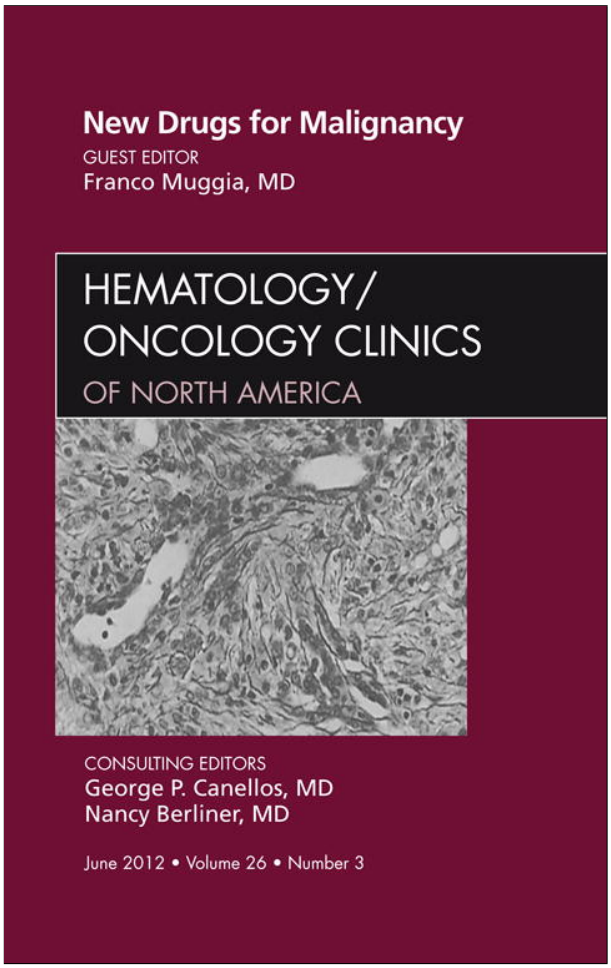

Therapeutic Antibodies Against Cancer

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KEYWORDS

• Therapeutics • Antibodies • Cancer • Immunogenicity • Safety • Efficacy

KEY POINTS

- Antibody-based therapeutics against cancer are highly successful in the clinic and currently enjoy unprecedented recognition of their potential.
- Hundreds of mAbs, including bispecific mAbs and multispecific fusion proteins, mAbs conjugated with small-molecule drugs, and mAbs with optimized pharmacokinetics, are in clinical trials.
- Challenges remain, and deeper understanding of mechanisms is needed to overcome major problems including resistance to therapy, access to targets, complexity of biological systems, and individual variations.

Antibody therapy has its roots thousands of years ago; early forms of vaccination against infectious diseases were developed in China as early as 200 BC. However, the history of true antibody therapy began much more recently with the discovery that serum from animals immunized with toxins, for example, diphtheria toxin or viruses, is an effective therapeutic against the disease caused by the same agent in humans. This discovery resulted in the development of the serum therapy, which saved thousands of lives; von Behring, who in the 1880s developed an antitoxin that did not kill the bacteria but neutralized the toxin released into the body by the bacteria, was awarded the first Nobel Prize in Medicine in 1901 for his role in the discovery and development of a serum therapy for diphtheria. Although historically success with antibody (serum) therapy was initially mostly in the treatment of patients with infectious diseases, currently there is only one monoclonal antibody (mAb) approved for treatment of any infectious disease (palivizumab [Synagis]), which is for prevention of the infection and not for treatment of the already established

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infection. Initial attempts with serum therapy to treat patients with cancer were not successful. It was not until several decades ago that several revolutionary scientific discoveries were made, which allowed the development of recombinant therapeutic antibodies leading to the approval of the first anticancer therapeutic antibody, the mAb rituximab, in 1997 (**Table 1**). Since then 13 mAbs have been approved for clinical use against cancer in the European Union and the United States, and 12 are on the market in August 2011; one of these, gemtuzumab ozogamicin (Mylotarg), has been withdrawn (see **Table 1**); by contrast, the first approved mAb-based therapeutic against an infectious disease (Synagis is for prevention) is still awaited. In 2010 sales of the top 4 recombinant therapeutic antibodies (bevacizumab, rituximab, trastuzumab, cetuximab) exceeded US$20 billion (**Table 2**).

Dating back to mummies and up to the recent successes with ipilimumab, it has become axiomatic that the human immune system has an inherent capacity for antitumor activity. This notion was bolstered in the 1900s by the finding of spontaneous remissions recorded, often in sparse anecdotal findings, in nearly every stage and form of cancer, by the more common observation of spontaneous regressions of melanoma and renal carcinoma, the success of nonspecific immune-stimulants such as bacillus Calmette-Guérin or Coley toxin, and the increasingly targeted use of antibodies against antigens more specific to certain cell types.[1] Indeed, the antibody specificity was perhaps the first and still the most powerful story supporting the ubiquitous catch-call of personalized medicine.

With all of the elegance of the specificity story and more than 35 years since the recipe for generating monoclonal antibodies by Kohler and Milstein,[2] the clinical promise has been largely disappointing. With rare exceptions, these molecular missiles have not annihilated their target tumors and have fallen far short of the marvel of the antibiotic revolution. The rarity of cures should not dampen the substantial, if incremental, progress that has been made. Even in the age of single-nucleotide etiologies there is a strong case that cancer, by the time of its clinical visibility, consists of many broken parts; hence the growing argument that targeted therapies may parallel the breakthrough to cure with chemotherapy in the 1970s with the move toward not one, but a cocktail of simultaneous, combined agents. As in the case of combination chemotherapy, antibody therapy may come to use different effector pathways in this assault.

Therapeutic mAbs and other therapeutic proteins have been reviewed previously (see recent reviews[1,3–15] and articles cited therein). Therefore, here the authors review the monoclonal antibodies used directly in treatment, shed some light on presumed primary mechanism of action, and survey use, from initial indication to the wider adoption based principally on clinical trials and trends. This line-up, with its wide spectrum of targets and mechanisms, may give some hope that the long trek may yet reach the originally envisioned summit. If not, these agents are undoubtedly part of the solution. This article focuses mainly on those native, unconjugated antibodies that directly affect solid tumors. Bevacizumab, though its antivascular action is indirect, has gained such wide application for solid tumors (and has been the subject of much controversy) that its inclusion seemed important. Although immune-conjugates have been well reviewed elsewhere[16–18] and are not the present focus, brentuximab vedontin, as the first new indication for Hodgkin lymphoma in 30 years, warranted special inclusion. Its success represents a partial rescue of a paradigm after the first approved antibody-drug conjugate, gemtuzumab, was withdrawn in 2010 because of lack of efficacy and increased mortality.[19] In the context of the present review it may also point to some limiting aspects of unconjugated tumor-directed antibodies which, as has been stated, have not delivered their quarter-century promise.

Table 1
Therapeutic monoclonal antibodies against cancer approved or in review in the European Union (EU) or United States (US) (information current as of August 2011)

Name	Trade Name	Type	Indication First Approved	First EU (US) Approval Year
Rituximab	MabThera, Rituxan	Anti-CD20; chimeric IgG1	Non-Hodgkin lymphoma	1998 (1997)
Trastuzumab	Herceptin	Anti-HER2; humanized IgG1	Breast cancer	2000 (1998)
Gemtuzumab ozogamicin	Mylotarg	Anti-CD33; humanized IgG4	Acute myeloid leukemia	NA (2000[a])
Alemtuzumab	MabCampath, Campath-1H	Anti-CD52; humanized IgG1	Chronic myeloid leukemia	2001 (2001)
Tositumomab + ^{131}I-tositumomab	Bexxar	Anti-CD20; murine IgG2a	Non-Hodgkin lymphoma	NA (2003)
Cetuximab	Erbitux	Anti-EGFR; chimeric IgG1	Colorectal cancer	2004 (2004)
Ibritumomab tiuxetan	Zevalin	Anti-CD20; murine IgG1	Non-Hodgkin lymphoma	2004 (2002)
Bevacizumab	Avastin	Anti-VEGF; humanized IgG1	Colorectal cancer	2005 (2004)
Panitumumab	Vectibix	Anti-EGFR; human IgG2	Colorectal cancer	2007 (2006)
Catumaxomab	Removab	Anti-EpCAM/CD3; rat/mouse bispecific mAb	Malignant ascites	2009 (NA)
Ofatumumab	Arzerra	Anti-CD20; human IgG1	Chronic lymphocytic leukemia	2010 (2009)
Ipilimumab	Yervoy	Anti-CTLA-4; human IgG1	Metastatic melanoma	2011 (2011)
Brentuximab vedotin	Adcetris	Anti-CD30; chimeric IgG1; immunoconjugate	Hodgkin lymphoma, systemic ALCL	NA (2011)

Abbreviations: ALCL, anaplastic large-cell lymphoma; CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte antigen 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; NA, not approved; VEGF, vascular endothelial growth factor.

[a] Voluntarily withdrawn from market in 2010.

Data from Dimitrov DS, Marks JD. Therapeutic antibodies: current state and future trends—is a paradigm change coming soon? Methods Mol Biol 2009;525:1–27.

Table 2
The 4 top-selling therapeutic antibodies in 2010 (in billion US$)

Rank (2009)	Name	Target	Type	Company	Sales
2 (3)	Bevacizumab	VEGF	Humanized IgG	Genentech Roche Chugai	6.973
3 (4)	Rituximab	CD20	Chimeric IgG	Genentech Biogen-IDEC Roche	6.859
6 (7)	Trastuzumab	HER2	Humanized IgG	Genentech Chugai Roche	5.859
18 (19)	Cetuximab	EGFR	Chimeric IgG	Eli Lilly BMS Merck Serono	1.791

The numbers denote ranking out of all therapeutic proteins in 2010; numbers in parentheses are for 2009. Currencies as of March 2012: 1 € = 1.33 US$; 1 CHF = 1.11 US$; 1 Yen = 0.01 US$; 1 DKK = 0.18 US$; 1 SEK = 0.15 US$.

Data from LaMerie Business Intelligence, Barcelona.

mAbs APPROVED FOR CLINICAL USE

At present 13 mAbs are approved for clinical use in the European Union or the United States (see **Table 1**). One of the approved mAbs, gemtuzumab ozogamicin (Mylotarg), was withdrawn from the market because of lack of clinical benefit and safety reasons after a clinical trial in which a greater number of deaths occurred in the group of patients with acute myeloid leukemia (AML) who received Mylotarg compared with those receiving chemotherapy alone. Mylotarg, catumaxumab (Removab) (not yet approved in the United States), and the two radiotherapeutic mAbs, tositumomab (Bexxar) and ibritumomab tiuxetan (Zevalin), are not reviewed here.

Rituximab

The first candidate remains in many ways the poster child for both specificity and efficacy. Rituximab (MabThera, Rituxan), initially developed in San Diego in the late 1980s, and father to that region's biotech explosion, was based on the finding of CD20 antigen on normal and malignant lymphocytes; it is not appreciably expressed at either pole of lymphocyte ontogeny—stem cells and plasma cells—or on other nonlymphoid cellular compartments. In contrast to many emerging cancer targets clearly connected with signal transduction circuitry, there is no clear consensus on the function of CD20. Nonetheless, the chosen antigen-antibody duo in CD20/rituximab rendered a striking clinical success and ushered in a continuing wave of similarly conceived agents, albeit with variant tactical goals and mechanisms of effect. It is interesting that only after many years afterward were clinical agents developed to target perhaps the ultimate tissue-specific bull's eye: the individual epitope of each B-lymphocyte population, separating the malignant fiend from more than a million brethren lymphocytes by one signature antigen expressed on one malignant subspecies.

