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Therapeutic antibodies against cancer

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Abstract

Antibody-based therapeutics against cancer are highly successful in clinic and currently enjoy unprecedented recognition of their potential; 13 monoclonal antibodies (mAbs) have been approved for clinical use in the European Union and in the United States (one, mylotarg, was withdrawn from market in 2010). Three of the mAbs (bevacizumab, rituximab, trastuzumab) are in the top six selling protein therapeutics with sales in 2010 of more than \$5 bln each. 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. However, challenges remain and it appears that deeper understanding of mechanisms is needed to overcome major problems including resistance to therapy, access to targets, complexity of biological systems and individual variations.

Keywords

therapeutics; antibodies; cancer; immunogenicity; safety; efficacy

1. Introduction

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 that the bacteria release into the body was awarded the first Nobel Prize in Medicine in 1901 for his role in the discovery and development of a serum therapy for diphtheria. Interestingly, although historically successes of antibody (serum) therapy were 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 (synagis) and it is for prevention of the infection not for therapy of already established infection. Initial attempts to treat cancer patients with serum therapy were not successful. It was not until several decades ago when a

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number of revolutionary scientific discoveries were made that allowed the development of recombinant therapeutic resulting in the approval of the first anti-cancer therapeutic antibody – 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 them, Gemtuzumab ozogamicin (Mylotarg), was withdrawn (Table 1); in contrast we still have to wait for the first approved mAb-based therapeutic against an infectious disease (synagis is for prevention). In 2010 sales of the top four recombinant therapeutic antibodies (bevacizumab, rituximab, trastuzumab, cetuximab) exceeded US\$ 20 bln (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 anti-tumor activity. This 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 non-specific immune-stimulants such as BCG or Coley's 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 Kohler and Milstein's recipe for generating monoclonal antibodies [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 1970's with the move to, not one, but a cocktail of simultaneous, combined agents. As in the case of combination chemotherapy, antibody therapy may come to utilize different effector pathways in this assault.

Therapeutic mAbs and other therapeutic proteins have been reviewed previously (see recent reviews [3–15] and articles cited there). Therefore, here, we 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 yet that the long trek may yet reach the originally envisioned summit. If not, these agents are undoubtedly part of the solution. We focus mainly on those native, unconjugated antibodies that directly impact solid tumors. Bevacizumab, though its anti-vascular action is indirect, has gained such wide application for solid tumors (and been subject of much controversy) that it seemed important to include. Finally, while immune-conjugates have been well reviewed elsewhere [16–18] and not the present focus brentuximab vedotin, as the first new indication for Hodgkin's 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 due to lack of efficacy and increased deaths [19]. In the context of the present review it may also point to some limiting aspects in unconjugated tumor-directed antibodies, which as has been stated, have not delivered their quarter-century promise.

2. mAbs approved for clinical use

Currently, (as of August 2011) 13 mAbs are approved for clinical use in the European Union (EU) or United States (US) (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 as well as removab which is not approved in the USA yet, and the two radiotherapeutic mAbs, Bexxar and Zevalin, will not be reviewed here.

2.1 Rituximab

The first candidate out of the starting box remains in many ways the poster child for both specificity and efficacy. Rituximab (MabThera, Rituxan), initially developed in San Diego in the late 1980's, and father to that regions biotech explosion, was based upon 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--nor on other non-lymphoid 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 to note 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 over a million brethren lymphocytes by one signature antigen expressed on one malignant subspecies.

In 1997 rituximab was approved by the US FDA for treatment of relapsed indolent B-cell non-Hodgkin's lymphoma. The antibody is a mouse-human chimera utilizing murine variable regions to effect anti-CD20 specificity and human IgG1k 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 upon increased idiotype-specific T cell response to follicular lymphoma [23], and upregulation of proapoptotic proteins such as Bax [24, 25].

Its 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]. Blackbox 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 non-Hodgkin's lymphoma, where combinations and optimizations, have sought to raise response rates and ultimately cure. Since its 1997 start with relapsed indolent non-Hodgkin's lymphoma (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 non-progressing (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].

