is undergoing evaluation in Phase III studies of breast cancer patients [5], and inotuzumab ozogamicin, a humanized anti-CD22 antibody conjugated to calicheamicin that is in a Phase III study as a treatment for follicular non-Hodgkin's lymphoma (NHL) [6]. In addition, at least two dozen ADCs are currently in early-stage clinical studies; these molecules comprise 15% of the anticancer mAbs currently in clinical study. Most (80%) of the ADCs were constructed using the drugs developed by either Seattle Genetics (monomethyl auristatin E or F) or ImmunoGen (DM1 or 4).

Bispecific antibodies, as the name suggests, are designed to bind two different targets. Bispecific antibodies began entering clinical study in the early 1990s [7,8]; however, the bispecific antibodies that entered clinical study in that period were ultimately terminated owing to poor safety and efficacy profiles [9], as well as production problems. Improvements in protein engineering and manufacturing methods, the expansion of biologics in the pharmaceutical industry pipeline and realization of the limitations of conventional IgGs have all led to a notable revival of interest in bispecific antibody formats. In 2009, the bispecific IgG catumaxomab was approved in Europe [10], although none of the other new bispecific antibody formats has progressed beyond Phase II studies. Numerous pairs of targets have now been clinically validated and bispecific molecules that might show enhanced efficacy compared with conventional IgGs are entering clinical study in increasing numbers [11]. Of those in the pipeline, recent results

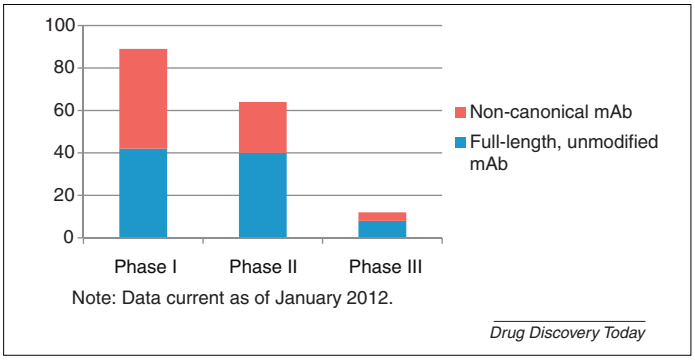

FIGURE 2

Number of novel anticancer antibodies at each phase of clinical development. *Note*: Data current as of January 2012.

have been reported for blinatumomab [12], a bispecific T-cell engager (BiTE) that targets CD19 and CD3. This candidate is undergoing evaluation in patients with B-precursor acute lymphoblastic leukemia and NHL.

The intense focus by researchers on bispecific antibodies has led to the development of a wide variety of bispecific formats [13], although most are in discovery or preclinical development. Several classes of these are IgG-like molecules, whereas others incorporate combinations of antigen-binding fragments. The scientific creativity and dedication of substantial resources to the development of these innovative molecules is laudable; however, knowledge gained about the properties of one type of bispecific antibody, for example regarding stability, immunogenicity, biological activity, manufacturability, might not inform development of others. Nevertheless, the increased functionality of bispecific antibodies compared with classical IgGs makes them attractive for development as therapeutic products. A total of ten bispecific mAb candidates are undergoing evaluation in clinical studies (seven in Phase I, three in Phase II). These molecules comprise 6% of the anticancer mAbs currently in clinical study.

Advances in protein- and glyco-engineering now enable the production of next-generation mAbs that are potentially more efficacious compared with first-generation versions [14]. For example, afucosylated mAbs are known to have enhanced effector functions [15,16], and protein sequence modifications have been shown to extend half-life [17,18]. The two mAbs in this category that have advanced the furthest in development are mogamulizumab and obinutuzumab, which are both glyco-engineered mAbs. Mogamulizumab (KW0761; AMG761) is a defucosylated humanized IgG1 that targets C–C chemokine receptor 4 [19]. The mAb is undergoing regulatory review in Japan as a potential treatment of adult T-cell leukemia–lymphoma (ATL). In a Phase II study of mogamulizumab, clinically meaningful antitumor activity was observed when ATL patients were administered 1 mg/kg doses [20]. Obinutuzumab (GA101) is a type II glyco-engineered anti-CD20 humanized IgG1 [21] that is undergoing evaluation in Phase III studies of patients with chronic lymphocytic leukemia (CLL) or NHL. In total, 17 protein- or glyco-engineered mAbs are currently undergoing evaluation in clinical studies (13 in Phase I, three in Phase II, one in Phase III). Of these, 11 (65%) were glyco-engineered and six (35%) were Fc-engineered mAbs. These candidates comprise 10% of the anticancer mAbs currently in clinical study.