In 1997 rituximab was approved by the US Food and Drug Administration (FDA) for the treatment of relapsed indolent B-cell non-Hodgkin lymphoma (NHL). The antibody is a mouse-human chimera using murine variable regions to effect anti-CD20 specificity and human immunoglobulin (Ig) G1k constant region to facilitate effector function, including complement-mediated lysis and antibody-directed cellular cytotoxicity.[20,21] Additional mechanisms include caspase activation,[22] a "vaccinal effect" based on increased idiotype-specific T-cell response to follicular lymphoma,[23] and upregulation of proapoptotic proteins such as Bax.[24,25]

Rituximab's well-known, early recognized, and sometimes fatal chief toxicity has been acute infusion reactions. Rare fatalities, occurring mainly during first infusion,

have been considered secondary to a cytokine reaction; generally associated with flu-like symptoms, they may progress to life-threatening hypotension, bronchospasm, and hypoxia, but can usually be controlled by stopping or adjusting of rates of infusion and proper premedication.[26] Black-box events include tumor lysis syndrome, severe mucocutaneous reactions, and progressive multifocal leukoencephalopathy (PML) resulting in death.[27,28]

Rituximab has demonstrated clinical activity across the spectrum of lymphoproliferative disorders, but the greatest impact has been in NHL, for which combinations and optimizations have sought to raise RRs and, ultimately, cure. Since its 1997 start with relapsed indolent NHL, rituximab has obtained the following additional indications for lymphoma per package insert: relapsed and refractory, follicular or low-grade, CD20-positive, B-cell NHL as single agent; previously untreated CD20-positive, follicular, B-cell NHL in combination with first-line chemotherapy; as single-agent maintenance therapy for patients achieving a partial or complete response to rituximab in combination with chemotherapy; for nonprogressing (including stable), CD20-positive, low-grade, B-cell NHL, as a single agent after first-line combination of cyclophosphamide, vincristine, and prednisone (CVP) chemotherapy; previously untreated CD20-positive, diffuse large B-cell NHL in combination with anthracycline-based chemotherapy, for example, in the workhorse, R-CHOP.[29] It also has an oft-used indication for treatment of previously treated or untreated patients with CD20 chronic lymphocytic leukemia (CLL) in combination with fludarabine and cyclophosphamide (FC).[30]

Rituximab has found off-label use in the clinic in all or nearly all malignant (and many nonmalignant) settings where B cells are presumed to participate in pathogenesis, and has been the subject of many scholarly reviews. Common use spans from aggressive to low-grade lymphoproliferative disorders including: combination with chemotherapy for induction in second-line therapy for relapsed lymphoma anticipating autologous transplant[31]; combination with chlorambucil for indolent and with bendamustine in treatment of relapsed or refractory CLL[32]; induction for Burkitt lymphoma; use for gastric and nongastric mucosa-associated lymphoid tissue (MALT tumors),[33,34] mantle cell tumor,[35] primary cutaneous B cell,[36] splenic marginal zone NHL,[37] Waldenström's macroglobulinemia/lymphoplasmacytic lymphoma.[38] Its uses have been tailored to mutational status of del(17p) and del(11q) with refractory CLL (National Comprehensive Cancer Network [NCCN] guidelines: http://www.nccn.org/index.asp) and combined in cocktail fashion with other antibodies such as alemtuzumab for refractory lymphoid malignancies.

The evolution of treatment for CLL mirrors, in many ways, that of NHL as it leads from purines to chemoimmunotherapy, and most recently to novel anti-CD20 antibodies. Conventional treatment of CLL evolved from alkylators to purine analogues when it was demonstrated that fludarabine (F) yielded greater efficacy with better complete response (CR), progression-free survival (PFS), and overall survival (OS) rates than with chlorambucil as primary therapy.[39] Subsequently, the combination of fludarabine with cyclophosphamide (FC) showed better CR and PFS than F.[40] Based on the activity of rituximab (R) alone as a front-line agent, it was added to FC (ie, FCR) and compared with FC alone; in a phase III randomized trial the combination FCR demonstrated better OR, CR, and PFS, establishing both the regimen and the concept of chemoimmunotherapy in this setting as the upfront standard of care.[41]

Ofatumumab

Unfortunately, the activity of rituximab as a single agent is only modest,[42] and duration of response in relapsed disease is generally measured in months.[43] This shortcoming was part of the impetus to develop newer anti-CD20 targeted antibodies with a goal of improving such characteristics as binding affinity, specificity and effector function, and

efficacy.[44] Ofatumumab (ofa), a fully human monoclonal IgG1 antibody, binds to a unique epitope,[45] induces considerably higher complement-dependent cytotoxicity (CDC) than rituximab,[46] and shows activity in rituxan-refractory B-cell lymphoma.[47]

Based on these potential biological advantages and modest early-phase clinical activity,[48] ofa was tested against CLL, which was either refractory to fludarabine and alemtuzumab or refractory to fludarabine with disease considered too bulky for efficacy with alemtuzumab.[49] The drug was well tolerated, though complicated by infections in 25% of the patients, but the impressive clinical results including median OS of 13.7 or 15.4 months, within 2 high-risk groups, respectively, contributed to the approval of ofa for disease refractory to fludarabine and for those who have failed alemtuzumab.[50,51]

Given the potential advantages of ofa versus rituximab and with FCR established as standard of care in the front line, substituting ofa for rituxan in the so-called O-FC regimen was tested in a multinational, randomized phase II trial in treatment-naïve patients.[52] Of the 2 tested doses, the higher dose arm yielded a CR rate of 50%. It remains unclear as to how this should be positioned with respect to such other findings as the initial randomized phase III trial that established FCR as standard of care. The precedent of combining permutations of purine analogues, alkylators, and antibodies including newer regimens such as ofa/bendamustine continues to inform ongoing studies.[53]

Ipilimumab

The novel treatment agents for melanoma, vemurafenib (a B-raf inhibitor) and ipilimumab (an antibody against cytotoxic T-lymphocyte antigen 4 [CTLA-4]), represent perhaps the most significant advance in oncology in several years. How they will fit into tactical treatment strategies and with respect to conventional dacarbazine, interleukin (IL)-2, and a new gp100-based vaccine is a welcome and exciting challenge after decades without appreciable progress.[54] Blockade of the CTLA-4 has been the subject of long and intensive investigation.[55,56]

Among the most active immune inhibitory pathways is the CD28/CTLA-4:B7-1/B7-2 receptor/ligand grouping, which modulates peripheral tolerance to tumors and outgrowth of immune-evasive clones. Inhibition is both toward the overexpressed self targets and via upregulation of inhibitory ligands on lymphocytes. Thus, blockade of CTLA-4 has potential for both monotherapy and in synergy with other therapies that enhance presentation of tumor epitopes to the immune system.[56] Genetic ablation of CTLA-4 leads to a massive and lethal lymphoproliferative disorder.[57] Antibody blockade of CTLA-4 induces potent antitumor activity through enhancing effector cells and concomitantly inhibiting T-cell regulatory activity.[58]

Given that this inhibition is not tumor specific, it is not surprising that other tumors including ovarian cancer, prostate cancer, and renal cell cancer have demonstrated durable remissions.[59]

In a recent phase III trial, patients with melanoma refractory to chemotherapy or IL-2 who received ipilimumab had improved OS compared with those receiving the gp100 peptide vaccine, and on this basis received FDA approval in 2011.[60]

Ipilimumab holds an FDA indication for the treatment of unresectable or metastatic melanoma, with NCCN guidelines that largely elucidate specific contexts consistent with this approval, including use as single agent for unresectable stage III in-transit metastases, local/satellite and/or in-transit unresectable recurrence, incompletely resected nodal recurrence, limited recurrence or metastatic disease, and disseminated recurrence of metastatic disease in patients with good performance status.

Based on its mechanism of unleashing the immune recognition and effector system, there was rationale to test the interactive effects with tumor-specific antigen. Specifically, the melanoma antigen, gp100, overexpressed on this tumor and among the antigens presented in the appropriate genetic major histocompatibility complex (MHC) context (HLA*A201), represented a prime vaccine candidate. In a phase III randomized trial, increased RRs were seen when vaccine was added to IL-2 compared with IL-2 alone (16% vs 6%, $P = .03$); PFS was also significantly improved with a trend toward improved OS.[61] Questions arose, nonetheless, as to whether gp100 vaccine was an appropriate control in the aforementioned phase III trial for ipilimumab. Another phase III randomized clinical trial treating previously untreated patients with metastatic melanoma compared ipilimumab (every 3 weeks for 4 doses followed by maintenance every 3 months) with and without dacarbazine as the standard control; improved OS was seen, including a difference at 3 years of nearly 21% versus 12%.[62]

The cluster of well-identified side effects induced by CTLA-4 inhibition has been referred to as immune-related adverse events (IRAEs). These unique adverse effects are likely a direct effect of impairing immune tolerance, and include colitis/diarrhea, dermatitis, hepatitis, uveitis, nephritis, inflammatory myopathy, and endocrinopathies. Although these reactions have gained a black-box designation for occasional severe and even fatal instances, they are generally manageable and reversible with treatment guidelines that include systemic corticosteroids.[63] These toxicities may be prolonged, suggestive of sustained release from immune tolerance, and perhaps a different response profile including long periods of stable disease, and correlation of toxicity with efficacy. In one report with high-risk melanoma, ipilimumab-treated patients who experienced high-grade IRAEs had a significantly higher rate of tumor regression than those without IRAEs (36% vs 5% of patients).[64]

Based on a mechanism of action clearly different from IL-2, which increases responsiveness to immune targets and is nonoverlapping with chemotherapy, earlier phase trials and future efforts will focus on combinations of vaccines, chemotherapy, and other immune modulators.[59] Furthermore, given the prolonged time course of side effects and the resulting requirement for prolonged steroids, timing of its use with respect to IL-2 and vaccines will be the subject of much attention.[65]

Trastuzumab

The human epidermal growth factor receptor 2 (HER2) is overexpressed in 20% to 30% of invasive breast cancers and is associated with a worse prognosis.[66] Trastuzumab is a humanized mAb targeting HER2, which was approved by the FDA in 1998 as the first monoclonal for a solid tumor indicated for patients with invasive breast cancer that overexpresses HER2. It is now a standard part of the treatment of HER2-positive tumors in both metastatic and adjuvant settings. Because tumors that overexpress HER2 receptor respond better across the range of studies, considerable effort has been expended to accurately assess receptor status.[67–69]

HER2 is part of a family of transmembrane tyrosine kinase receptors that normally regulate cell growth and survival, differentiation, and migration.[70] It consists of an extracellular binding domain, a transmembrane segment, and an intracellular tyrosine kinase domain. The receptor is activated generally by homodimerization or heterodimerization, but not always activated through ligand binding; it can dimerize and thus activate, independent of ligand[71] through either overexpression or mutation.[72] Thus activated by overexpression, signal-transduction cascades act to promote a host of progrowth activities including proliferation, survival, and invasion. Such signal transduction is mediated through the RAS-MAKP pathway, inhibiting cell death through

the m-TOR pathway.[73] In addition, it inhibits the PI3K pathway, reducing PTEN phosphorylation and AKT dephosphorylation and thus increasing cell death.[74,75]

The human IgG1 is capable of inducing antibody-dependent cell-mediated cytotoxicity (ADCC) in vitro[76] and of recruitment of effector cells in animal studies.[77] An immune mechanism is suggested by the increased lymphoid infiltration into tumor after preoperative administration of trastuzumab.[78] There is also evidence that it causes regression of vasculature by modulating angiogenic factors.[79]

As a single agent in metastatic breast cancer, and receptor status using earlier immunohistochemistry (IHC) expression criteria, trastuzumab produced RRs of 11% to 26%.[80] From the earliest studies, though time has sharpened the assessment, it has been clear that the best results occur in tumors that overexpress HER2. The breakthrough trial for trastuzumab in metastatic disease came in a randomized phase III trial when it was used in combination with chemotherapy for HER2-positive patients.[81] As first-line therapy for metastatic disease, patients were given either chemotherapy alone or in combination. Patients were given an anthracycline and cyclophosphamide or, paclitaxel (if they had previous anthracycline in an adjuvant setting). Results showed not only improvement in response rate (RR) and progression-free interval but also in OS. Trastuzumab was subsequently showed to have efficacy and safety with a variety of other chemotherapeutics including docetaxel,[82] vinorelbine,[83] and doxil[84] in nonrandomized trials.

As for the adjuvant setting, large randomized trials established significant benefits from the addition of trastuzumab to both anthracycline and nonanthracycline regimens for early breast cancer.[85] Four major adjuvant trials including more than 13,000 women with HER2-positive early breast cancer used different adjuvant regimens with trastuzumab; in these studies overall, trastuzumab reduced the 3-year risk of recurrence by about half in this population.[86] On this basis, trastuzumab has become part of standard adjuvant therapy. Both the international Consensus Group and NCCN recommend its use for women with HER2-positive, node-positive tumors as well as for node-negative disease when the primary tumor is larger than 1 cm.

Trastuzumab combined with chemotherapy has also shown improvement in pathologic responses and event-free survival when used in the neoadjuvant setting before surgery.[87] In a randomized phase III trial, patients with advanced gastroesophageal and gastric adenocarcinoma tumors that overexpressed HER2 showed a significant increase in OS when trastuzumab was added to their chemotherapy.[88] Trastuzumab now has an FDA indication for use in combination with cisplatin and fluorouracil (5FU) or capecitabine for first-line treatment of gastric and gastroesophageal tumors that overexpress HER 2.

The most significant toxicity associated with trastuzumab is cardiomyopathy, ranging from subclinical decreases in left ventricular ejection fraction to cardiac failure manifesting as congestive heart failure. The risk is greatest when administered concurrently with anthracyclines.[81] Use following anthracyclines was associated commonly with asymptomatic cardiac dysfunction, but most severe decreases recovered with time.[89] Close monitoring of clinical status and cardiac function, sequential rather than concomitant use, and development of nonanthracycline regimens[90,91] have all been objectives.