It has found off-label use in the clinic in all or nearly all malignant (and many non-malignant) settings where B-cells are presumed to participate in pathogenesis and 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's, use for gastric and non-gastric 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" 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 chemo-immunotherapy and most recently to novel antiCD20 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 and overall survival (PFS and OS) rates than chlorambucil as primary therapy [39]. Subsequently, the combination of fludarabine with cyclophosphamide (FC) showed better CR and PFS than F [40]. Based upon the activity of rituximab (R) alone as a front line agent, it was added to FC and compared to 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 chemo-immunotherapy in this setting as the upfront standard of care [41].

2.2 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 was part of the impetus to develop newer anti-CD20 targeted antibodies with a goal to improve such characteristics as binding affinity, specificity and effector function, and efficacy [44]. Ofatumumab (ofa), a fully human monoclonal IgG1 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].

On the basis of 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 two high risk groups, respectively, contributed to the approval of ofa for disease refractory to fludara and for those who have failed alemtuzumab [50, 51].

Given the potential advantages of ofa versus rituximab and FCR established as standard of care in front line, substituting ofa for rituxan in the so-call O-FC regimen was tested in a multinational, randomized phase II trial in treatment naïve patients [52]. Of the two tested doses, the higher dose arm yielded a CR rate of 50%. It remains unclear how to position this

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 like Ofa/bendamustine continues to inform ongoing studies [53].

2.3 Ipilimumab

The novel treatment agents for melanoma, vemurafenib (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 in tactical treatment strategies, and with respect to conventional dacarbazine, 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 modulate peripheral tolerance to tumors and outgrowth of immune-evasive clones. Inhibition is both toward the overexpressed self targets via upregulation of inhibitory ligands on lymphocytes. Thus blockade of CTLA-4 has potential for both mono-therapy 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 lympho-proliferative disorder [57]. Antibody blockade of CTLA-4 induces potent anti-tumor activity through enhancing effector cells and concomitantly inhibiting T 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 overall survival compared to 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 or metastatic disease in patients with good performance status.

Based upon 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 response rates were seen when vaccine was added to IL-2 compared to IL-2 alone (16% versus 6%, $P=0.03$); progression free survival was also significantly improved with a trend toward improved overall survival [61]. Questions arose, nonetheless, 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 four doses followed by 'maintenance' every three months) with and without dacarbazine as the standard control; improved OS was seen including a difference at 3 years of nearly 21% vs. 12% [62].

The cluster of well-identified side effects induced by CTLA-4 inhibition have been referred to as “immune-related adverse events” (IRAEs). These unique adverse effects are likely a direct effect of impairing immune tolerance. They include colitis/diarrhea, dermatitis, hepatitis, uveitis, nephritis, inflammatory myopathy and endocrinopathies. While these reactions have gained a blackbox designation for occasional severe and even fatal instances they are generally manageable and reversible with treatment guidelines which 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% versus 5% of patients) [64].

Based upon a mechanism of action clearly different from IL-2, which increases responsiveness to immune targets, and is non-overlapping with chemotherapy, earlier phase trials and future efforts will focus upon combinations of vaccines, chemo, 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].

2.4 Trastuzumab

HER2 is overexpressed in 20–30 % of invasive breast cancers and associated with a worse prognosis [66]. Trastuzumab is a humanized monoclonal antibody targeting the human epidermal growth factor receptor 2 (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 treatment for HER2 positive tumors in both metastatic and adjuvant settings. Since, across the range of studies, tumors that overexpress HER2 receptor respond better, considerable effort has been expended to accurately assess receptor status [67–69].

HER2 is part of a family of transmembrane tyrosine kinase receptors which 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 by homo- or hetero- dimerization generally, 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 pro-growth 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]. Additionally, it inhibits P13K 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 response rates 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 Her-2 positive patients [81]. As first line therapy for metastatic disease patients were given either chemo alone or in combination. Patients were given an anthracycline and cyclophosphamide or, paclitaxel (if they had previous anthracycline in adjuvant setting). Results showed not only improvement in response rate (RR) and progression free interval but in overall survival. 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 non-anthracycline early breast cancer [85]. Four major adjuvant trials including >13,000 women with HER2-positive early breast cancer utilized 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 is >1cm.