Antibody-based therapeutics composed of fragments or domains have potential advantages, for example better penetration of tumors, compared with full-length IgG molecules as treatments for cancer. The category includes single chain variable fragments (scFvs), antigen-binding fragments (Fab), Nanobodies® and TandAbs®. A total of 16 antibody fragments and/or domains (five in Phase I, ten in Phase II, one in Phase II/III) in the pipeline were identified. These molecules comprise 10% of the total number of anticancer mAbs in clinical study. The majority of the molecules are scFv. Because of the lack of an Fc region, antibody fragments and/or domains can have reduced biological activity unless they are modified, for example include a cytotoxic payload [22,23]. Eight of the 16 molecules (50%) are conjugated to either a cytotoxin or radiolabel, and six (38%) are bispecific. The antibody fragment currently undergoing Phase II/III studies is naptumomab estafenatox, which is a fusion protein composed of an anti-5T4 Fab conjugated to a mutated variant of the superantigen Staphylococcal enterotoxin A/E-120 [24]. The safety and effectiveness of the mAb when administered with interferon-alpha is being evaluated in patients with advanced renal cell carcinoma.

Antibody targets

Although it is suggested that mAbs in clinical study target only a few antigens [25], examination of the pipeline suggests otherwise. At least 92 distinct antigens are targeted by the anticancer mAbs in clinical study, with 65 unique to a single mAb. The remaining 27 antigens are targeted by an average of three mAbs. It is important to note that greater risk is associated with targeting antigens that are not well-validated (i.e. those for which there is limited evidence of relevant clinical response), whereas risk is mitigated if the clinical effect of targeting an antigen with a mAb is known. A frequency analysis of mAb targets does show that the well-validated antigens EGFR, HER2 and CD20 (Table 1) are among the top five most frequently targeted. These three antigens are targets for a total of 18 mAbs in clinical study, as well as eight marketed mAbs.

TABLE 1

Frequent targets for anticancer mAbs in clinical study

Target	Number of mAbs in pipeline
Epidermal growth factor receptor (EGFR)	7
Human epidermal growth factor receptor (HER)2	6
CD20	5
Angiopoietin 2	5
CD19	5
CD22	4
HER3	4
CD38	3
CD70	3
Carcinoembryonic antigen	3
Fibronectin	3
GD2	3
Insulin-like growth factor receptor 1	3
PD-1	3

Nevertheless, the top five antigens are targets for only 17% of the total number of anticancer mAbs currently in clinical study.

Examination of the data for the marketed and investigational mAbs targeting EGFR, HER2 and CD20 suggests that companies are using the versatility of mAbs to their advantage by developing novel antibodies that are improvements, at least theoretically, over previously developed versions.

MAbs targeting EGFR

EGFR is the target for three marketed anticancer mAbs: cetuximab (Erbitux®), panitumumab (Vectibix®), and nimotuzumab (TheraCIM®) [26,27], and seven mAbs are currently undergoing clinical study. The composition of each marketed mAb is slightly different from the others in ways that potentially affect the immunogenicity, toxicity and efficacy of the products, but all three antibodies bind a closely related, overlapping epitope on EGFR domain III [28].

Cetuximab is a chimeric IgG1 that was first approved in the USA in 2004, panitumumab is a human IgG2 that was first approved in the USA in 2006, whereas nimotuzumab is a humanized IgG1 that is not approved in Europe, the USA or Japan, but was approved in several other countries (e.g. India, China) from 2006. In addition to differences in the sequence source and isotype, the binding affinities of the three mAbs to the EGFR target vary, with dissociation constants of the order $\sim 1 \times 10^{-8}$, 1×10^{-10} and 1×10^{-11} for nimotuzumab, cetuximab and panitumumab, respectively [29,30]. Compared with cetuximab and panitumumab, nimotuzumab appears to have lower incidences of side effects, which has been attributed to the lower binding affinity of nimotuzumab to EGFR [31,32]. Cetuximab induces hypersensitivity reactions in patients with pre-existing IgE antibodies that cross-react with carbohydrate residues on the Fab portion of the product [33]. As an IgG2, panitumumab is thought to function primarily through blocking ligand–receptor interactions, and has decreased likelihood of damaging normal EGFR-positive cells because the molecule has reduced effector functions compared with the two IgG1 mAbs. Panitumumab-induced antibody-dependent cell-mediated cytotoxicity (ADCC) is mediated by myeloid effector cells only and it is affected by the FcγRIIa-R131H polymorphism [34]. The marketed anti-EGFR mAbs are approved for a variety of EGFR-expressing cancers: cetuximab is approved as a treatment for metastatic colorectal cancer and squamous cell carcinoma of the head and neck; panitumumab is approved for the treatment of metastatic colorectal cancer; and nimotuzumab is approved for glioma, as well as head and neck, nonsmall-cell lung and esophageal cancers.