Bevacizumab

The discussion of bevacizumab here is asymmetric in bulk and breadth compared with the other antibodies, owing to its conceptual and actual application in many tumor types, its unique mechanism, and toxicity profile. Bevacizumab is a humanized IgG1 mAb that binds to and neutralizes the ligand vascular endothelial growth factor (VEGF) rather than binding the cell-surface receptor. In fact many tissues and most

malignancies produce VEGF whose native function, whether acting from a distance or in an autocrine loop, operates through binding and activation of the VEGF receptor.[91] The latter includes an extracellular binding domain and a cytoplasmic kinase domain. Following VEGF binding, the otherwise inactive monomer receptor undergoes dimerization, autophosphorylation of the tyrosine kinase domain, and downstream activation of many of the usual signal transduction suspects including MAPK and protein kinase C pathways, which mediate proliferative events—in this setting, endothelial proliferation and angiogenesis[92]; such neoangiogenesis is required by tumors once they grow to greater than 2 mm.[93]

Many of this antibody's common toxicities are related to its impact on microvasculature, including hypertension, proteinuria, rare bowel perforation, impaired wound healing, and bleeding.[94] Other than the rare bowel perforation, these can generally be managed without necessitating cessation of therapy. Although there will naturally be some specificity of side effects and adverse reactions dependent on the drugs with which bevacizumab is paired, with some notable exceptions toxicities are generally neither drug-combination specific nor tumor specific.

More severe and fatal consequences of bevacizumab have been the subject of several meta-analyses and reports of large-institution experience. In perhaps the largest of these, fatal adverse events (FAEs) were considered in a meta-analysis of more than 10,000 patients with various solid tumor types, comparing regimens with and without the addition of bevacizumab. The overall incidences of FAEs were 2.5%, and among these nearly a quarter were attributable to hemorrhage, about half related to neutropenia, and a smaller amount to perforation. There was increased RR attributable to combining bevacizumab with taxanes or platinum but not with other agents, nor were there significant tumor-specific increases. In another large meta-analysis bevacizumab was associated with high-grade congestive heart failure in breast cancer, with an overall incidence of 1.6%.[95] Yet a third large meta-analysis identified a 12% risk of thromboembolic events.[96] Of note, a pooled analysis of phase II and phase III trials did not show an increase in venous thromboembolic events (VTEs), which is important to recognize given a baseline of tumor-associated VTEs of around 10% with or without this agent.[97] Massive hemoptysis, which has been linked to large central lesions at risk for cavitation,[98] was avoided in these circumstances, and more generally in squamous cancer where this risk is increased. Bowel perforation occurred with an incidence of less than 2% in a large institution with a treated population of more than 1400 patients; it was generally managed without the need for surgical intervention.[99]

Bevacizumab demonstrated no[100] to small[101] RRs as monotherapy and, with such exceptions as maintenance regimens and single-agent use with recurrent glioblastoma, its predominant clinical role lies in combination with chemotherapy. In 2004, based on improvement of RRs, PFS, and OS, bevacizumab, when combined with chemotherapy in metastatic colorectal cancer,[102] became the first antiangiogenic agent approved for clinical use. Since then it has gained indications for metastatic breast cancer, metastatic renal cancer, metastatic (as well as advanced or recurrent) non–small cell lung cancer (NSCLC), and glioblastoma. Increasing use of bevacizumab is also being seen with hepatocellular and ovarian cancer.

Colorectal cancer

At this time bevacizumab has an indication in metastatic colorectal cancer in both first-line and second-line settings. The initial approval followed its use with bolus irinotecan, 5FU, and leucovorin (IFL) whereby addition of bevacizumab significantly improved RR and median survival (20 vs 16 months) compared with chemotherapy only.[102] While

bolus IFL has fallen out of general use because of its toxicity profile, studies have supported the value of bevacizumab in combination with more widely used treatments including FOLFIRI (FOL, leucovorin plus F, 5FU and IRI, irinotecan [Camptosar]),[103,104] and 3 oxaliplatin-containing regimens.[105] In addition, when bevacizumab was added to 5FU/leucovorin in the absence of irinotecan or oxaliplatin, RRs were approximately doubled and median survival improved in comparison with chemotherapy alone.[106,107]

Efforts to apply bevacizumab in the adjuvant setting for colorectal cancer moved from initial enthusiasm to disappointment. As already noted, bevacizumab had shown favorable impact in metastatic disease in several settings including in combination with IFL (irinotecan, 5FU, and leucovorin) for metastatic colorectal cancer. Borrowing the prevailing paradigm for chemotherapy, which attempts to apply results in metastatic disease to adjuvant use on the presumption of potential elimination of micrometastases, bevacizumab was studied in the adjuvant setting for colorectal cancer. Two recently published phase III trials, unfortunately, did not show the sought-for benefit. When bevacizumab was added (for 12 months) to FOLFOX (folinic acid [FOL], fluorouracil [F], and oxaliplatin [OX]) (for 6 months), it failed to meet its primary end point of improving 3-year disease-free survival.[108] In a second phase III trial the combination of bevacizumab with FOLFOX actually led to a slight but significant decrease in OS.[109]

Non–small cell lung cancer

The role of bevacizumab in NSCLC was initially established in a phase III trial as first-line therapy for advanced, nonsquamous NSCLC including those with malignant effusions and metastatic disease.[110] Patients received paclitaxel and carboplatin with or without bevacizumab; those patients receiving bevacizumab then continued it as monotherapy for an additional 6 cycles unless disease progressed. The objective RR more than doubled, and there was an increase in PFS and OS. At 2 years, the survival rate was 23% in the group treated with bevacizumab versus 15% without. In another phase III trial with a similarly defined patient population, on the addition of bevacizumab to gemcitabine and cisplatin (also with maintenance bevacizumab in the concurrent bevacizumab group) an increase in PFS did not translate into improved OS[111]; the investigators suggested this may have been due to the wide availability of secondary therapies. Testing with a current standard, pemetrexate, is under way but has not yet ripened to a point to give clinical guidance.[112,113]

The story for use of bevacizumab in advanced metastatic breast cancer (MBC) has been tumultuous, tracking a course from early excitement and widespread use to an FDA withdrawal; understandably this raised public furor from a highly engaged population. In the first phase III trial to assess impact in newly diagnosed patients with MBC, bevacizumab was either added to chemotherapy (weekly paclitaxel) or not; the bevacizumab arm doubled the PFS and significantly improved RR.[114] These striking results led to accelerated FDA approval and its wide adoption. Unfortunately, neither of 2 phase III postapproval studies, one trial with docetaxel and the other with capecitabine, a taxane, or an anthracycline, confirmed this magnitude of benefit, and no trial has shown an improvement in OS.[115,116]

Renal cancer

For metastatic renal cancer, 2 phase III trials demonstrated improved OS when bevacizumab was added to interferon-α in first-line treatment.[117,118] In one of these trials the initially reported PFS with bevacizumab of 10.2 months was nearly doubled,[117] but only a nonsignificant and clinically small difference of OS was reported in the final analysis.[119] In the second phase III trial, with a similar dose schedule as the first,

bevacizumab plus interferon-α improved RR and PFS compared with monotherapy with interferon, but did not reach significance for OS.[118,120]

Glioblastoma

For recurrent glioblastoma, adding bevacizumab to irinotecan increased RR.[121,122] Nevertheless, in the notoriously difficult setting of recurrent glioblastoma both alone and in combination with irinotecan, bevacizumab showed respectable RRs of 28% and 38%, respectively[123]; it holds an indication for use as monotherapy in this setting despite the absence of a demonstrated improvement in OS.

Ovarian cancer

The benefit of bevacizumab in ovarian cancer was assessed in the setting of first-line use with paclitaxel and carboplatin in a large trial for stage III or IV epithelial ovarian, primary peritoneal, or fallopian tube cancer following maximal cytoreduction.[124] Of the 3 arms in this phase III trial, representing chemotherapy only, concurrent bevacizumab and chemotherapy, and concurrent bevacizumab and chemotherapy followed by maintenance bevacizumab, the latter improved PFS but not OS. First-line use for advanced and high-risk early-stage disease treated with paclitaxel and carboplatin, with and without bevacizumab, showed significant improvement in median survival without improving OS; a subset analysis suggested that adding bevacizumab may be more beneficial among women with a poorer prognosis.[125]

Small studies in hepatocellular with bevacizumab alone[126] or with gemcitabine and oxaliplatin[127] showed RRs sufficient to generate further interest and more definitive study.

Bevacizumab has a unique profile of toxicities and adverse reactions. Some preclinical studies had suggested that VEGF-targeted therapies could unfavorably alter the biology of the neoplasms, for example, by upregulating proinflammatory pathways and factors that are associated with metastasis,[128] but a pooled meta-analysis of 5 randomized phase III trials did not show altered disease progression following bevacizumab.[129] Although the clinical data are too scant to explain the unpredicted disappointments such as failure in the adjuvant setting for colorectal cancer, numerous hypotheses such as the foregoing, some readily testable, have been suggested.[130] As in most contexts in oncology, risk/benefit analysis is important to decision making, and the risk in some clinical settings where bevacizumab is considered often pits treatment against the prospect and probability of imminent death. It is notable, therefore, that while a recent meta-analysis of 16 randomized trials in advanced cancer showed nearly a 1.5-fold increase in fatal adverse events, the absolute values were 2.5% versus 1.7% in the respective presence or absence of bevacizumab.[131] Nevertheless, these same numbers gather increased clinical sway in adjuvant settings where the risks and benefits are markedly different.

Cetuximab

Cetuximab is a recombinant chimeric antibody that derives specificity from its murine Fv portion and effector functions from human IgG1 constant regions. The primary mechanism of impact is through disruption of the signal transduction pathway of the endothelial growth factor receptor (EGFR).[132] Nevertheless, selection based on IHC expression of EGFR expression or somatic mutations[133,134] of the EGFR tyrosine kinase domain,[135] as in the response of NSCLC to small-molecule tyrosine kinase inhibitors, do not predict response of colorectal cancer to EGFR antibodies. Wild-type K-ras, on the other hand, is necessary for effect.[136]

Cetuximab has been studied alone and in combination, predominantly with colorectal cancer and head and neck cancer. In colorectal cancer, cetuximab as monotherapy showed improvement in OS compared with best supportive care (BSC) in patients previously treated with a fluoropyrimidine, irinotecan, and oxaliplatin.[137] This study also demonstrated improved quality of life and the association of rash with a favorable outcome. Cetuximab as monotherapy or combined with irinotecan both showed clinically significant activity in patients with metastatic disease who were refractory to irinotecan, but the combination showed superior RR, time to progression, and median survival.[138] In another study, the combination of cetuximab and irinotecan also showed improvement in RR and PFS in patients previously treated with oxaliplatin and fluoropyrimidines for metastatic disease.[139] In combination with FOLFIRI as first-line therapy for metastatic disease it showed increased OS in patients with wild-type K-ras.[140] The data showing advantage in the first line when combined with oxaliplatin are not as clear. In one study the addition of cetuximab to FOLFOX showed significant improvement of RR only in the wild type K-ras subpopulation[141] but in another, more recent trial, no advantages were shown when added to oxaliplatin, even in the wild-type K-ras group.[142]

In squamous cell head and neck cancer, cetuximab showed improvement in OS when added to radiation compared with radiation alone for locally and regionally advanced disease.[143,144] The advantage did not extend to those with marked functional compromise or who were older than 65 years. Here, too, response was improved in those with acneiform rash.

As first-line treatment in patients with recurrent or metastatic squamous cell carcinoma of the head and neck, cetuximab plus platinum-5FU chemotherapy improved OS compared with platinum-based chemotherapy plus fluorouracil alone.[145]

Despite 2 recent phase III trials in NSCLC, the role of cetuximab in lung cancer remains unclear. These randomized trials compared doublets of standard chemotherapy with and without cetuximab in the first-line setting for metastatic disease, and may suggest different clinical guidance. In the FLEX trial, a randomized phase III multinational study, patients with IIIB (malignant pleural effusion) and IV, who expressed EGFR, received cisplatin and vinorelbine with or without cetuximab. Patients who received cetuximab had significant but clinically modest increased OS at 11.3 months versus 10.3 months with chemotherapy alone.[146] First-cycle rash in this study was substantially associated with OS, with the median with rash at 15 months compared with 8.8 months without the rash.[147] In another phase III randomized trial studying same-stage patients in first-line treatment, without restrictions on EGFR expression, cetuximab combined with taxol/carboplatin did not improve PFS compared with chemotherapy alone; a small increase in OS for cetuximab of less than 2 months did not reach statistical significance.[148]

Panitumumab

Panitumumab, an IgG2 class antibody to the EGFR receptor, was the first fully human antibody to be approved by the FDA in 2006 for the treatment of patients with EGFR-expressing, metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. It received regulatory approval for use as monotherapy in refractory disease based on prolonging disease-free survival.[149] Given the similarities to cetuximab, efforts have focused on where to place each and in which clinical contexts and sequences, although they have notably not been compared in a face-to-face randomized phase III trial. The close relation to cetuximab, both biological and clinical, provides a helpful context for review. Like cetuximab, panitumumab binds to the receptor, the dyad is

internalized, and the downstream signal transduction is blunted. Its activity cannot be reliably shown to depend on the overexpression of EGFR.[150–152] However, downstream signal transduction by constitutively activated K-ras abrogates its effect, and its use according to American Society of Clinical Oncology guidelines, consistent with clinical trials,[141,153] is limited to tumors with wild-type K-ras. There is more recent evidence that mutations in B-raf may also predict no response to either cetuximab or panitumumab.[154] Biologically, its difference from cetuximab in being fully human may underlie its significant reduction in infusion reactions. Despite its design to be more activating of cell-mediated cytotoxicity and ADCC, neither of these activities nor its efficacy compared with cetuximab has been demonstrated.[1] Its toxicities, which include a predictable rash in almost all cases, as well as frequent diarrhea and malaise, parallel those of cetuximab, as does the positive association of rash with clinical impact.[153]

Panitumumab has shown activity when used both in monotherapy and in combination. A large phase III trial showed improved RR and decreased tumor progression when used as monotherapy compared with BSC in patients refractory to oxaliplatin and irinotecan-based therapies.[155] In combination chemotherapy with FOLFOX, panitumumab in the first line improved both RR and PFS[156] in contrast to cetuximab, which had mixed results as previously noted. When combined with FOLFIRI versus FOLFIRI alone after failure with 5FU-based chemotherapy (the majority with oxaliplatin), addition of panitumumab significantly improved PFS.[157]

Brentuximab Vedotin

The first-in-class antibody drug conjugate (ADC), brentuximab vedontin, received accelerated FDA approval in August 2011 for treatment of relapsed or refractory Hodgkin lymphoma and systemic anaplastic large-cell lymphoma (ALCL). Approval was based on impressive RR rather than demonstrable survival improvement in rather dire clinical circumstances: in Hodgkin lymphoma after failure of at least 2 prior systemic regimens in autologous stem cell candidates, and for ALCL after failure of at least 1 multiagent regimen.