Trastuzumab, combined with chemotherapy has also shown improvement in pathological responses and event free survival when used in the neoadjuvant setting prior to surgery [87]. In a randomized phase III trial patients with advanced gastroesophageal and gastric adenocarcinoma tumors which overexpressed HER2 showed significant increase in overall survival 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 tumor which overexpress HER 2.

The most significant toxicity associated with trastuzumab is cardiomyopathy ranging from subclinical decreases in left ventricular ejection fraction (LVEF) to cardiac failure manifesting as congestive heart failure (CHF). 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 non-anthracycline regimens [90] [91] have all been objectives.

2.5 Bevacizumab

The discussion of bevacizumab (bev) here will be asymmetric in bulk and breadth compared to 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 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 and

without necessitating cessation of therapy. While there will naturally be some specificity of side effects and adverse reactions dependent upon the drugs with which bevacizumab is paired, with some notable exceptions, toxicities are generally neither drug combination nor tumor specific.

More severe and fatal consequences of bevacizumab have been the subject of a number of 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 over 10,000 patients with various solid tumor types, comparing regimens with and without the addition of bevacizumab. The overall incidences of fatal adverse events were 2.5% and among these the highest percentages were nearly a quarter attributable to hemorrhage, about half of that related to neutropenia, and a smaller amount to perforation. There was increased relative risk 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--important to recognize with a baseline of tumor--associated venous thromboembolic events (VTEs) of around 10% with or without this agent [97]. Massive hemoptysis has been linked to large central lesions at risk for cavitation [98], and avoided in these circumstances, and more generally in squamous cancer where this risk is increased. Bowel perforation occurred with an incidence under 2% in a large institution with a treated population of over 1400 patients; it was generally managed without the need for surgical intervention [99].

Bevacizumab demonstrated nil [100] to small [101] response rates as mono-therapy 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 upon improvement of response rates progression-free survival, and overall survival, bevacizumab, when combined with chemotherapy in metastatic colorectal cancer [102], became the first anti-angiogenic agent approved for clinical use. Since then it has gained indications for metastatic breast, metastatic renal cancer, metastatic (as well as advanced or recurrent) non-small cell lung cancer, and glioblastoma. Increasing use of bevacizumab is also being seen with hepatocellular and ovarian cancer.

2.5.1 Colorectal cancer—At this time bevacizumab has an indication in metastatic colorectal cancer in both first and second line settings. The initial approval followed its use with bolus irinotecan, fluorouracil, and leucovorin (IFL) where addition of bevacizumab significantly improved response rate and median survival (20 versus 16 months) compared to chemo only [102]. While bolus IFL has fallen out of general use due to its toxicity profile, studies have supported the value of bevacizumab in combination with more widely used treatments including FOLFIRI (FOL – leucovorin plus F – fluorouracil (5-FU) and IRI – irinotecan (Camptosar)) [103, 104], and three oxaliplatin-containing regimens [105]. In addition, when bevacizumab was added to 5FU/leucovorin in the absence of irinotecan or oxaliplatin, response rates were approximately doubled and median survival improved compared to chemo alone [106, 107].

Efforts to apply bevacizumab in the adjuvant setting for colorectal cancer moved from initial enthusiasm to disappointment. As noted above, bevacizumab had shown favorable impact in metastatic disease in a number of 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 micro-metastases - 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 (for 6 months), it failed to meet its primary endpoint of improving 3-year disease free survival [108]. In a second phase III trial the combination of bevacizumab with FOLFOX (Folinic Acid (FOL), Fluorouracil (F) and Oxaliplatin (OX)) actually led to a slight but significant decrease in overall survival [109].

2.5.2 Non-small cell lung cancer (NSCLC)—The role of bevacizumab in non-small cell lung cancer was initially established in a phase III trial as first line therapy for advanced, non-squamous 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 response rate more than doubled, and there was an increase in progression free and overall survival. At two 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, the addition of bevacizumab to gemcitabine and cisplatin (also with maintenance bevacizumab in the concurrent bevacizumab group) an increase in progression-free survival did not translate into improved overall survival [111]; the authors suggested this may have been due to the wide availability of secondary therapies. Testing with a current standard, pemetrexate is underway but 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 metastatic breast cancer bevacizumab was either added to chemotherapy (weekly paclitaxel) or not; the bevacizumab arm doubled the progression-free survival and significantly improved response rate [114]. These striking results led to accelerated FDA approval and its wide adoption. Unfortunately, neither of two phase III post-approval 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 overall survival [115, 116].