The seven investigational anti-EGFR mAbs achieve EGFR inhibition through use of: (i) conventional, single unmodified IgG1; (ii) glyco-engineered mAbs; (iii) mixtures of mAbs; or (iv) dual specific IgG1 (Table 2). The approaches to treatment and the MOA of necitumumab and ABT806 are closely related to those of the anti-EGFR mAbs currently marketed. Necitumumab is being developed by ImClone, the same company that developed cetuximab. As a human mAb, necitumumab has the potential for reduced incidence of side effects compared with the chimeric cetuximab. The two mAbs have similar binding affinities to the target and

TABLE 2

MAb product candidates targeting validated antigens or biological pathways

mAb INN or code name	Target	Description	Clinical phase
Necitumumab, IMC11F8	EGFR	Human IgG1, phage display derived	Phase III
ABT806	EGFR	Chimeric IgG1	Phase I
RO5083945, RG7160, GA201	EGFR	Glyco-engineered humanized IgG1	Phase II
GTMAB 5.2 GEX, CetuGEX	EGFR	Glyco-engineered chimeric mAb	Phase I
SYM004	EGFR	Mixture of two chimeric IgG1	Phase II
MM151	EGFR	Mixture of three human mAbs	Phase I
MEHD7945A, RG7597	EGFR	Dual-targeting (anti-EGFR and -HER3) IgG1	Phase I
Pertuzumab	HER2	Humanized IgG1	Regulatory review
Trastuzumab emtansine	HER2	Antibody–drug conjugate, humanized IgG1	Phase III
MM111	HER2	Bispecific (anti-HER2 and -HER3) human scFv	Phase I
MGAH22	HER2	Fc-optimized mAb	Phase I
GTMAB7.3 GEX	HER2	Glyco-engineered human mAb	Phase I
MM302	HER2	scFv-targeted liposome containing doxorubicin	Phase I
Veltuzumab	CD20	Humanized IgG1	Phase II
AME133v, LY2469298	CD20	Fc engineered, humanized IgG1	Phase I/II
Ublituximab, LFBR603	CD20	Chimeric IgG1 with low fucose content	Phase I
FBTA05, lymphomun	CD20	Murine bispecific (anti-CD20 and -CD3)	Phase I/II
Obinutuzumab, GA101, RO5072759	CD20	Glyco-engineered type II humanized IgG1	Phase III
IMC3C5	VEGFR-3	Human IgG1	Phase I
Icrucumab	VEGFR-1	Human IgG1	Phase II
Ramucirumab	VEGFR-2	Human IgG1	Phase III

Abbreviations: EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; Ig, immunoglobulin; mAb, monoclonal antibody; VEGFR, vascular endothelial growth factor receptor.

probably share the same MOA [35]. Necitumumab has advanced to Phase III studies as a first-line treatment of patients with stage IV squamous nonsmall-cell lung cancer in combination with gemcitabine–cisplatin; however, a second Phase III study of necitumumab as first-line treatment of patients with stage IV nonsquamous nonsmall-cell lung cancer in combination with pemetrexed–cisplatin was stopped owing to safety concerns related to blood clots during drug-injection [36]. The humanized ABT806 targets de2–7 EGFR (also known as EGFRvIII), a naturally occurring extracellular truncation of the EGFR, and is undergoing evaluation in a Phase I study of patients with advanced solid tumors. A chimeric predecessor, ch806, was evaluated in a Phase I study [37].