The antibody is a chimerized IgG, which targets CD30 and thus delivers its antimitotic payload, monomethyl auristatin (vedontin). CD30 is only minimally expressed in normal tissue but densely expressed in both Hodgkin lymphoma and ALCL.[158]

As optimistically as progress with treatment of Hodgkin disease (HD) is viewed, approximately 15% to 30% of patients do not achieve long-term remissions on conventional therapy, and despite autologous stem cell transplantation (ASCT) many of these subsequently perish while still in young adulthood.[159]

In the pivotal phase I[160] and subsequent phase II trials[161] RR data were rightly greeted with excitement. In the phase II trial, for patients with HD, all with prior transplants, 75% achieved an objective response including 32% complete responders who have not yet reached median duration of response. For 58 patients with relapsed or refractory systemic ALCL, a CR was reached in 56% of patients the median duration of which, likewise, has not yet been reached. Moreover, retreatment has been successfully used to maintain complete remissions[162] and, though the number of patients was small, a retrospective look across 3 studies demonstrated provocatively high responses with retreatment.[163]

It has been suggested that these impressive results may include several mechanisms, from apoptosis by ligating CD30, to cytotoxic, to the bystander effect on surrounding tissue.[160,164] The remarkable results with HD were particularly impressive considering the minimal responses achieved using the unconjugated anti-CD30 antibody. Beyond the direct impact on the malignant cell, it has been suggested that the

local effect on the tumor-supporting cellular milieu was also a factor. By bulk, malignant Reed-Sternberg cells represent a minority of the masses in HD, which otherwise consist largely of inflammatory cells recruited by chemokines that in turn support the tumor cells that recruited them.[165] The shrinkage of these masses might thus be understood, in part, to be due to the bystander effect caused by local diffusion of the cytotoxic agent into this local environment. Although other differences no doubt exist, this may be one factor to explain greater responses than achieved for similar antibody-toxin conjugates, for example, with trastuzumab in HER2-positive cancer[166] where tumor masses are predominantly composed of malignant cells.

Alemtuzumab

Alemtuzumab is a humanized anti-CD52 IgG1 monoclonal antibody. Early studies demonstrated its efficacy in refractory disease, leading initially to approval by the FDA in 2001 for treatment of fludaribine-refractory CLL; subsequent trials demonstrated its use as front-line monotherapy for B-cell CLL.[167] Antibodies to CD52 induce complement-mediated lysis and antibody-directed cellular toxicity through this target that is not only expressed on CLL and lymphomas but also on both normal B cells and normal T cells, neutrophils, and monocytes.[168] This large spectrum of targets accounts not only for positive aspects such as off-label uses with T-cell lymphoproliferative disorders such as peripheral T-cell lymphoma, T-cell prolymphocytic leukemia, cutaneous T-cell lymphoma,[169–171] mycosis fungoides, and Sezary syndrome,[172] but also for negative consequences such as heightened infusion reactions and significant vulnerability to opportunistic infections.

Based on increased acute toxicity and prolonged myelosuppression, alemtuzumab has not supplanted the more B-cell–specific rituximab either as monotherapy or in combination with cytotoxic chemotherapy. First-line treatment for CLL generally uses fludaribine as the cornerstone, often in combination with cyclophosphamide and rituximab.[173] Second-line therapy with alemtuzumab added to fludarabine and cyclophosphamide demonstrated substantial efficacy in a recently reported phase II trial.[174] Response rates have ranged in the area of 30% to 50% in the relapsed setting and 80% to 90% in previously untreated patients with CLL.[1] In a large multicenter study, patients with refractory or relapsed CLL, previously exposed to alkylating agents and having failed fludarabine, had an overall RR of about one third, nearly all partial responses; median survival for responders was 32 months.[49] Alemtuzumab has received particular attention in high-risk settings, including 17p deletions and p53 defects[175,176] known to be resistant to standard agents including chlorambucil, purine analogues and rituximab. One study demonstrated nearly 50% overall RRs and favorable OS[177] in the 17p-deletion cytogenetic group. Alemtuzumab has been shown to achieve CR in the setting of p53 mutation and resistance to chemotherapy,[178] and in one study of fludarabine-refractory disease, even within a small subset with the presence of p53 mutations or deletions (predictors of poor response to conventional therapy), responses occurred in 40% with a median response duration of 8 months.[179] In a phase II trial with subcutaneous alemtuzumab, efficacy with fludarabine-refractory CLL did not vary with 17p deletion, mutated p53, 11q deletion, or mutated p53.[180]

Combination of alemtuzumab with rituximab has not gained traction, based on results of FCR that are hard to compete with and significant infectious complications. In a study of 32 patients with relapsed or refractory disease, for example, while slightly more than 50% showed response a similar percentage also developed infections, including 27% cytomegalovirus antigenemia.[181] In a recent phase II trial, alemtuzumab was added to conventional FCR yielding 70% CR and 18% partial response in

high-risk patients, results considered comparable with historic FCR-treated high-risk patients. Based on nearly 60% CRs in the subset with 17p deletion it was suggested, however, that this may nonetheless have a useful front-line role before allogeneic stem cell transplantation.[182]

The general use of alemtuzumab for consolidation in the community setting cannot yet be recommended, although the question remains to be settled and is the subject of significant investigation.[183] A phase III trial in which alemtuzumab was used as consolidation to fludarabine ± cyclophosphamide was stopped prematurely because of severe infections; nevertheless, minimal residual disease was durably reduced by consolidation and PFS was significantly improved after median follow-up of 48 months.[184] Although there was a trend toward shorter response duration in comparison with historic groups receiving intravenous alemtuzumab, patients receiving subcutaneous treatment showed reliable decreases in graded measures of residual disease.[185] Although alemtuzumab consolidation improved both CR and minimal residual disease (MRD)-negative rates, in a study of 102 patients initially treated with induction fludarabine and rituxan there were 5 deaths from infection, and 2-year PFS and OS were not improved.[186] Efforts have been under way over the past decade to unravel genomic complexity in CLL.[187,188] Such understanding will inform trial design and, undoubtedly, the value of consolidation will depend on identification of molecular diagnostic settings where improvements of MRD-negative status translate into improved OS.

mAbs IN CLINICAL AND PRECLINICAL DEVELOPMENT

Hundreds of mAbs are in thousands of clinical trials[14]; 2239 entries for planned, ongoing, or completed clinical trials were retrieved from http://www.clinicaltrials.gov by searching with cancer AND therapy AND monoclonal antibodies as of August 2011, of which 270 are in phase III. A significant number of all new medicines are mAbs against cancer (see also http://www.phrma.org/research/new-medicines). At least 1 to 3 different antibodies are being developed at different companies for each relevant therapeutic target. However, some molecules are targeted by many more mAbs; for example, the insulin-like growth factor receptor type I (IGF-IR) is targeted by more than 10 different mAbs.[189] During the last decade and especially in the last several years, the number of clinical trials with therapeutic antibodies has increased dramatically. However, this increase has been largely due to an increase in the number of indications for the same antibodies, especially in combination with other therapeutics. The number of targets and corresponding antibodies in preclinical development and in the discovery phase has also increased significantly during the past decade.

Second- and third-generation mAbs are being developed against already validated targets. The improvement of already existing antibodies also includes an increase (to a certain extent) of their binding to Fc receptors for enhancement of ADCC and half-life, selection of appropriate frameworks to increase stability and yield, decrease of immunogenicity by using in silico and in vitro methods, and conjugation to small molecules and various fusion proteins to enhance cytotoxicity. A major lesson from the current state of antibody-based therapeutics is that gradual improvement in the properties of existing antibodies and identification of novel antibodies and novel targets is likely to continue in the foreseeable future.

One area where one could expect conceptually novel antibody-based candidate therapeutics, even though within the current paradigm, is going beyond traditional antibody structures. At present, all FDA-approved anticancer therapeutic antibodies (see **Table 1**) and the vast majority of those in clinical trials are full-size antibodies,

mostly in IgG1 format of about 150 kDa. A fundamental problem for such large molecules is their poor penetration into tissues (eg, solid tumors) and poor or absent binding to regions on the surface of some molecules (eg, on the human immunodeficiency virus envelope glycoprotein) that are accessible by molecules of smaller size. Therefore much work, especially during the last decade, has been aimed at developing novel scaffolds of much smaller size and higher stability (see, eg, recent reviews in Refs.[11,190,191]). Such scaffolds are based on various human and nonhuman molecules of high stability and could be divided into 2 major groups for the purposes of this review: antibody-derived and others. Here the advantages of antibody-derived scaffolds, specifically those derived from antibody domains, and binders selected from libraries based on engineered antibody domains (eAds) are briefly discussed; an excellent recent review describes the second group.[190]

First, their size (12–15 kDa) is about an order of magnitude smaller than the size of an IgG1 (about 150 kDa). The small size leads to relatively good penetration into tissues and the ability to bind into cavities or active sites of protein targets that may not be accessible to full-size antibodies. Second, eAds may be more stable than full-size antibodies in the circulation and can be relatively easily engineered to further increase their stability. For example, some eAds with increased stability could be taken orally or delivered via the pulmonary route, or may even penetrate the blood-brain barrier, and retain activity even after being subjected to harsh conditions such as freeze-drying or heat denaturation. In addition, eAds are typically monomeric, of high solubility, and do not significantly aggregate or can be engineered to reduce aggregation. Their half-life in the circulation can be relatively easily adjusted from minutes or hours to weeks by making fusion proteins of varying size and changing binding to the neonatal Fc receptor (FcRn). In contrast to conventional antibodies, eAds are well expressed in bacterial, yeast, and mammalian cell systems. Finally, the small size of eAds allows for higher molar quantities per gram of product, which should provide a significant increase in potency per dose and reduction in overall manufacturing cost. However, despite all these advantages there is still no candidate therapeutic based on such scaffolds in phase III clinical trial as of August 2011.

Research on novel antibody-derived scaffolds continues. The authors identified a scaffold based on the variable region of heavy chain that is stable and highly soluble.[192] It was used for construction of a large-size (20 billion clones) eAd phage library by grafting complementarity-determining regions (CDR3s and CDR2s) from 5 of the authors' other Fab libraries and randomly mutagenizing CDR1. It was also proposed to use engineered antibody constant domains (C_H2 of IgG, IgA and IgD, and C_H3 of IgE and IgM) as scaffolds for construction of libraries.[193] Because of their small size and the domain's role in antibody effector functions, these have been termed nanoantibodies, the smallest fragments that could be engineered to exhibit simultaneous antigen binding and effector functions. Several large libraries (up to 50 billion clones) were constructed and antigen-specific binders successfully identified.[194] The authors have recently engineered C_H2-based scaffolds with high stability by introducing an additional disulfide bond[195] and by shortening C_H2.[196] It is possible that these and other novel scaffolds under development could provide new opportunities for identification of potentially useful therapeutics.

SAFETY, EFFICACY, AND QUALITY OF CANDIDATE THERAPEUTIC mAbs

The success of antibody-based therapeutics is mostly attributable to the use of concepts and methodologies developed during a paradigm change decades ago that resulted in dramatic improvement of 3 key features in candidate therapeutics

required for FDA approval: safety, efficacy, and quality. These factors are critical for the success of any drug, and are discussed in more detail as regards antibody-based therapeutics.