2.5.3 Renal cancer—For metastatic renal cancer two phase III trials demonstrated improved overall survival when bevacizumab was added to interferon alfa in first line treatment [117, 118]. In one of these trials the initially reported progression-free survival with bevacizumab of 10.2 months was nearly double [117] but only a non-significant and clinically small difference of overall survival was reported in the final analysis [119]. In the second phase III trial, with a similar dose schedule as the first, bevacizumab plus interferon alfa improved response rate and progression-free survival compared to mono-therapy with interferon but did not reach significance for overall survival [118, 120].

2.5.4 Glioblastoma—For recurrent glioblastoma, adding bevacizumab to irinotecan increased response rate [121, 122]. Nevertheless, in the notoriously difficult setting of recurrent glioblastoma, both alone and in combination with irinotecan, bevacizumab showed respectable response rates of 28% and 38%, respectively [123]; it holds an indication for use as mono-therapy in this setting despite the absence of a demonstrated improvement in overall survival.

2.5.5 Ovarian cancer—The benefit of bevacizumab in ovarian was assessed in 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 three arms in this Phase III trial, representing chemo only, concurrent bevacizumab and chemo, and concurrent bevacizumab and chemo 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 overall survival; 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 response rates 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 pro-inflammatory pathways and factors that are associated with metastasis [128] but a pooled meta-analysis of five randomized phase III trials did not show altered disease progression following bevacizumab [129]. While the clinical data is scant to explain the unpredicted disappointments like failure in adjuvant setting for colorectal, 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 in which 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% vs. 1.7% in the respective presence or absence of bevacizumab [131]. Nevertheless, these same numbers gather increased clinical sway adjuvant settings where the risks and benefits are markedly different.

2.6 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 EGF receptor [132]. Nevertheless, selection based upon 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 TKIs, 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. In colorectal cancer cetuximab as monotherapy showed improvement in overall survival compared to 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 response rate, time to progression and median survival [138]. In another study, the combination of cetuximab and irinotecan also showed improvement in response rate and progression free survival 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 overall survival in patients with K-ras wild type [140]. The data showing advantage in first line when combined with oxaliplatin are not as clear. In one study the addition of cetuximab to FOLFOX showed significant improvement of response rate only in Kras wild type subpopulation [141] but in another,

more recent trial, no advantages were shown when added to oxaliplatin, even in the Kras wild type group [142].

In squamous cell head and neck cancer cetuximab showed improvement in overall survival when added to radiation compared to radiation alone for locally and regionally advanced disease [143, 144]. The advantage did not extend to those with marked functional compromise or over 65 years of age. 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-fluorouracil chemotherapy improved overall survival compared to platinum-based chemotherapy plus fluorouracil alone [145].

Despite two 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, and 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 overall survival at 11.3 months vs 10.3 months in chemotherapy alone [146]. First cycle rash in this study was substantially associated with overall survival with the median with rash at 15 months compared to 8.8 months without the rash [147]. In another phase III randomized trial studying the same stage patients in first line treatment, without restrictions on EGFR expression, cetuximab combined with taxol/carboplatin did not improve PFS compared to chemo only; a small increase in OS for cetuximab of less than 2 months did not reach statistical significance [148].