The three approaches that are alternatives to conventional antibodies are designed to provide improved efficacy compared with that achieved by single IgG molecules: (i) glyco-engineering; (ii) use of antibody mixtures; and (iii) dual-targeting. Glyco-engineering is used to enhance the ability of mAbs to induce an immune response against cancer cells. Compared with cetuximab and panitumumab, the glyco-engineered RO5083945, also known as RG7160 or GA201, demonstrated increased binding affinity for all FcγRIIIa variants expressed on immune effector cells, and improved activity in ADCC assays and *in vivo* models [38]. In a Phase I study in 75 patients with advanced solid tumors, RO5083945 demonstrated an acceptable safety profile and promising efficacy [39]. It is undergoing evaluation in two Phase II studies in patients with advanced or recurrent nonsmall-cell lung cancer or with metastatic colorectal cancer. CetuGEX™ (Glycotope), which is glyco-engineered to have human glycosylation, demonstrated up to 200-fold higher ADCC activity *in vitro* using PBMCs from donors with various FcγIIIa-receptor polymorphisms [40]. CetuGEX™ is in a Phase I study evaluating the safety, tolerability and pharmacokinetics of the mAb in patients with EGFR-positive, locally advanced or metastatic solid cancers.

Improvements in efficacy might also be achieved through use of antibody mixtures that target different epitopes or single antibodies that are designed to bind to two different targets. The investigational anti-EGFR mAbs that exploit these approaches have only recently entered clinical studies; therefore, clinical data are limited. In July 2011, Sym004, a mixture of two chimeric anti-EGFR IgG1s, entered Phase II evaluation in patients with recurrent or metastatic squamous cell carcinoma of the head and neck who have failed anti-EGFR mAb-based therapy [41]. Sym004 has been found to trigger EGFR internalization and degradation and exhibited more-pronounced growth inhibition *in vitro* and superior efficacy *in vivo* compared with reference anti-EGFR mAbs [42]. MM151, a mixture of three human mAbs that bind nonoverlapping epitopes of EGFR, is being evaluated in a Phase I study of patients with refractory advanced solid tumors. Initiated in January 2012, the study has an estimated completion date of February 2014 [43].

MEHD7945A, a so-called dual-action mAb, is a humanized IgG1 that binds EGFR and HER3 [44,45]. The rationale for this combination of targets stems from data suggesting that transactivation of ErbB3 by EGFR can lead to resistance to EGFR inhibitors. MEHD7945A thus might be effective in patients who have acquired resistance to EGFR inhibitors. A Phase I study of MEHD7945A in patients with incurable, locally advanced or metastatic epithelial malignancies that have progressed despite standard therapy or for which no standard therapy exists was initiated in September 2010.

MAbs targeting HER2

HER2 is the target of one marketed mAb, trastuzumab (Herceptin®), and six investigational mAbs (Table 2). Overexpression of HER2 and EGFR is associated with aggressive tumor growth caused by the increased potential for dimerization (i.e. homo- or heterodimerization) that activates signaling pathways within the tumor cells [46]. Heterodimerization of EGFR with HER2 had been found to induce a more potent activation of EGFR compared with EGFR homodimerization; this observation has spurred investigation of combinations of anti-EGFR and anti-HER2 agents in patients with various tumor types.

The humanized IgG1 trastuzumab is FDA-approved as a treatment for HER2-overexpressing breast cancer and metastatic gastric or gastroesophageal junction adenocarcinoma. The investigational anti-HER2 candidates are proposed to have advantages over trastuzumab; only one (i.e. pertuzumab) is a conventional IgG molecule. As was seen with the anti-EGFR mAbs in clinical study, numerous approaches to improve the safety and efficacy of the molecules compared with the marketed therapeutic have been taken. Like trastuzumab, pertuzumab binds HER2 with nanomolar affinity and interferes with the dimerization of HER2, but pertuzumab targets a unique epitope [47]. Pertuzumab has been or is being evaluated in at least 24 clinical studies, including two Phase III studies in breast cancer patients and a Phase I/II study in combination with cetuximab in cetuximab-refractory metastatic colorectal cancer. Marketing applications for pertuzumab were submitted to regulatory agencies in Europe and the USA in December 2011.