Safety

Side effects caused by therapeutic antibodies may be divided into 2 large groups: (1) interactions with intended targets and (2) interactions with unintended targets. Binding to an intended target can lead to undesirable side effects, for example, by immunomodulatory antibodies that could be suppressory or stimulatory. Administration of suppressory therapeutic antibodies could lead to a wide range of side effects related to decreased function of the immune system. An important example is the use of the best-selling antibody-based therapeutics targeting tumor necrosis factor α (TNFα) (infliximab, certolizumab pegol, and adalimumab), which can lead to infectious complications.[197] The overstimulation of the immune system can also produce life-threatening illness. In one case, which gained wide publicity, administration of a single dose of the stimulatory anti-CD28 mAb TGN1412 resulted in induction of a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines in all 6 volunteers, leading to critical illness within 12 to 16 hours.[198] One important difference between antibody-based therapeutics containing Fc and other therapeutic proteins (not conjugated with toxic molecules) is that the antibody effector functions including ADCC and CDC could lead to toxicities after binding to intended target molecules, but on tissues other than those intended. An example of this is the trastuzumab-associated cardiotoxicity that is potentiated when the antibody is used concurrently or sequentially with an anthracycline.[199]

Interactions with unintended targets can lead to a wide range of side effects, in many cases with poorly understood mechanisms. An important example is the adverse acute infusion reactions after administration of antibodies whereby cytokine release plays a pivotal role but whereby other not fully explained mechanisms could also be involved; such reactions were reported for many antibodies including infliximab, rituximab, cetuximab, alemtuzumab, trastuzumab, and panitumumab.[200] Infusion side effects for rituximab can result from release of cellular contents from lysed malignant B cells.[201] Administration of antibodies can also lead to hypersensitivity reactions, including anaphylactic shock and serum sickness.[197] Preexisting IgEs that cross-react with therapeutic antibodies can increase the number and severity of such reactions, which can occur even with the first protein infusion. A notable example of this occurred with administration of cetuximab.[200] Hypersensitivity is frequently associated with immunogenicity.

Immunogenicity

Immunogenicity of antibodies can be a significant issue in safety and efficacy.[197,202–207] For example, the success of the mAb-based therapeutics was critically related to the development of less immunogenic proteins. Murine mAbs were used initially as candidate therapeutics in the 1980s, but their high immunogenicity resulted in high titers of human antimouse antibodies (HAMAs), and related toxicities and low potency. Development of the less immunogenic chimeric mAbs, which contain human Fc fragments, and humanized mAbs, which contain mouse CDRs grafted into a human antibody framework, was critical for the clinical success of the products. Human antibodies exhibit low immunogenicity on average, and are currently the favored type of antibody in development, although most of the therapeutic antibodies approved for clinical use are still chimeric and humanized mAbs.

Immunogenicity can be influenced by factors related to protein structure, composition, posttranslational modifications, impurities, heterogeneity, aggregate formation, degradation, formulation, storage conditions, as well as properties of its interacting partner, the patient's immune system and disease status, concomitant medications, dose, route, and time and frequency of administration, especially when administered as multiple doses over prolonged periods.[203] Even human proteins can elicit human antihuman antibodies. In one of the most studied cases of anti-TNFα mAbs, treatment with the human mAb adalimumab resulted in antibodies against the therapeutic that varied from less than 1% to up to 87% for different cohorts of patients, protocols, diseases, and methods of measurement.[208]

A likely mechanism for the immunogenicity of human mAbs involves the unique antibody sequences that confer antigen binding and specificity, but may appear foreign. Human therapeutic proteins can also break immune tolerance, and aggregation can be a major determinant of antibody elicitation.[203] Aggregation can result in repetitive structures that may not require T-cell help.[209] Antibody immunogenicity may also affect efficacy through either the pharmacokinetic or neutralizing effects of the antibody responses that are dependent on several factors, including the affinity, specificity, and concentration of the induced antibodies.[202] Because immunogenicity is an important factor in both safety and efficacy, significant efforts to predict and reduce immunogenicity of therapeutic antibodies are ongoing.[204–207]

Individual immune responses to therapeutic antibodies vary widely. A key, and largely unanswered, question is what determines these variations. Despite extensive laboratory and clinical studies that were instrumental in delineating general concepts about critical factors involved in immunogenicity, it is impossible to predict the extent to which a novel therapeutic protein will be immunogenic in human patients. Little is known about the individual antibodies that compose the polyclonal response to therapeutic proteins. The germline antibody repertoire at any given time could be a major determinant of individual differences, so knowledge of large portions of antibodies generated by the human immune system, preferably the complete set, that is, the antibodyome,[8] could ultimately help to predict individual immune responses to therapeutic antibodies.

Despite the possibility for immunogenicity and other side effects, antibody therapeutics are relatively safe, primarily because of their high specificity. This advantage is fundamental in comparison with small-molecule drugs, which on average are less specific and can bind nonspecifically to a large number of molecules. However, in some cases there are significant side effects, and safety concerns can lead to the withdrawal of therapeutic antibodies from the market (eg, Mylotarg). Thus, choosing the most appropriate animal model for toxicity testing is very important and species cross-reactivity should be included when identifying new candidate mAb therapeutics. If such a model does not exist, transgenic animals expressing the human target and surrogate protein that is cross-reactive with the human homologous target in relevant animals can be used.[210]

Efficacy

After safety, efficacy is the most important parameter considered by the FDA for approval. Many therapeutic antibodies are highly effective in vivo and have revolutionized the treatment of cancer (eg, rituximab for NHL).[201] Alemtuzumab plays an important role in therapy for hematological malignancies.[211] Another example is trastuzumab as adjuvant systemic therapy for HER2-positive breast cancer.[212] Results from 6 trials randomizing more than 14,000 women with HER2-positive early breast cancer to trastuzumab versus nontrastuzumab-based adjuvant chemotherapy

demonstrate that the addition of trastuzumab reduces recurrence by approximately 50% and improves OS by 30%.[213]

On average, the efficacy of therapeutic mAbs is not high, and there is substantial individual variability. One prominent example is trastuzumab (Herceptin), which has clearly revolutionized the treatment of HER2-positive patients; however, half of the patients still have nonresponding tumors, and disease progression occurs within a year in most cases.[214] For patients with disease progression, combination with small molecules could be useful; for example, the addition of a the dual tyrosine kinase inhibitor of EGFR and HER2 lapatinib to capecitabine was shown to provide superior efficacy for women with HER2-positive, advanced breast cancer progressing after treatment with anthracycline-, taxane-, and trastuzumab-based therapy.[215] Current data do not support the use of trastuzumab for more than 1 year; the appropriate length of treatment, optimum timing, and administration schedule are not known.[212] Like other therapeutic proteins, trastuzumab does not appear to efficiently cross the blood-brain barrier, and it is unclear whether the current practice of local therapy for the central nervous system and continued trastuzumab is optimal.[214]

Antiangiogenic therapies that target the VEGF, for example, bevacizumab, and the VEGF receptor (VEGFR), are effective adjuncts for the treatment of solid tumors, and are commonly administered in combination with cytotoxic chemotherapy. However, at least half of patients fail to respond to antiangiogenic treatment of gliomas, and the response duration is modest and variable.[216] The use of bevacizumab plus paclitaxel as a first-line treatment for patients with MBC doubled median PFS (11.8 months vs 5.9 months; hazard ratio = 0.60; $P<.001$) compared with paclitaxel alone; however, a statistically significant improvement in OS was not provided by the addition of bevacizumab, although a post hoc analysis demonstrated a significant increase in 1-year survival for the combination arm.[217]

The anti-EGFR mAbs cetuximab and panitumumab, either as single agents or in combination with chemotherapy, have demonstrated clinical activity against metastatic colorectal cancer, but seem to benefit only select patients with predictive markers of efficacy, including EGFR overexpression, development of rash, and the absence of a K-ras mutation.[218] In general, as single agents or in combination, therapeutic mAbs and other proteins have produced only modest clinical responses in solid tumors.[219] There are no mAbs approved for the treatment of several tumors (eg, prostate cancer). However, for prostate cancer there are 30 candidates in the pipeline (16 vaccines and 14 antibodies), and one FDA-approved prostate cancer vaccine (Provenge); of these candidates, 19 are in phases II and III (9 vaccines and 10 antibodies) and 8 are in phase I clinical trials.

The mechanisms underlying the relatively low efficacy of some therapeutic antibodies and the high variability of responses to treatment are not well known, but are likely to involve multiple factors. Preexisting resistance or development of resistance is a fundamental problem for any therapeutic. Various mechanisms, including mutations, activation of multidrug transporters, and overexpression or activation of signaling proteins, are exemplified as EGFR-targeted therapies.[220] Another major problem is poor penetration into tissues (eg, solid tumors).

New approaches are being developed to increase efficacy of mAbs, including enhanced effector functions, improved half-life, increased tumor and tissue accessibility, and greater stability; the methods used involve both protein engineering and glycoengineering, and results to date are encouraging.[221,222] mAbs that do not engage the innate immune system's effector functions are being developed when binding is sufficient.[223] Multitargeted antibodies are being developed and tested in clinical trials, for example, an antibody targeting HER2/neu and CD3 with preferential binding to

activating Fcγ type I/III receptors, resulting in the formation of tri-cell complexes between tumor cells, T cells, and accessory cells.[224] A similar bispecific (targeting CD3 and EpCAM) trifunctional mAb, catumaxomab, was approved in the European Union for the treatment of malignant ascites in 2009 (see **Table 1**), and is the first bispecific mAb approved for clinical use. This antibody binds to cancer cells expressing epithelial cell adhesion molecule (EpCAM) on their surface via one arm, to a T-lymphocyte–expressing CD3 via the other arm and to an antigen-presenting cell like a macrophage, a natural killer cell, or a dendritic cell via the Fc. This process initiates an immunologic reaction leading to the removal of cancer cells from the abdominal cavity, thus reducing the tumor burden that is considered the cause of ascites in cancer patients. Bispecific and multispecific mAbs and other therapeutic proteins are currently being developed to be aimed at several targets.

A promising line of enquiry is the modulation of immune responses by mAbs targeting regulators of T-cell immune responses. The cytotoxic T-lymphocyte antigen 4 (CTLA-4), present on activated T cells, is an inhibitory regulator of such responses. Human antibodies and Fc fusion proteins that abrogate the function of CTLA-4 have been tested in the clinic and have been found to have clinical activity against melanoma.[225,226] It appears that CTLA-4 blockade also enhanced the cancer-testis antigen NY-ESO-1–specific B-cell and T-cell immune responses in patients with durable objective clinical responses and stable disease, suggesting immunotherapeutic designs that combine NY-ESO-1 vaccination with CTLA-4 blockade.[226] Ipilimumab, which targets CTLA-4, was approved by the FDA in 2011 for the treatment of metastatic melanoma (see **Table 2**). Therapeutic mAbs that mimic the natural ligand, for example, the TNF-related apoptosis inducing ligand (TRAIL), have also been developed.[227,228]

Second- and third-generation mAbs against already validated targets, for example, HER2, CD20, and TNFα, are currently under clinical study or already approved. Various approaches have been used to discover novel, relevant targets, but progress has been slow. Modifications of the standard panning procedures have been reported, including enhanced selection of cross-reactive antibodies by sequential antigen planning[229] and competitive antigen panning for focused selection of antibodies targeting a specific protein domain or subunit.[230,231] To ensure better tissue penetration and hidden epitope access, a variety of small engineered antibody domains (about 10-fold smaller than IgG) are being developed.[191,192] Knowledge of antibodyomes could be used for generation of semisynthetic libraries for selection of high-affinity binders of small size and minimal immunogenicity.[8]

A major lesson from the current state of antibody-based therapeutics is that gradual improvement in the properties of existing therapeutic proteins and identification of novel proteins and targets is likely to continue in the foreseeable future. A fundamental challenge has been to increase dramatically the efficacy of therapeutic antibodies and to apply them to many more diseases. Other major challenges are the development of effective personalized antibody-based therapeutics, and prediction of toxicity or potentially low efficacy in vivo.

Quality

Quality is a very important parameter for approval of any drug by the FDA. A specific fundamental feature that distinguishes mAbs and other biologics from small-molecule drugs is their heterogeneity. Heterogeneity of mAbs is due to modifications such as incomplete disulfide bond formation, glycosylation, N-terminal pyroglutamine cyclization, C-terminal lysine processing, deamidation, isomerization, oxidation, amidation of the C-terminal amino acid, and modification of the N-terminal amino acids by maleuric

acid, as well as noncovalent associations with other molecules, conformational diversity, and aggregation.[232] Tens of thousands of variants with the same sequence may coexist.

Development of high-quality protein therapeutics with minimal heterogeneity and contamination is essential for their safety and approval by the FDA. Process development for production of a therapeutic protein is a complex operation involving recombinant DNA technologies, verification of a strong expression system, gene amplification, characterization of a stable host-cell expression system, optimization and design of the mammalian cell-culture fermentation system, and development of an efficient recovery process resulting in high yields and product quality.[233] Titers in the range of 5 to 10 g/L or even higher, cell densities of more than 20 million cells/mL, and specific productivity of more than 20 pg/cell/d (even up to 100 pg/cell/d) have been achieved.[234]

Genetic delivery of therapeutic antibodies by in vivo production offers a new way of increasing quality and reducing cost. Three approaches can be used for the stable long-term expression and secretion of therapeutic proteins in vivo: (1) direct in vivo administration of integrating vectors carrying the gene, (2) grafting of ex vivo genetically modified autologous cells, and (3) implantation of an encapsulated antibody producing heterologous or autologous cells. Another promising direction is the prospect of using molecular farming methods to create relatively low-cost therapeutic proteins in plants, for example, in genetically engineered tobacco leaves.