2.7 Panitumumab

Panitumumab, an IgG2 class antibody to the EGFR receptor was the first fully human antibody to be approved by 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 for use in refractory disease based upon prolonging disease free survival [149]. Given the similarities to cetuximab efforts have focused on where to place which in clinical contexts and sequences though they have notably not been compared in a face to face randomized phase III trial. The close relation to cetuximab, both biological and clinical, provide a helpful context for review. Like cetuximab it binds to the receptor, the dyad is internalized, and the downstream signal transduction blunted. Its activity can not be reliably shown to depend upon the overexpression of EGFR [150–152]. However, downstream signal transduction by constitutively activated K-ras abrogates its effect and its use according to ASCO guidelines, consistent with clinical trials [141, 153] is limited to tumors with wild type Kras. 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 CMC and ADCC neither of these activities nor its efficacy compared to cetuximab has been demonstrated [1]. Its toxicities, which include a predictable rash in almost all cases, as well as frequent diarrhea and malaise parallel cetuximab, as does the positive association of rash with clinical impact [153].

Panitumumab has shown activity used both in monotherapy and in combination. A large Phase III showed improved response rate and decreased tumor progression when used as monotherapy compared to best supportive care (BSC) in patients refractory to oxaliplatin,

and irinotecan-based therapies[155]. In combination chemotherapy with FOLFOX panitumumab in first line improved both response rate and PFS[156] in contrast to cetuximab which had mixed results as previously noted. When combined with FOLFIRI vs FOLFIRI alone after failure with 5FU-based chemotherapy (the majority with oxaliplatin) addition of panitumumab significantly improved PFS [157].

2.8 Brentuximab vedotin

The first in class antibody drug conjugate (ADC), brentuximab vedotin, received accelerated FDA approval in August 2011 for treatment of relapsed or refractory Hodgkin's lymphoma and systemic anaplastic large cell lymphoma. Approval was based on impressive response rate rather than demonstrable survival improvement in rather dire clinical circumstances: in Hodgkin's after failure of at least two prior systemic regimens in autologous stem cell candidates and for anaplastic large cell lymphoma after failure of at least one multi-agent regimen.

The antibody is a chimerized IgG which targets CD30 and thus delivers its anti-mitotic payload, monomethyl auristatin (vedotin). CD30 is only minimally expressed in normal tissue but densely expressed in both Hodgkin's and ALCL [158].

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

In the pivotal phase I [160] and subsequent phase II trials [161] response rate 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 anaplastic large cell lymphoma (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 three studies demonstrated provocatively high responses with retreatment [163].

It has been suggested that these impressive results may include a number of 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 upon 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 Hodgkin's disease which otherwise consist largely of inflammatory cells recruited by chemokines, in turn, support the tumor cells that recruited them [165]. The shrinkage of these masses might thus be understood, in part, as due to the bystander effect caused by local diffusion of the cytotoxic agent into this local environment. While other differences no doubt exist, this may be one factor explaining greater responses than similar antibody-toxin conjugates, for example with trastuzumab in HER2 positive cancer [166] where tumor masses are predominantly composed of malignant cells.

2.9 Alemtuzumab

Alemtuzumab is a humanized anti-CD52 IgG1 monoclonal antibody. Early studies demonstrated its efficacy in refractory disease, leading initially to approval by FDA in 2001 for treatment of fludarabine-refractory chronic lymphocytic leukemia; 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 chronic lymphocytic leukemia and lymphomas but also on both normal B 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, and cutaneous T cell lymphoma [169–171], mycosis fungoides, and Sezary syndrome [172] but also for negative consequences like heightened infusion reactions and significant vulnerability to opportunistic infections.

Based upon increased acute toxicity and prolonged myelosuppression, alemtuzumab has not supplanted the more B-cell specific rituximab either as mono-therapy or in combination with cytotoxic chemotherapy. First line treatment for CLL generally uses fludarabine 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 30–50% in the relapsed setting and 80–90% in previously untreated patients with CLL [1]. In a large multi-center study patients with refractory or relapsed CLL, previously exposed to alkylating agents and having failed fludarabine had an overall response rate of about 1/3, nearly all PR's; 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 analogs and rituximab. One study demonstrated nearly 50% overall response rates 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% of 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 p 53 [180].

Combination of alemtuzumab with rituximab has not gained traction based upon 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 over 50% showed response a similar percentage also developed infections including 27% CMV antigenemia [181]. In a recent phase II trial alemtuzumab was added to conventional FCR yielding 70% CR, 18% PR in high-risk patients—results considered comparable to historic FCR-treated high risk patients. Based upon nearly 60% CRs in the subset with 17p deletion it was suggested, however, that this may nonetheless have a useful frontline role prior to allogeneic stem cell transplant [182].