The ADC trastuzumab emtansine is composed of trastuzumab linked to DM1, a maytansinoid drug that inhibits tubulin polymerization. Trastuzumab emtansine is undergoing evaluation in three Phase III studies, including one evaluation of the efficacy and safety of trastuzumab emtansine alone or in combination with pertuzumab in patients with HER2-positive metastatic breast cancer. Anti-HER2 mAbs that use other formats and MOA are in Phase I studies, including a bispecific scFv (MM111), two mAbs that were engineered to improve activity (MGAH22, GTMAB7.3GEX) and a scFv-targeted, doxorubicin-filled liposome (MM302).

MAbs targeting CD20

CD20 is the target of four marketed mAbs (rituximab, ofatumumab, ibritumomab tiuxetan, ^{131}I tositumomab) and five mAbs currently in clinical study (Table 2). Ibritumomab tiuxetan (Zevalin®; IDEC) and ^{131}I tositumomab (Bexxar®; Corixa) are radiolabeled mAbs that have not been extensively used. Market forces are commonly cited for this, for example the mAbs are generally administered in hospital settings, which requires oncologists to refer their patients to treatment centers [48]. In addition, the treatment regimens are complicated. For Zevalin®, patients first receive indium-111 ibritumomab tiuxetan as a diagnostic, then yttrium-90 ibritumomab tiuxetan as a therapeutic dose one week after the diagnostic dose [49]. Treatment with Bexxar® involves dosimetric and therapeutic doses seven to 14 days apart. Each dose consists of a sequential infusion of tositumomab followed by iodine-131 tositumomab; the dosimetric step is required to ensure

a consistent radiation dose by adjusting for the individual patient's rate of clearance of the mAb [50]. Zevalin® was first FDA-approved in 2002 for treatment of patients with relapsed or refractory low-grade, follicular or transformed B-cell NHL, including patients with rituximab-refractory follicular NHL, and received a supplemental approval in 2009 for treatment of previously untreated follicular NHL in patients who achieve a partial or complete response to first-line chemotherapy. Bexxar® was first approved in 2003 for treatment of patients with CD20-positive, follicular NHL, with and without transformation, whose disease is refractory to rituximab and have relapsed following chemotherapy; the indication was expanded in 2004 to include patients with relapsed or refractory, low-grade, follicular or transformed CD20-positive NHL who have not received rituximab.

The chimeric IgG1 rituximab (Rituxan®) and the human IgG1 ofatumumab (Arzerra®) target different CD20 epitopes. Ofatumumab binds a membrane-proximal epitope that is thought to position complement activation close to the cell surface [51]. Compared with rituximab, ofatumumab is more active *in vitro* against low-level CD20-expressing tumors, has a slower off-rate and has enhanced complement-dependent cytotoxicity (CDC) [52,53]. Rituximab was first approved by the FDA in 1997 for treatment of patients with relapsed or refractory low-grade or follicular B-cell NHL. It subsequently received three supplemental approvals: (i) in 2006 for adult patients with moderately to severely active rheumatoid arthritis; (ii) in 2010 for treatment of patients previously treated or previously untreated for CD20-positive CLL in combination with fludarabine and cyclophosphamide (FC); and (iii) in 2011 for use in combination with glucocorticoids for the treatment of patients with Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA). Ofatumumab was first approved by the FDA in 2009 for treatment of CLL refractory to alemtuzumab and fludarabine and, as of January 2012, had not received supplemental approvals.

Of the five anti-CD20 mAbs in the clinical pipeline, only veltuzumab is an unmodified IgG1. The mAb is humanized and uses similar mechanisms to rituximab, but has a slower off-rate and higher CDC activity [54]. Veltuzumab is currently undergoing evaluation in a total of six Phase I/II studies in patients with chronic immune thrombocytopenic purpura or various types of leukemia or lymphoma, as well as one dose-ranging Phase II study in patients with moderate-to-severe rheumatoid arthritis. AME133/LY2469298 is a humanized IgG1 that has been optimized through engineering of the Fc domain. It binds to the low-affinity variant of FcγRIIIa with a higher affinity than rituximab, which gives AME133 increased ADCC activity. Results of two Phase I studies indicated AME133/LY2469298 was well-tolerated and showed encouraging clinical activity at the doses administered to patients with follicular lymphoma [55,56].