BIOSIMILAR AND BIOBETTER THERAPEUTIC ANTIBODIES

A major goal of current activity is to develop therapeutic antibodies that are similar but cheaper than the currently existing ones, or are better in terms of efficacy and safety. By 2015 biologics worth $60 billion in annual sales will lose patent protection, bolstering hopes for the rapid growth of the biosimilars as generics companies elbow their way into a large new market. Rituxan/MabThera and Remicade are on the top of the list for biosimilars. Sandoz, for example, which is leading the pack of generic companies angling to get into the market, expects to see biosimilar revenue jump from $250 million in 2011 to $20 billion by 2020. Over the next 5 years, the market for biosimilars will increase to $10 billion, but only a handful of big pharmaceutical companies and world-class R&D facilities will be able to take part, meaning that most small and medium-sized drug developers will never have a chance of getting into the new market for follow-on biologics.

The niche for most small biotech companies is taking a preclinical or very early-stage candidate to proof of concept, at which point they can make the sale to bigger companies. With biosimilars, the developer will start with proof-of-concept data and then ramp up the most expensive stage of clinical development, with the added charge of running a likely comparison study with the marketed therapeutic; such a process will not be cheap. It could take 8 years to run a biosimilar program, with development costs sliding from $100 million to $150 million. With that much time and money at stake, most biotech companies may never be competitive.

SUMMARY

The rapid progress made in the last few decades toward the development of potent therapeutic antibodies raises several questions for the future directions of this field. A key question is whether there are any indications of a paradigm change that could lead to radically different therapeutics, as occurred 2 to 3 decades ago, and which resulted in an explosion of antibody therapeutics approved for clinical use during

the last decades. If history provides an answer and such a paradigm shift occurs, it will probably take decades before the fruition of such a shift in terms of new licensed protein therapeutics is witnessed. Meanwhile, gradual improvements in the characteristics of existing antibody therapeutics, discovery of novel antibody-based drugs and novel targets, combining therapeutics and conjugating them with drugs, nanoparticles, and other reagents, using integrative approaches based on cell biology, bioengineering and genetic profiling as well as use of predictive tools to narrow down which candidate molecules could be successfully developed as therapeutics, and developing novel protein-based scaffolds with superior properties to those already in use will be major areas of research and development in the coming decades. A decade from now it is likely that many antibody-based therapeutics based on different scaffolds will be approved for clinical use, with hundreds more in preclinical and clinical development.

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REFERENCES

1. Dillman RO. Cancer immunotherapy. Cancer Biother Radiopharm 2011;26(1): 1–64.
2. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–7.
3. Carter PJ. Potent antibody therapeutics by design. Nat Rev Immunol 2006;6(5): 343–57.
4. Schrama D, Reisfeld RA, Becker JC. Antibody targeted drugs as cancer therapeutics. Nat Rev Drug Discov 2006;5(2):147–59.
5. Waldmann TA. Immunotherapy: past, present and future. Nat Med 2003;9(3): 269–77.
6. Casadevall A, Dadachova E, Pirofski LA. Passive antibody therapy for infectious diseases. Nat Rev Microbiol 2004;2(9):695–703.
7. Carter PJ. Introduction to current and future protein therapeutics: a protein engineering perspective. Exp Cell Res 2011;317(9):1261–9.
8. Dimitrov DS. Therapeutic antibodies, vaccines and antibodyomes. MAbs 2010; 2(3):347–56.
9. Walsh G. Biopharmaceutical benchmarks 2010. Nat Biotechnol 2010;28(9): 917–24.
10. Leader B, Baca QJ, Golan DE. Protein therapeutics: a summary and pharmacological classification. Nat Rev Drug Discov 2008;7(1):21–39.
11. Dimitrov DS, Marks JD. Therapeutic antibodies: current state and future trends—is a paradigm change coming soon? Methods Mol Biol 2009;525:1–27.
12. Ashkenazi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. Nat Rev Drug Discov 2008;7(12):1001–12.
13. Beck A, Reichert JM. Therapeutic Fc-fusion proteins and peptides as successful alternatives to antibodies. MAbs 2011;3(5):415–6.
14. Reichert JM. Antibody-based therapeutics to watch in 2011. MAbs 2011;3(1): 76–99.
15. Reichert JM. Metrics for antibody therapeutics development. MAbs 2010;2(6): 695–700.

16. Teicher BA, Chari RV. Antibody conjugate therapeutics: challenges and potential. Clin Cancer Res 2011;17(20):6389–97.
17. Pasquetto MV, Vecchia L, Covini D, et al. Targeted drug delivery using immunoconjugates: principles and applications. J Immunother 2011;34(9):611–28.
18. Ricart AD. Immunoconjugates against solid tumors: mind the gap. Clin Pharmacol Ther 2011;89(4):513–23.
19. Ricart AD. Antibody-drug conjugates of calicheamicin derivative: gemtuzumab ozogamicin and inotuzumab ozogamicin. Clin Cancer Res 2011;17(20): 6417–27.
20. Grillo-Lopez AJ. Rituximab: an insider's historical perspective. Semin Oncol 2000;27(6 Suppl 12):9–16.
21. Maloney DG. Mechanism of action of rituximab. Anticancer Drugs 2001; 12(Suppl 2):S1–4.
22. Byrd JC, Kitada S, Flinn I, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002;99(3):1038–43.
23. Hilchey SP, Hyrien O, Mosmann TR, et al. Rituximab immunotherapy results in the induction of a lymphoma idiotype-specific T-cell response in patients with follicular lymphoma: support for a "vaccinal effect" of rituximab. Blood 2009;113(16): 3809–12.
24. Mathas S, Rickers A, Bommert K, et al. Anti-CD20- and B-cell receptor-mediated apoptosis: evidence for shared intracellular signaling pathways. Cancer Res 2000;60(24):7170–6.
25. Shan D, Ledbetter J, Press O. Signaling events involved in anti-CD20-induced apoptosis of malignant human B cells. Cancer Immunol Immunother 2000;48:673–83.
26. Dillman RO, Hendrix CS. Unique aspects of supportive care using monoclonal antibodies in cancer treatment. Support Cancer Ther 2003;1(1):38–48.
27. Allison M. PML problems loom for Rituxan. Nat Biotechnol 2010;28(2):105–6.
28. Paues J, Vrethem M. Fatal progressive multifocal leukoencephalopathy in a patient with non-Hodgkin lymphoma treated with rituximab. J Clin Virol 2010;48(4):291–3.
29. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346(4):235–42.
30. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol 2005;23(18):4079–88.
31. Harting R, Venogopal P, Gregory SA, et al. Efficacy and safety of rituximab combined with ESHAP chemotherapy for the treatment of relapsed/refractory aggressive B-cell non-Hodgkin lymphoma. Clin Lymphoma Myeloma 2007; 7(6):406–12.
32. Fischer K, Cramer P, Busch R, et al. Bendamustine combined with rituximab in patients with relapsed and/or refractory chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol 2011;29(26):3559–66.
33. Datta YH, Kampalath B, Binion DG. Rituximab-induced remission of a gastric MALT lymphoma. Leuk Lymphoma 2004;45(6):1297–9.
34. Chong EA, Svoboda J, Cherian S, et al. Regression of pulmonary MALT lymphoma after treatment with rituximab. Leuk Lymphoma 2005;46(9):1383–6.
35. Kluin-Nelemans HC, Doorduijn JK. Treatment of elderly patients with mantle cell lymphoma. Semin Hematol 2011;48(3):208–13.

36. Fenot M, Quereux G, Brocard A, et al. Rituximab for primary cutaneous diffuse large B-cell lymphoma-leg type. Eur J Dermatol 2010;20(6):753–7.
37. Bennett M, Schechter GP. Treatment of splenic marginal zone lymphoma: splenectomy versus rituximab. Semin Hematol 2010;47(2):143–7.
38. Dimopoulos MA, Kastritis E, Roussou M, et al. Rituximab-based treatments in Waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma 2009;9(1):59–61.
39. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med 2000; 343(24):1750–7.
40. Flinn IW, Neuberg DS, Grever MR, et al. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J Clin Oncol 2007; 25(7):793–8.
41. Robak T, Dmoszynska A, Solal-Céligny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol 2010;28(10):1756–65.
42. McLaughlin P. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998;16:2825–33.
43. Huhn D, von Schilling C, Wilhelm M, et al. Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. Blood 2001;98(5):1326–31.
44. Nabhan C, Kay NE. The emerging role of ofatumumab in the treatment of chronic lymphocytic leukemia. Clin Med Insights Oncol 2011;5:45–53.
45. Teeling JL, Mackus WJ, Wiegman LJ, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol 2006;177(1):362–71.
46. Pawluczkowycz AW, Beurskens FJ, Beum PV, et al. Binding of submaximal C1q promotes complement-dependent cytotoxicity (CDC) of B cells opsonized with anti-CD20 mAbs ofatumumab (OFA) or rituximab (RTX): considerably higher levels of CDC are induced by OFA than by RTX. J Immunol 2009;183(1):749–58.
47. Li B, Zhao L, Guo H, et al. Characterization of a rituximab variant with potent antitumor activity against rituximab-resistant B-cell lymphoma. Blood 2009; 114(24):5007–15.
48. Coiffier B, Lepretre S, Pedersen LM, et al. Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: a phase 1-2 study. Blood 2008;111(3):1094–100.
49. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 2002;99(10):3554–61.
50. Wierda WG, Kipps TJ, Mayer J, et al. Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. J Clin Oncol 2010;28(10):1749–55.
51. Lemery SJ, Zhang J, Rothmann MD, et al. U.S. Food and Drug Administration approval: ofatumumab for the treatment of patients with chronic lymphocytic leukemia refractory to fludarabine and alemtuzumab. Clin Cancer Res 2010; 16(17):4331–8.
52. Wierda WG, Kipps TJ, Durig J, et al. Chemoimmunotherapy with O-FC in previously untreated patients with chronic lymphocytic leukemia. Blood 2011; 117(24):6450–8.

53. Haskova Z, Whitacre MN, Dede KA, et al. Combination therapy with ofatumumab and bendamustine in xenograft model of chronic lymphocytic leukaemia. Br J Haematol 2012;156(3):402–4.
54. Sondak VK, Flaherty LE. Targeted therapies: improved outcomes for patients with metastatic melanoma. Nat Rev Clin Oncol 2011;8(9):513–5.
55. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996;271(5256):1734–6.
56. Peggs KS, Quezada SA, Allison JP. Cell intrinsic mechanisms of T-cell inhibition and application to cancer therapy. Immunol Rev 2008;224:141–65.
57. Tivol EA, Borriello F, Schweitzer AN, et al. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 1995;3(5):541–7.
58. Peggs KS, Quezada SA, Chambers CA, et al. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med 2009;206(8):1717–25.
59. Weber J. Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. Oncologist 2007;12(7):864–72.
60. Hodi FS. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.
61. Schwartzentruber DJ. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med 2011;364:2119–27.
62. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011;364(26):2517–26.
63. Di Giacomo AM, Biagioli M, Maio M. The emerging toxicity profiles of anti-CTLA-4 antibodies across clinical indications. Semin Oncol 2010;37(5):499–507.
64. Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 2005;23(25):6043–53.
65. Sondak VK, Smalley KS, Kudchadkar R, et al. Ipilimumab. Nat Rev Drug Discov 2011;10(6):411–2.
66. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235(4785):177–82.
67. Pauletti G, Dandekar S, Rong H, et al. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. J Clin Oncol 2000;18(21):3651–64.
68. Dowsett M, Bartlett J, Ellis IO, et al. Correlation between immunohistochemistry (HercepTest) and fluorescence in situ hybridization (FISH) for HER-2 in 426 breast carcinomas from 37 centres. J Pathol 2003;199(4):418–23.
69. Perez EA, Suman VJ, Davidson NE, et al. HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol 2006;24(19):3032–8.
70. Yarden Y. The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities. Eur J Cancer 2001;37(Suppl 4):S3–8.
71. Cho HS, Mason K, Ramyar KX, et al. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature 2003;421(6924):756–60.
72. Bashey A, Medina B, Corringham S, et al. CTLA4 blockade with ipilimumab to treat relapse of malignancy after allogeneic hematopoietic cell transplantation. Blood 2009;113(7):1581–8.

73. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2(2):127–37.
74. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004;6(2):117–27.
75. Junttila TT, Akita RW, Parsons K, et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. Cancer Cell 2009;15(5):429–40.
76. Sliwkowski MX, Lofgren JA, Lewis GD, et al. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). Semin Oncol 1999;26(4 Suppl 12):60–70.
77. Weiner LM, Adams GP. New approaches to antibody therapy. Oncogene 2000; 19(53):6144–51.
78. Gennari R, Menard S, Fagnoni F, et al. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. Clin Cancer Res 2004;10(17):5650–5.
79. Kumar R, Yarmand-Bagheri R. The role of HER2 in angiogenesis. Semin Oncol 2001;28(5 Suppl 16):27–32.
80. Hudis CA. Trastuzumab–mechanism of action and use in clinical practice. N Engl J Med 2007;357(1):39–51.
81. Slamon D. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344: 783–92.
82. Esteva FJ, Valero V, Booser D, et al. Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. J Clin Oncol 2002;20(7):1800–8.
83. Burstein HJ, Harris LN, Marcom PK, et al. Trastuzumab and vinorelbine as first-line therapy for HER2-overexpressing metastatic breast cancer: multicenter phase II trial with clinical outcomes, analysis of serum tumor markers as predictive factors, and cardiac surveillance algorithm. J Clin Oncol 2003;21(15): 2889–95.
84. Chia S, Clemons M, Martin LA, et al. Pegylated liposomal doxorubicin and trastuzumab in HER-2 overexpressing metastatic breast cancer: a multicenter phase II trial. J Clin Oncol 2006;24(18):2773–8.
85. Costa RB, Kurra G, Greenberg L, et al. Efficacy and cardiac safety of adjuvant trastuzumab-based chemotherapy regimens for HER2-positive early breast cancer. Ann Oncol 2010;21(11):2153–60.
86. Baselga J, Perez EA, Pienkowski T, et al. Adjuvant trastuzumab: a milestone in the treatment of HER-2-positive early breast cancer. Oncologist 2006;11(Suppl 1):4–12.
87. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 2010;375(9712):377–84.
88. Jorgensen JT. Targeted HER2 treatment in advanced gastric cancer. Oncology 2010;78(1):26–33.
89. Kelly H, Kimmick G, Dees EC, et al. Response and cardiac toxicity of trastuzumab given in conjunction with weekly paclitaxel after doxorubicin/cyclophosphamide. Clin Breast Cancer 2006;7(3):237–43.
90. Perez EA, Suman VJ, Rowland KM, et al. Two concurrent phase II trials of paclitaxel/carboplatin/trastuzumab (weekly or every-3-week schedule) as first-line

therapy in women with HER2-overexpressing metastatic breast cancer: NCCTG study 983252. Clin Breast Cancer 2005;6(5):425–32.
91. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol 2005;23(9):1147–57.
92. Banerjee S, Flores-Rozas H. Monoclonal antibodies for targeted therapy in colorectal cancer. Cancer Biol Ther 2010;9(8):563–71.
93. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992; 3(2):65–71.
94. Eskens FA, Verweij J. The clinical toxicity profile of vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR) targeting angiogenesis inhibitors; a review. Eur J Cancer 2006;42(18):3127–39.
95. Choueiri TK, Mayer EL, Je Y, et al. Congestive heart failure risk in patients with breast cancer treated with bevacizumab. J Clin Oncol 2011;29(6):632–8.
96. Nalluri SR, Chu D, Keresztes R, et al. Risk of venous thromboembolism with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis. JAMA 2008;300(19):2277–85.
97. Hurwitz HI, Saltz LB, Van Cutsem E, et al. Venous thromboembolic events with chemotherapy plus bevacizumab: a pooled analysis of patients in randomized phase II and III studies. J Clin Oncol 2011;29(13):1757–64.
98. Sandler AB, Schiller JH, Gray R, et al. Retrospective evaluation of the clinical and radiographic risk factors associated with severe pulmonary hemorrhage in first-line advanced, unresectable non-small-cell lung cancer treated with Carboplatin and Paclitaxel plus bevacizumab. J Clin Oncol 2009;27(9):1405–12.
99. Badgwell BD, Camp ER, Feig B, et al. Management of bevacizumab-associated bowel perforation: a case series and review of the literature. Ann Oncol 2008; 19(3):577–82.
100. Gordon MS, Margolin K, Talpaz M, et al. Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol 2001;19(3):843–50.
101. Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003;349(5):427–34.
102. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350:2335–42.
103. Fuchs CS, Marshall J, Mitchell E, et al. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. J Clin Oncol 2007; 25(30):4779–86.
104. Fuchs CS, Marshall J, Barrueco J. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: updated results from the BICC-C study. J Clin Oncol 2008;26(4):689–90.
105. Hochster HS, Hart LL, Ramanathan RK, et al. Safety and efficacy of oxaliplatin and fluoropyrimidine regimens with or without bevacizumab as first-line treatment of metastatic colorectal cancer: results of the TREE Study. J Clin Oncol 2008;26(21):3523–9.
106. Vincenzi B, Santini D, Russo A, et al. Bevacizumab in association with de Gramont 5-fluorouracil/folinic acid in patients with oxaliplatin-, irinotecan-, and cetuximab-refractory colorectal cancer: a single-center phase 2 trial. Cancer 2009;115(20):4849–56.

107. Kabbinavar FF, Schulz J, McCleod M, et al. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. J Clin Oncol 2005;23(16):3697–705.
108. Allegra CJ, Yothers G, O'Connell MJ, et al. Phase III trial assessing bevacizumab in stages II and III carcinoma of the colon: results of NSABP protocol C-08. J Clin Oncol 2011;29(1):11–6.
109. de Gramont A, Van Cutsem E, Tabernero J, et al. AVANT: Results from a randomized, three-arm multinational phase III study to investigate bevacizumab with either XELOX or FOLFOX4 versus FOLFOX4 alone as adjuvant treatment for colon cancer. J Clin Oncol 2011;29(Suppl 4):[abstract: 362].
110. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355(24):2542–50.
111. Reck M, von Pawel J, Zatloukal P, et al. Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). Ann Oncol 2010;21(9):1804–9.
112. Patel JD, Hensing TA, Rademaker A, et al. Phase II study of pemetrexed and carboplatin plus bevacizumab with maintenance pemetrexed and bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer. J Clin Oncol 2009;27(20):3284–9.
113. Patel JD, Bonomi P, Socinski MA, et al. Treatment rationale and study design for the pointbreak study: a randomized, open-label phase III study of pemetrexed/carboplatin/bevacizumab followed by maintenance pemetrexed/bevacizumab versus paclitaxel/carboplatin/bevacizumab followed by maintenance bevacizumab in patients with stage IIIB or IV nonsquamous non-small-cell lung cancer. Clin Lung Cancer 2009;10(4):252–6.
114. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357(26):2666–76.
115. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. J Clin Oncol 2010;28(20):3239–47.
116. Robert NJ, Dieras V, Glaspy J, et al. RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. J Clin Oncol 2011;29(10):1252–60.
117. Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 2007;370(9605):2103–11.
118. Rini BI, Halabi S, Rosenberg JE, et al. Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. J Clin Oncol 2008;26(33):5422–8.
119. Escudier B, Bellmunt J, Negrier S, et al. Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): final analysis of overall survival. J Clin Oncol 2010;28(13):2144–50.
120. Rini BI, Halabi S, Rosenberg J, et al. Bevacizumab plus interferon-alpha versus interferon-alpha monotherapy in patients with metastatic renal cell carcinoma: results of overall survival for CALGB 90206. J Clin Oncol 2009;27:18s.
121. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, et al. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. Clin Cancer Res 2007;13(4):1253–9.

122. Desjardins A, Reardon DA, Herndon JE 2nd, et al. Bevacizumab plus irinotecan in recurrent WHO grade 3 malignant gliomas. Clin Cancer Res 2008;14(21): 7068–73.
123. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 2009;27(28):4733–40.
124. Burger R, Brady MF, Bookman MA, et al. Phase III trial of bevacizumab (BEV) in the primary treatment of advanced epithelial ovarian cancer (EOC), primary peritoneal cancer (PPC), or fallopian tube cancer (FTC): a Gynecologic Oncology Group study. J Clin Oncol 2010;28(Suppl):18s: p. LBA1.
125. Kristensen G, Perren T, Qian W, et al. Result of interim analysis of overall survival in the GCIG ICON7 phase III randomized trial of bevacizumab in women with newly diagnosed ovarian cancer. J Clin Oncol 2011;29(Suppl):[abstract: LBA5006].
126. Siegel AB, Cohen EI, Ocean A, et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol 2008;26(18):2992–8.
127. Zhu AX, Blaszkowsky LS, Ryan DP, et al. Phase II study of gemcitabine and oxaliplatin in combination with bevacizumab in patients with advanced hepatocellular carcinoma. J Clin Oncol 2006;24(12):1898–903.
128. Xu L, Duda DG, di Tomaso E, et al. Direct evidence that bevacizumab, an anti-VEGF antibody, up-regulates SDF1alpha, CXCR4, CXCL6, and neuropilin 1 in tumors from patients with rectal cancer. Cancer Res 2009;69(20):7905–10.
129. Miles D, Harbeck N, Escudier B, et al. Disease course patterns after discontinuation of bevacizumab: pooled analysis of randomized phase III trials. J Clin Oncol 2011;29(1):83–8.
130. Kerr DJ, Young AM. Targeted therapies: bevacizumab—has it reached its final resting place? Nat Rev Clin Oncol 2011;8(4):195–6.
131. Ranpura V, Hapani S, Wu S. Treatment-related mortality with bevacizumab in cancer patients: a meta-analysis. JAMA 2011;305(5):487–94.
132. Mendelsohn J. Blockade of receptors for growth factors: an anticancer therapy–the fourth annual Joseph H. Burchenal American Association of Cancer Research Clinical Research Award Lecture. Clin Cancer Res 2000;6(3):747–53.
133. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005;23(9):1803–10.
134. Lenz HJ, Van Cutsem E, Khambata-Ford S, et al. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. J Clin Oncol 2006;24(30): 4914–21.
135. Tsuchihashi Z, Khambata-Ford S, Hanna N, et al. Responsiveness to cetuximab without mutations in EGFR. N Engl J Med 2005;353(2):208–9.
136. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009;27(12):2091–6.
137. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007;357(20):2040–8.
138. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351(4):337–45.

139. Sobrero AF, Maurel J, Fehrenbacher L, et al. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. J Clin Oncol 2008;26(14):2311–9.
140. Van Cutsem E, Kohne CH, Lang I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol 2011;29(15):2011–9.
141. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26(10):1626–34.
142. Maughan TS, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet 2011; 377(9783):2103–14.
143. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354(6):567–78.
144. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 2010;11(1):21–8.
145. Vermorken JB, Herbst RS, Leon X, et al. Overview of the efficacy of cetuximab in recurrent and/or metastatic squamous cell carcinoma of the head and neck in patients who previously failed platinum-based therapies. Cancer 2008; 112(12):2710–9.
146. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet 2009;373(9674):1525–31.
147. Gatzemeier U, von Pawel J, Vynnychenko I, et al. First-cycle rash and survival in patients with advanced non-small-cell lung cancer receiving cetuximab in combination with first-line chemotherapy: a subgroup analysis of data from the FLEX phase 3 study. Lancet Oncol 2011;12(1):30–7.
148. Lynch TJ, Patel T, Dreisbach L, et al. Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter phase III trial BMS099. J Clin Oncol 2010;28(6):911–7.
149. Giusti RM, Shastri KA, Cohen MH, et al. FDA drug approval summary: panitumumab (Vectibix). Oncologist 2007;12(5):577–83.
150. Berlin J, Posey J, Tchekmedyian S, et al. Panitumumab with irinotecan/leucovorin/5-fluorouracil for first-line treatment of metastatic colorectal cancer. Clin Colorectal Cancer 2007;6(6):427–32.
151. Martinelli E, De Palma R, Orditura M, et al. Anti-epidermal growth factor receptor monoclonal antibodies in cancer therapy. Clin Exp Immunol 2009;158(1):1–9.
152. Hecht JR, Mitchell E, Neubauer MA, et al. Lack of correlation between epidermal growth factor receptor status and response to panitumumab monotherapy in metastatic colorectal cancer. Clin Cancer Res 2010;16(7):2205–13.
153. Peeters M, Siena S, Van Cutsem E, et al. Association of progression-free survival, overall survival, and patient-reported outcomes by skin toxicity and KRAS status in patients receiving panitumumab monotherapy. Cancer 2009; 115(7):1544–54.
154. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 2008;26(35):5705–12.

155. Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007;25(13):1658–64.
156. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol 2010;28(31):4697–705.
157. Peeters M, Price TJ, Cervantes A, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol 2010;28(31):4706–13.
158. Chiarle R, Podda A, Prolla G, et al. CD30 in normal and neoplastic cells. Clin Immunol 1999;90(2):157–64.
159. Sureda A, Constans M, Iriondo A, et al. Prognostic factors affecting long-term outcome after stem cell transplantation in Hodgkin's lymphoma autografted after a first relapse. Ann Oncol 2005;16(4):625–33.
160. Younes A, Bartlett NL, Leonard JP, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med 2010;363(19):1812–21.
161. Chen R, Gopal A. Results of a pivotal phase 2 study of brentuximab vedontin (SGN-35) in patients with relapsed or refractory Hodgkin's lymphoma. Blood 2010;116:128–9.
162. Foyil KV, Kennedy DA, Grove LE, et al. Extended retreatment with brentuximab vedotin (SGN-35) maintains complete remission in patient with recurrent systemic anaplastic large-cell lymphoma. Leuk Lymphoma 2012;53(3):560–7.
163. Bartlett N, Grove L. Objective responses with brentuximab vedontin retreatment in CD30-positive hematologic malignancies: a case series. J Clin Oncol 2010; 28(Suppl 15):[abstract: 8062].
164. Katz J, Janik JE, Younes A. Brentuximab vedotin (SGN-35). Clin Cancer Res 2011;17(20):6428–36.
165. Aldinucci D, Gloghini A, Pinto A, et al. The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape. J Pathol 2010;221(3):248–63.
166. Lorusso PM, Weiss D, Guardino E, et al. Trastuzumab emtansine: a unique antibody-drug conjugate in development for human epidermal growth factor receptor 2-positive cancer. Clin Cancer Res 2011;17(20):6437–47.
167. Kaufman M, Rai KR. Alemtuzumab in the up-front setting. Ther Clin Risk Manag 2008;4(2):459–64.
168. Dyer MJ. The role of CAMPATH-1 antibodies in the treatment of lymphoid malignancies. Semin Oncol 1999;26(5 Suppl 14):52–7.
169. Dearden C. The role of alemtuzumab in the management of T-cell malignancies. Semin Oncol 2006;33(2 Suppl 5):S44–52.
170. Dearden CE, Matutes E. Alemtuzumab in T-cell lymphoproliferative disorders. Best Pract Res Clin Haematol 2006;19(4):795–810.
171. Kimby E. Management of advanced-stage peripheral T-cell lymphomas. Curr Hematol Malig Rep 2007;2(4):242–8.
172. Lundin J, Hagberg H, Repp R, et al. Phase 2 study of alemtuzumab (anti-CD52 monoclonal antibody) in patients with advanced mycosis fungoides/Sezary syndrome. Blood 2003;101(11):4267–72.
173. Ricci F, Tedeschi A, Morra E, et al. Fludarabine in the treatment of chronic lymphocytic leukemia: a review. Ther Clin Risk Manag 2009;5(1):187–207.

174. Montillo M, Tedeschi A, Petrizzi VB, et al. An open-label, pilot study of fludarabine, cyclophosphamide, and alemtuzumab in relapsed/refractory patients with B-cell chronic lymphocytic leukemia. Blood 2011;118(15):4079–85.
175. Grever MR, Lucas DM, Dewald GW, et al. Comprehensive assessment of genetic and molecular features predicting outcome in patients with chronic lymphocytic leukemia: results from the US Intergroup Phase III Trial E2997. J Clin Oncol 2007;25(7):799–804.
176. Stilgenbauer S, Zenz T. Understanding and managing ultra high-risk chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program 2010;2010:481–8.
177. Fiegl M, Erdel M, Tinhofer I, et al. Clinical outcome of pretreated B-cell chronic lymphocytic leukemia following alemtuzumab therapy: a retrospective study on various cytogenetic risk categories. Ann Oncol 2010;21(12):2410–9.
178. Stilgenbauer S, Dohner H. Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. N Engl J Med 2002;347(6):452–3.
179. Lozanski G, Heerema NA, Flinn IW, et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood 2004;103(9):3278–81.
180. Stilgenbauer S, Zenz T, Winkler D, et al. Subcutaneous alemtuzumab in fludarabine-refractory chronic lymphocytic leukemia: clinical results and prognostic marker analyses from the CLL2H study of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol 2009;27(24):3994–4001.
181. Faderl S, Thomas DA, O'Brien S, et al. Experience with alemtuzumab plus rituximab in patients with relapsed and refractory lymphoid malignancies. Blood 2003;101(9):3413–5.
182. Parikh SA, Keating MJ, O'Brien S, et al. Frontline chemoimmunotherapy with fludarabine, cyclophosphamide, alemtuzumab, and rituximab for high-risk chronic lymphocytic leukemia. Blood 2011;118(8):2062–8.
183. Hainsworth JD, Vazquez ER, Spigel DR, et al. Combination therapy with fludarabine and rituximab followed by alemtuzumab in the first-line treatment of patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a phase 2 trial of the Minnie Pearl Cancer Research Network. Cancer 2008; 112(6):1288–95.
184. Schweighofer CD, Ritgen M, Eichhorst BF, et al. Consolidation with alemtuzumab improves progression-free survival in patients with chronic lymphocytic leukaemia (CLL) in first remission: long-term follow-up of a randomized phase III trial of the German CLL Study Group (GCLLSG). Br J Haematol 2009;144(1):95–8.
185. Wierda WG, Kipps TJ, Keating MJ, et al. Self-administered, subcutaneous alemtuzumab to treat residual disease in patients with chronic lymphocytic leukemia. Cancer 2011;117(1):116–24.
186. Lin TS, Donohue KA, Byrd JC, et al. Consolidation therapy with subcutaneous alemtuzumab after fludarabine and rituximab induction therapy for previously untreated chronic lymphocytic leukemia: final analysis of CALGB 10101. J Clin Oncol 2010;28(29):4500–6.
187. Kipps TJ. Genomic complexity in chronic lymphocytic leukemia. Blood 2008; 112(5):1550.
188. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343(26):1910–6.
189. Feng Y, Dimitrov DS. Monoclonal antibodies against components of the IGF system for cancer treatment. Curr Opin Drug Discov Devel 2008;11(2): 178–85.

190. Skerra A. Alternative non-antibody scaffolds for molecular recognition. Curr Opin Biotechnol 2007;18(4):295–304.
191. Chen W, Dimitrov DS. Human monoclonal antibodies and engineered antibody domains as HIV-1 entry inhibitors. Curr Opin HIV AIDS 2009;4(2):112–7.
192. Chen W, Zhu Z, Feng Y, et al. Construction of a large phage-displayed human antibody domain library with a scaffold based on a newly identified highly soluble, stable heavy chain variable domain. J Mol Biol 2008;382(3):779–89.
193. Dimitrov DS. Engineered CH2 domains (nanoantibodies). MAbs 2009;1(1):26–8.
194. Xiao X, Feng Y, Vu BK, et al. A large library based on a novel (CH2) scaffold: identification of HIV-1 inhibitors. Biochem Biophys Res Commun 2009;387(2): 387–92.
195. Gong R, Vu BK, Feng Y, et al. Engineered human antibody constant domains with increased stability. J Biol Chem 2009;284(21):14203–10.
196. Gong R, Wang Y, Feng Y, et al. Shortened engineered human antibody CH2 domains: increased stability and binding to the human neonatal receptor. J Biol Chem 2011;286(31):27288–93.
197. Descotes J, Gouraud A. Clinical immunotoxicity of therapeutic proteins. Expert Opin Drug Metab Toxicol 2008;4(12):1537–49.
198. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med 2006;355(10): 1018–28.
199. Ewer SM, Ewer MS. Cardiotoxicity profile of trastuzumab. Drug Saf 2008;31(6): 459–67.
200. Chung CH. Managing premedications and the risk for reactions to infusional monoclonal antibody therapy. Oncologist 2008;13(6):725–32.
201. Winter MC, Hancock BW. Ten years of rituximab in NHL. Expert Opin Drug Saf 2009;8(2):223–35.
202. Pendley C, Schantz A, Wagner C. Immunogenicity of therapeutic monoclonal antibodies. Curr Opin Mol Ther 2003;5(2):172–9.
203. Schellekens H. How to predict and prevent the immunogenicity of therapeutic proteins. Biotechnol Annu Rev 2008;14:191–202.
204. Onda M. Reducing the immunogenicity of protein therapeutics. Curr Drug Targets 2009;10(2):131–9.
205. Baker MP, Jones TD. Identification and removal of immunogenicity in therapeutic proteins. Curr Opin Drug Discov Devel 2007;10(2):219–27.
206. Stas P, Lasters I. Strategies for preclinical immunogenicity assessment of protein therapeutics. IDrugs 2009;12(3):169–73.
207. De Groot AS, McMurry J, Moise L. Prediction of immunogenicity: in silico paradigms, ex vivo and in vivo correlates. Curr Opin Pharmacol 2008;8(5):620–6.
208. Emi Aikawa N, de Carvalho JF, Artur Almeida Silva C, et al. Immunogenicity of anti-TNF-alpha agents in autoimmune diseases. Clin Rev Allergy Immunol 2010;38(2–3):82–9.
209. Hangartner L, Zinkernagel RM, Hengartner H. Antiviral antibody responses: the two extremes of a wide spectrum. Nat Rev Immunol 2006;6(3):231–43.
210. Dixit R, Coats S. Preclinical efficacy and safety models for mAbs: the challenge of developing effective model systems. IDrugs 2009;12(2):103–8.
211. Castillo J, Winer E, Quesenberry P. Newer monoclonal antibodies for hematological malignancies. Exp Hematol 2008;36(7):755–68.
212. Mariani G, Fasolo A, De Benedictis E, et al. Trastuzumab as adjuvant systemic therapy for HER2-positive breast cancer. Nat Clin Pract Oncol 2009;6(2): 93–104.

213. Bedard PL, Piccart-Gebhart MJ. Current paradigms for the use of HER2-targeted therapy in early-stage breast cancer. Clin Breast Cancer 2008; 8(Suppl 4):S157–65.
214. Hall PS, Cameron DA. Current perspective—trastuzumab. Eur J Cancer 2009; 45(1):12–8.
215. Cameron D, Casey M, Press M, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat 2008;112(3):533–43.
216. Norden AD, Drappatz J, Wen PY. Novel anti-angiogenic therapies for malignant gliomas. Lancet Neurol 2008;7(12):1152–60.
217. Sachdev JC, Jahanzeb M. Evolution of bevacizumab-based therapy in the management of breast cancer. Clin Breast Cancer 2008;8(5):402–10.
218. Patel DK. Clinical use of anti-epidermal growth factor receptor monoclonal antibodies in metastatic colorectal cancer. Pharmacotherapy 2008;28(11 Pt 2): 31S–41S.
219. Tassev DV, Cheung NK. Monoclonal antibody therapies for solid tumors. Expert Opin Biol Ther 2009;9(3):341–53.
220. Hopper-Borge EA, Churchill T, Paulose C, et al. Mechanisms of tumor resistance to EGFR-targeted therapies. Expert Opin Ther Targets 2009;13(3):339–62.
221. Presta LG. Molecular engineering and design of therapeutic antibodies. Curr Opin Immunol 2008;20(4):460–70.
222. Jefferis R. Glycosylation as a strategy to improve antibody-based therapeutics. Nat Rev Drug Discov 2009;8(3):226–34.
223. Labrijn AF, Aalberse RC, Schuurman J. When binding is enough: nonactivating antibody formats. Curr Opin Immunol 2008;20(4):479–85.
224. Kiewe P, Thiel E. Ertumaxomab: a trifunctional antibody for breast cancer treatment. Expert Opin Investig Drugs 2008;17(10):1553–8.
225. Weber J. Ipilimumab: controversies in its development, utility and autoimmune adverse events. Cancer Immunol Immunother 2009;58(5):823–30.
226. Yuan J, Gnjatic S, Li H, et al. CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. Proc Natl Acad Sci U S A 2008;105(51):20410–5.
227. Bellail AC, Ling Q, Mulligan P, et al. TRAIL agonists on clinical trials for cancer therapy: the promises and the challenges. Rev Recent Clin Trials 2009;4(1): 34–41.
228. Feng Y, Xiao X, Zhu Z, et al. Identification and characterization of a novel agonistic anti-DR4 human monoclonal antibody. MAbs 2010;2(5):565–70.
229. Zhang MY, Shu Y, Phogat S, et al. Broadly cross-reactive HIV neutralizing human monoclonal antibody Fab selected by sequential antigen panning of a phage display library. J Immunol Methods 2003;283(1–2):17–25.
230. Choudhry V, Zhang MY, Sidorov IA, et al. Cross-reactive HIV-1 neutralizing monoclonal antibodies selected by screening of an immune human phage library against an envelope glycoprotein (gp140) isolated from a patient (R2) with broadly HIV-1 neutralizing antibodies. Virology 2007;363(1): 79–90.
231. Zhang MY, Dimitrov DS. Novel approaches for identification of broadly cross-reactive HIV-1 neutralizing human monoclonal antibodies and improvement of their potency. Curr Pharm Des 2007;13(2):203–12.
232. Liu H, Gaza-Bulseco G, Faldu D, et al. Heterogeneity of monoclonal antibodies. J Pharm Sci 2008;97(7):2426–47.

233. Birch JR, Racher AJ. Antibody production. Adv Drug Deliv Rev 2006;58(5–6): 671–85.
234. Zhou JX, Tressel T, Yang X, et al. Implementation of advanced technologies in commercial monoclonal antibody production. Biotechnol J 2008;3(9–10): 1185–200.