The general use of alemtuzumab for consolidation in the community setting can not yet be recommended though the question remains to be settled and is the subject of significant investigation [183]. A phase III trial where alemtuzumab was used as consolidation to fludarabine +/- cyclophosphamide was stopped prematurely due to severe infections; nevertheless minimal residual disease was durably reduced by consolidation and progression-free survival was significantly improved after median follow-up of 48 months [184]. Though there was a trend toward shorter response duration compared to historic groups receiving i.v. alemtuzumab patients receiving subcutaneous treatment showed reliable decreases in graded measures of residual disease [185]. While 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; two year PFS and OS were not improved [186]. Efforts have been underway over the past decade to unravel genomic complexity in chronic lymphocytic

leukemia [187, 188]. Such understanding will inform trial design and, undoubtedly, the value of consolidation will depend upon identification of molecular diagnostic settings in which improvements of minimal residual disease-negative status translates to improved overall survival.

3. 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 3. A significant number of all new medicines are mAbs against cancer (see also <http://www.phrma.org/research/new-medicines>). At least one to three different antibodies are being developed at different companies for each relevant therapeutic target. However, some molecules are targeted by many more mAbs, e.g., 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 generations 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. Currently, all FDA approved anti-cancer therapeutic antibodies (Table 1) and the vast majority of those in clinical trials are full-size antibodies mostly in IgG1 format of about 150 kDa size. A fundamental problem for such large molecules is their poor penetration into tissues (e.g. solid tumors) and poor or absent binding to regions on the surface of some molecules (e.g. on the HIV envelope glycoprotein) which are accessible by molecules of smaller size. Therefore, a large amount of work especially during the last decade has been aimed at developing novel scaffolds of much smaller size and higher stability (see, e.g., recent reviews [11, 190, 191]). Such scaffolds are based on various human and non-human molecules of high stability and could be divided into two major groups for the purposes of this review – antibody-derived and others. Here we will briefly discuss advantages of antibody-derived scaffolds, specifically those derived from antibody domains, and binders selected from libraries based on engineered antibody domains (eAds); an excellent recent review describes the second group [190].

Firstly, 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 which may not be accessible to full size antibodies. Secondly, 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, in spite of 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 scaffold continues. We identified a VH based scaffold which is stable and highly soluble [192]. It was used for construction of a large-size (20 billion clone) eAd phage library by grafting CDR3s and CDR2s from five of our other Fab libraries and randomly mutagenizing CDR1. It was also proposed to use engineered antibody constant domains (CH2 of IgG, IgA and IgD, and CH3 of IgE and IgM) as scaffolds for construction of libraries [193]. Because of their small size and the domains role in antibody effector functions, these have been termed nanoantibodies, the smallest fragments that could be engineered to exhibit simultaneously antigen binding and effector functions. Several large libraries (up to 50 billion clones) were constructed and antigen-specific binders successfully identified [194]. We have recently engineered CH2-based scaffolds with high stability by introducing an additional disulfide bond [195] and by shortening CH2 [196]. It is possible that these and other novel scaffolds under development could provide new opportunities for identification of potentially useful therapeutics.

4. Safety, efficacy and quality of candidate therapeutic mAbs

The success of antibody-based therapeutics is mostly due to the use of concepts and methodologies developed during a paradigm change decades ago that resulted in dramatic improvement of three key features in candidate therapeutics required for FDA approval: safety, efficacy and quality. They are critical for the success of any drug and will be discussed in more detail below for antibody-based therapeutics.

4.1 Safety

Side effects due to therapeutic antibodies could be divided into two large groups: i) interactions with intended targets and ii) interactions with unintended targets. Binding to an intended target can lead to undesirable side effects, e.g., by immunomodulatory antibodies that could be suppressory or stimulatory. Administration of suppressory therapeutic antibodies could lead to 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 TNF α (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 pro-inflammatory cytokines in all six volunteers, leading to critical illness in 12 to 16 hours [198]. One important difference between antibody-based therapeutic containing Fc and other therapeutic proteins (not conjugated with toxic molecules) is that the antibody effector functions including antibody dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (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 where cytokine release plays a pivotal role, but other not fully explained mechanisms could 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]. Pre-existing 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.