Ublituximab is a chimeric IgG1 derived from a host cell line capable of producing antibodies with low fucose content, and, therefore, it has high affinity for FcγRIIIa and high ADCC activity [57]. A Phase I study to evaluate the safety, pharmacokinetics and preliminary efficacy of ublituximab in patients with relapsed or refractory CLL who have received at least one prior fludarabine-containing regimen is on-going. Preliminary results indicated that ublituximab is clinically active in patients with relapsed CLL and induces partial remissions; the clinical efficacy of an escalating eight-dose regimen is currently under evaluation. FBTA05 is a bispecific, trifunctional mAb capable of simultaneously targeting B cells (via binding to CD20), T cells (via binding to CD3) and recruiting FcγR-positive accessory immune cells via its Fc region. Preclinical data demonstrated that FBTA05-induced cytotoxicity exceeded that of rituximab [58]. Obinutuzumab (GA101, RO5072759), a humanized IgG1 containing a modified hinge region, was optimized using GlycArt's glyco-engineering technology. It has improved binding affinity to FcγRIII and higher ADCC, but lower CDC activity, compared with rituximab [59,60]. GA101 is currently undergoing evaluation in eight clinical studies, including a total of four Phase III studies in patients with previously untreated CLL, untreated advanced indolent NHL, previously untreated CD20-positive diffuse large-B-cell lymphoma or rituximab-refractory, indolent NHL. These studies have estimated completion dates between January 2015 and August 2023.

Theoretical versus actual improvement

Comparison of the investigational and marketed mAbs that target EGFR, HER2 and CD20 reveals that companies are devoting substantial resources to the development of a wide variety of mAbs that are designed to be safer and more efficacious compared with the products of their competitors. This raises the question of whether the 'next generation' investigational candidates really are better. The anti-EGFR, anti-HER2 and anti-CD20 mAbs discussed here differ in many ways, including source of antibody sequence (e.g. chimeric, humanized, human), isotype, carbohydrate residues, target epitope, binding affinities to target or Fc receptors, design (e.g. naked, ADC, bispecific), indications studied and specific disease states of patients in studies. Preclinical data are available for some of the mAbs, but many of the investigational candidates entered clinical study recently and therefore clinical study results are lacking. Thus, it is still too early to determine whether the differences in preclinical results (e.g. improved ADCC) observed for the 'next generation' mAbs are actually clinically relevant.

Alternate targets in validated pathways

An alternate approach to targeting a validated antigen with a novel antibody designed for improved safety or efficacy is targeting an alternate antigen in a validated biological pathway. For example, as the target of bevacizumab (Avastin®), VEGF is considered a validated target. Although there are currently no anti-VEGF mAbs for cancer indications in the pipeline, there are three human IgG1s that target receptors for VEGF (Table 2). The VEGF tyrosine kinase signaling pathway, which includes VEGF-A and three receptors VEGFR-1, -2 and -3, is crucial for tumor neovascularization [61].

Anti-VEGF-A bevacizumab was first approved by the FDA in 2004 for use in combination with intravenous 5-fluorouracil-based chemotherapy for the first-line treatment of patients with metastatic carcinoma of the colon and rectum. The product subsequently received three supplemental approvals: (i) in 2006 as first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic nonsquamous, nonsmall-cell lung cancer in combination with carboplatin and paclitaxel; (ii) in 2008 for use in combination with paclitaxel for the treatment of patients who have not received chemotherapy for metastatic HER2-negative breast cancer; and (iii) in 2009 for treatment of glioblastoma with progressive disease following prior therapy and metastatic renal

TABLE 3

MAb product candidates that generate anticancer immune responses

mAb INN or code name	Target, description	Clinical phase
Tremelimumab	CTLA-4, human IgG2	Phase II
PF05082566	4-1BB agonist, human IgG2	Phase I
BMS663513	4-1BB agonist, human IgG4	Phase I
IPH2101	KIR, human IgG4	Phase II
MDX1105, BMS936559	PD-L1, human PD-L1	Phase I
MK3475, SCH900475	PD-1	Phase I
MDX1106, ONO4538, BMS936558	PD-1, human IgG4	Phase II
CT011, CTACTIBODY	PD-1, humanized IgG1	Phase II

Abbreviations: CTLA-4, cytotoxic T lymphocyte associated antigen-4; KIR, killer-cell immunoglobulin-like receptor; PD, programmed death; scFv, single chain variable fragment.

cell carcinoma in combination with interferon-alpha. The supplemental approval for breast cancer proved controversial, and in June 2011 an FDA advisory committee unanimously recommended that the approval be rescinded.