4.2 Immunogenicity

Immunogenicity of antibodies can be a significant safety and efficacy issue [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 anti-mouse 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 complementarity determining regions (CDRs) grafted into 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, time and frequency of administration especially when administered as multiple doses over prolonged periods [203]. Even human proteins can elicit human anti-human antibodies. In one of the most studied cases of anti-TNF α mAbs, treatment with the human mAb adalimumab resulted in antibodies against the therapeutic that varied from <1% to up to 87% for different cohorts of patients, protocols, disease 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 a number of 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 on-going [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 composing the polyclonal response to therapeutic proteins. The germline antibody repertoire at any given time could be a major determinant of individual differences, and so knowledge of large portions of antibodies generated by the human immune system, preferably the complete set, i.e., the antibodyome [8], could ultimately help to predict individual immune responses to therapeutic antibodies.

In spite of the possibility for immunogenicity and other side effects antibody therapeutics are relatively safe due primarily to their high specificity. This is a fundamental advantage compared to small molecule drugs which on average are less specific and can bind nonspecifically to 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, e.g., 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 doesn't 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].

4.3 Efficacy

After safety, efficacy is the most important parameter considered by FDA for approval. Many therapeutic antibodies are highly effective in vivo and have revolutionized treatment of cancer, e.g., rituximab for non-Hodgkin lymphoma [201]. Alemtuzumab plays an important role in the therapy of hematological malignancies [211]. Another example is trastuzumab as adjuvant systemic therapy for human epidermal growth factor receptor type 2 (HER2)-positive breast cancer [212]. Results from six trials randomizing more than 14,000 women with HER2-positive early breast cancer to trastuzumab versus non-trastuzumab-based adjuvant chemotherapy demonstrate that the addition of trastuzumab reduces recurrence by approximately 50% and improves overall survival 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 non-responding tumors, and disease progression occurs within a year in the majority of cases [214]. For patients with disease progression, combination with small molecules could be useful, e.g., the addition of a the dual tyrosine kinase inhibitor of epidermal growth factor receptor (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 one 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 if the current practice of local therapy of the central nervous system and continued trastuzumab is optimal [214].

Anti-angiogenic therapies that target the vascular endothelial growth factor (VEGF), e.g., bevacizumab, and the VEGF receptor (VEGFR) are effective adjuncts for treatment of solid tumors, and are commonly administered in combination with cytotoxic chemotherapy. However, at least half of patients fail to respond to anti-angiogenic treatment of gliomas, and the response duration is modest and variable [216]. The use of bevacizumab plus paclitaxel as a first-line treatment of patients with metastatic breast cancer doubled median progression-free survival (PFS; 11.8 months vs. 5.9 months; hazard ratio = 0.60; $P < .001$) compared with paclitaxel alone; however, a statistically significant improvement in overall

survival was not provided by the addition of bevacizumab, although a post hoc analysis demonstrated a significant increase in one-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 appear to benefit only select patients with predictive markers of efficacy, including EGFR overexpression, development of skin 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 treatment of a number of tumors, e.g., 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 (nine vaccines and 10 antibodies) and eight 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. Pre-existing 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 operating as exemplified for EGFR-targeted therapies [220]. Another major problem is poor penetration into tissues, e.g., solid tumors.

New approaches are being developed to increase efficacy of mAb, including enhanced effector functions, improved half-life, increased tumor and tissue accessibility, and greater stability; the methods used involve both protein- and glyco-engineering, 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]. Multi-targeted antibodies are being developed and tested in clinical trials, e.g., an antibody targeting HER2/neu and CD3 with preferential binding to activating Fc γ type I/III-receptors, resulting in the formation of tri-cell complexes between tumor cells, T cells and accessory cells [224]. Similar bispecific (targeting CD3 and EpCAM) trifunctional mAb, catumaxomab, was approved in the European union for therapy of malignant ascites in 2009 (Table 1); the first bispecific mAb approved for clinical use. This antibody binds to cancer cells expressing EpCAM (epithelial cell adhesion molecule) 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 initiates an immunological reaction leading to the removal of cancer cells from the abdominal cavity thus reducing the tumor burden which is seen as the cause for ascites in cancer patients. Bispecific and multispecific mAbs and other therapeutic proteins are currently being developed to a number of targets.

A promising direction 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 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 US FDA in 2011 for therapy of metastatic melanoma (Table 2). Therapeutic mAbs that mimic the natural ligand, e.g., the tumor necrosis factor-related apoptosis inducing ligand (TRAIL), have also been developed [227, 228].

Currently second and third generation mAbs against already validated targets, e.g., HER2, CD20 and TNF α , are in clinical studies 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 panning [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.

4.4 Quality

Quality is a very important parameter for approval of any drug by FDA. A specific fundamental feature that distinguishes mAb 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 co-exist.

Development of high quality protein therapeutics with minimal heterogeneity and contamination is essential for their safety and approval by FDA. Process development for production of a therapeutic proteins is a very 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 5–10 g/L or even higher, cell densities of more than 20 million cells/ml, and specific productivity of over 20 pg/cell/day (even up to 100 pg/cell per day) have been achieved [234].

Genetic delivery of therapeutic antibodies by in vivo production offers a new direction to increase quality and reduce 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 prospects for using molecular farming methods to create relatively low-cost therapeutic proteins in plants, e.g., in genetically engineered tobacco leaves.

5. Biosimilar and biobetter therapeutic antibodies

A major direction of current activity is to develop therapeutic antibodies that are similar but cheaper than the currently existing 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 big new

market. Rituxan/MabThera and Remicade are on the top of the list for biosimilars. Sandoz, e.g., 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 five 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. And that means that most small- and medium-size 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 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 to the marketed therapeutic. That will not be cheap. It could take eight 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.

6. Conclusions

The rapid progress made in the last few decades toward the development of potent therapeutic antibodies raises a number of 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 2–3 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 we witness the fruition of such a shift in terms of new licensed protein therapeutics. Meanwhile, gradual improvements in the characteristics of existing antibody therapeutics, discovery of novel antibody-based drugs and novel targets, combining therapeutics, conjugating them with drugs, nanoparticles and other reagents, using integrative approaches based on cell biology, bioengineering and genetic profiling, as well as 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 we will see many antibody-based therapeutics based on different scaffolds approved for clinical use and hundreds more in preclinical and clinical development.

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1. Antibody-based therapeutics against cancer are highly successful in clinic and currently enjoy unprecedented recognition of their potential
2. 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.
3. Challenges remain and it appears that deeper understanding of mechanisms is needed to overcome major problems including resistance to therapy, access to targets, complexity of biological systems and individual variations.

Table 1

Therapeutic monoclonal antibodies against cancer approved or in review in the European Union or United States. Information current as of August 2011. Updated and modified from Janice M. Reichert, Editor, mAbs, and Dimitar S. Dimitrov, Therapeutic proteins, in press in Methods Molecular Biology.

Name	Trade name	Type	Indication first approved	First EU (US) approval year
Rituximab	MabThera, Rituxan	Anti-CD20; Chimeric IgG1	Non-Hodgkin's 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#2010)
Alemtuzumab	MabCampat h, 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	Erbix	Anti-EGFR; Chimeric IgG1	Colorectal cancer	2004 (2004)
Ibritumomab tiuxetan	Zevalin	Anti-CD20; Murine IgG1	Non-Hodgkin's 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)

#Voluntarily withdrawn from market. **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; **ALCL**, anaplastic large cell lymphoma.

Table 2

The four top-selling therapeutic antibodies in 2010 (in bln US\$) (modified from LaMerie Business Intelligence, Barcelona). The numbers denotes place out of all therapeutic proteins in 2010, in parentheses numbers are for year 2009

# (09)	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

Currencies as of March 2, 2011: 1 €= 1.37726 US\$; 1 CHF = 1.07917 US\$; 1 Yen = 0.0121955 US\$; 1 DKK = 0.184739 US\$; 1 SEK = 0.157766 US\$