Inhibition of the VEGF pathway can also be achieved by blocking the VEGF receptors, which share structural features but are selective for the ligands that they bind. All three mAbs currently in clinical studies that target the VEGF receptors are being developed by ImClone Systems, a subsidiary of Eli Lilly. The anti-VEGFR-2 IgG1 ramucirumab (IMC1121B) has been, or is being, evaluated in at least 27 clinical studies sponsored by ImClone or Eli Lilly. Six of these are Phase III studies in the following indications: (i) metastatic gastric adenocarcinoma; (ii) metastatic gastric or gastroesophageal junction adenocarcinoma; (iii) stage IV nonsmall-cell lung cancer; (iv) hepatocellular carcinoma; (v) metastatic colorectal carcinoma; (vi) HER2-negative, unresectable, locally recurrent or metastatic breast cancer. The six studies have estimated completion dates between January 2013 and December 2015. Clinical results have been published for one Phase I study in which objective antitumor activity and antiangiogenic effects were observed in patients with advanced solid malignancies who were administered weekly doses of ramucirumab ranging from 2 to 16 mg/kg [62].

The anti-VEGF-R1 IgG1 icrucumab (IMC18F1) [63] is currently undergoing evaluation in three Phase II studies of patients with: (i) unresectable, locally advanced or metastatic breast cancer; (ii) metastatic colorectal cancer; (iii) metastatic transitional cell carcinoma of the bladder, urethra, ureter or renal pelvis. These studies have estimated completion dates between September 2014 and July 2015. A Phase I study of anti-VEGFR-3 IMC3C5 [64] was initiated in April 2011. The study will evaluate escalating doses of IMC3C5 administered intravenously weekly or every other week to patients with advanced solid tumors refractory to standard therapy or for which no standard therapy is available.

Anticancer mAbs with indirect mechanisms of action

Most pipeline and approved anticancer mAbs kill cells in a direct manner (i.e. by binding to an antigen associated with a tumor cell and inducing cell death via effector functions, cytotoxic payloads or blockade of signals required for growth) but approaches that harness indirect MOA such as agonism of immune activation receptors or antagonism of immune inhibitory receptors are also being explored (Table 3). To date, only one anticancer mAb with such a MOA is marketed. Ipilimumab (Yervoy®), an anticytotoxic T lymphocyte antigen-4 (CTLA-4) human IgG1, was approved in the USA and the European Union in 2011 for the treatment of unresectable or metastatic melanoma [65]. The costimulatory molecule CTLA-4 is expressed on the surface of helper T cells and transmits an inhibitory signal; blocking the antigen thus enables an active immune response from the cells. Tremelimumab, a human IgG2 mAb that also targets CTLA-4 [66,67], has been evaluated in at least 17 clinical studies sponsored by Pfizer; MedImmune announced October 2011 that they had in-licensed the candidate.

Unlike CTLA-4, which is a negative costimulatory molecule, 4-1BB (also known as CD137) is a positive costimulatory molecule that sustains T-cell responses [68]. PF05082566, a human IgG2 anti-4-1BB mAb with agonist activity [69], is undergoing evaluation in a Phase I study as a single agent in patients with solid tumors or B-cell lymphomas, and in combination with rituximab in patients with CD20-positive NHL. The estimated study completion date is June 2013. BMS663513 also targets 4-1BB, and it is currently in Phase I studies of cancer patients with advanced or metastatic tumors. In a Phase I study of patients with advanced cancer, the mAb was tolerable at doses in the range of 0.3–15.0 mg/kg [70]. A Phase II study of BMS663513 as a second-line monotherapy in melanoma patients was completed, but results have not yet been reported.

Killer-cell Ig-like receptors (KIRs) are expressed on natural killer (NK) cells; the KIRs interact with HLA class I on target cells and inhibit NK cytolytic activity. IPH2101 facilitates activation of NK cells by blocking the interaction between KIRs and their ligands [71]. The mAb is undergoing evaluation in a Phase II study in patients with multiple myeloma in stable partial response after a first-line therapy, a Phase II study in patients with smoldering multiple myeloma and a Phase I/II study combined with lenalidomide in patients with multiple myeloma experiencing a first relapse.

A total of four mAbs that target the immunoinhibitory receptor PD-1 or its ligand PD-L1 are currently in clinical studies. PD-1 is expressed by activated T cells, B cells and myeloid cells; interaction with PD-L1 leads to inhibition of proliferation and cytokine secretion by the cells [72]. MDX1105, which targets the ligand PD-L1, is undergoing evaluation in a Phase I study of patients with selected advanced or recurrent solid tumors. Three pipeline mAbs (MK3475, MDX1106, CT011) target the PD-1 receptor and are undergoing evaluation in patients with various types of cancer. In a dose-escalating Phase I study of patients with advanced metastatic melanoma, colorectal cancer, prostate cancer, non-small-cell lung cancer or renal cell carcinoma administration of

TABLE 4

MAb product candidates that recruit T cells

mAb INN or code name	Targets, description	Clinical phase
FBTA05, lymphomun	CD20xCD3, bispecific	Phase I/II
Blinatumomab	CD19xCD3, bispecific tandem scFv	Phase II
MT111, MEDI565	CEAxCD3, bispecific tandem scFv	Phase I
MT110	EpCAMxCD3, bispecific tandem scFv	Phase I
IMCgp100	gp100xCD3, bispecific scFv	Phase I

Abbreviations: CD, cluster of differentiation; CEA, carcinoembryonic antigen; EpCAM, epithelial cell adhesion molecule; gp, glycoprotein; scFv, single chain variable fragment.

up to 10 mg/kg of MDX1106 was well-tolerated and evidence of antitumor activity was observed [73]. Results of a Phase I safety and PK study of CT011 indicted that the mAb was safe and well-tolerated in patients with advanced hematologic malignancies administered at a single dose of 0.2–6.0 mg/kg [74].

Anticancer mAbs that recruit T cells

Cytotoxic T cells are not involved in typical antibody-mediated cell-killing mechanisms such as ADCC, CDC or antibody-dependent cellular phagocytosis, but bispecific antibodies have been designed to engage T cells via CD3 and bring the T cells in proximity to a tumor cell (Table 4). One such bispecific antibody, the murine catumaxomab (Removab®), was approved in 2009 in the European Union for treatment of malignant ascites [75,10]. Catumaxomab targets epithelial cell adhesion molecule (EpCAM) and CD3. The anti-CD20xCD3 FBTA05 is designed to have the same type of MOA. Catumaxomab and FBTA05 are full-length bispecific, trifunctional, murine mAbs that bind tumor cells and T cells via the two variable regions and recruit Fcγ-positive immune cells via the Fc region.

The BiTEs use an alternate format but the same approach. The three BiTE molecules in clinical study (blinatumomab, MT111, MT110) are composed of two linked scFvs. Blinatumomab has been, or is being, evaluated in three Phase II studies of patients with acute lymphoblastic leukemia, and a Phase I study of patients with NHL. MT111, which targets carcinoembryonic antigen and CD3, is being evaluated in a Phase I study of patients with advanced gastrointestinal cancers. MT110, which targets EpCAM and CD3 [76], is being evaluated in a Phase I study of patients with advanced solid tumors, including lung cancer, gastric cancer or adenocarcinoma of the gastroesophageal junction, colorectal cancer, breast cancer, hormone-refractory prostate cancer and ovarian cancer.

IMCgp100 (Immunocore) is composed of a high-affinity T-cell receptor specific to a peptide sequence from the gp100, a glycoprotein antigen presented on melanoma cells by HLA-A2, fused to an anti-CD3 scFv. The candidate is undergoing evaluation in a Phase I study in patients with malignant melanoma. The estimated study completion date is June 2013.

Future prospects

Although most mAbs currently on the market as cancer drugs are canonical (i.e. full-length and unmodified IgG molecules), our examination of the commercial pipeline of anticancer mAbs revealed trends toward the development of a wide variety of noncanonical mAbs, including ADCs, bispecific antibodies, engineered antibodies and antibody fragments and/or domains. In total, the noncanonical versions now comprise half of the pipeline. We also observed notable trends toward targeting antigens that are not well-validated and exploration of innovative approaches such as the use of indirect MOA or recruitment of cytotoxic T cells. Many of the mAb candidates derived from the noncanonical formats and those that use alternate MOA have only recently entered clinical studies; thus, the data for most are not yet available. As results are released in the future, the understanding about how the composition and MOA of these innovative mAbs affects clinical outcomes should improve. The pipeline of 165 mAbs currently undergoing evaluation as cancer treatments might therefore yield innovative medicines in the next decade, and it could also serve to direct research much further into future.

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