One target, different effects: a comparison of distinct therapeutic antibodies against the same targets

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Abbreviations: ADC, antibody-drug conjugate; ADCC, antibody-dependent cellular cytotoxicity; CD20, cluster of differentiation 20; CDC, complement dependent cytotoxicity; CLL, chronic lymphocytic leukemia; ECD, extracellular domain; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FcγR, Fc gamma receptor; FDA, Food and Drug Administration; HACA, human anti-chimeric antibody; HAHA, human anti-human antibody; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; JAK, Janus kinase; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MAPK, mitogen-activated protein kinase; mCRC, metastatic colorectal cancer; NHL, non-Hodgkin's lymphoma; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; RA, rheumatoid arthritis; RTK, receptor tyrosine kinase; SCCHN, squamous cell carcinoma of the head and neck; STAT, signal transducer and activator of transcription; TNFR, tumor necrosis factor receptor; VEGF, vascular endothelial growth factor

Abstract

To date, more than 30 antibodies have been approved worldwide for therapeutic use. While the monoclonal antibody market is rapidly growing, the clinical use of therapeutic antibodies is mostly limited to treatment of cancers and immunological disorders. Moreover, antibodies against only five targets (TNF- α , HER2, CD20, EGFR, and VEGF) account for more than 80 percent of the worldwide market of therapeutic antibodies. The shortage of novel, clinically proven targets has resulted in the development of many distinct therapeutic antibodies against a small number of proven targets, based on the premise that different antibody molecules against the same target antigen have distinct biological and clinical effects from one another. For exam-

ple, four antibodies against TNF- α have been approved by the FDA – infliximab, adalimumab, golimumab, and certolizumab pegol – with many more in clinical and preclinical development. The situation is similar for HER2, CD20, EGFR, and VEGF, each having one or more approved antibodies and many more under development. This review discusses the different binding characteristics, mechanisms of action, and biological and clinical activities of multiple monoclonal antibodies against TNF- α , HER-2, CD20, and EGFR and provides insights into the development of therapeutic antibodies.

Keywords: antibodies, monoclonal; antigens, CD20; pharmacology; receptor, epidermal growth factor; receptor, erbB-2; tumor necrosis factor- α

Introduction

The therapeutic potential of monoclonal antibodies had been well recognized by the pharmaceutical industry, and just one decade after the development of hybridoma technology by Milstein and Köhler (Köhler and Milstein, 1975), the first therapeutic monoclonal antibody (muromonab, Orthoclone OKT3) was approved for clinical use in 1986. Subsequent technological advances such as chimerization/humanization of murine antibodies, transgenic mice, and antibody phage display (Clark, 2000) have enabled the discovery, engineering, and development of monoclonal antibodies with high efficacy and low side effects, especially in terms of immunogenicity. Recent advancements in this area include antibody-drug conjugates (ADCs) (Carter and Senter, 2008), bispecific antibodies (Müller and Kontermann, 2010), and Fc engineering for longer half-life and greater effector functions (Kaneko and Niwa, 2011). Using currently available technological platforms, it is now possible to produce highly functional antibodies against virtually any antigen or epitope. However, until recently, the number of clinically successful target antigens to which these technologies can be applied was surprisingly small. As a result, only a handful of therapeutically relevant antigens, including cell-surface proteins HER2, CD20 and EGFR, and soluble ligands TNF- α and VEGF, have been targeted by multiple antibodies,

Figure 1. Mechanisms of action for therapeutic antibodies. Antibodies against soluble ligands, such as anti-TNF- α antibodies infliximab, adalimumab, golimumab and certolizumab pegol, interfere with ligand-receptor interaction (A). Anti-EGFR antibodies cetuximab, panitumumab and nimotuzumab inhibit ligand binding to the receptor (A) and thus stabilize the inactive conformation of EGFR (B). HER2 is in a constitutively active conformation, and anti-HER2 antibodies trastuzumab and pertuzumab block homo- and heterodimerization of HER2 with ErbB receptors (C). For antibodies targeting CD20, which does not have a known ligand and probably is not a receptor, the major mechanisms of action is Fc-mediated effector functions (D). Most of other antibodies, especially of IgG1 subtype, that bind a cell surface antigen can also mediate ADCC/CDC for effective cell killing. See text for various other possible mechanisms not shown in this figure, such as receptor internalization and sensitization of the target cells.

with great clinical and commercial success. While these antibodies target the same antigen, their biological and clinical characteristics, as well as their modes of action in many cases, differ widely from one another, hence justifying attempts to develop new candidate antibodies against antigens that have already been targeted by other approved antibody drugs. Detailed comparisons of antibodies that target the same antigen (TNF- α , HER2, EGFR or CD20) are given in this review, with emphases on their biochemical/biophysical properties and mechanisms of action (Figure 1).

TNF- α

TNF- α is the single most successful antibody target molecule, worth more than 15 billion USD in combined worldwide sales in 2010 alone. There are three anti-TNF- α IgG1 antibodies (infliximab/Remicade, adalimumab/Humira, and golimumab/Simponi), one pegylated antibody fragment (certolizumab pegol/Cimzia), and an antibody-like Fc-fusion protein (etanercept/Enbrel) approved for the treatment of various autoimmune disorders. The approved indications for these molecules include rheumatoid arthritis, psoriasis, psoriatic arthritis, Crohn's disease, ulcerative colitis, and anky-

losing spondylitis (Williams *et al.*, 2007). TNF- α is expressed as a homotrimeric transmembrane protein on activated macrophages and T lymphocytes. Proteolytic cleavage of the extracellular domain releases soluble trimeric TNF- α , and both membranous and soluble TNFs are able to bind TNF receptors (TNFR1 and TNFR2). Upon binding to TNFR, TNF- α mediates apoptosis and inflammation and regulates immune functions by activating NF- κ B, the MAPK pathways, and death signaling. As a master pro-inflammatory cytokine, TNF- α plays a protective role against infection and injury in normal immune responses; however, chronically elevated levels of TNF- α have also been associated with the pathogenesis of many autoimmune and inflammatory diseases (Feldmann *et al.*, 1996; Ritchlin *et al.*, 1998). While there are many TNF- α antagonistic antibodies, their modes of action are basically the same, i.e., inhibition of the TNF-TNFR interaction. The efficacies of these agents, therefore, are mostly determined by factors other than their modes of action, such as affinity, immunogenicity, tissue penetration, and serum half-life. While there is no head-to-head, direct comparison clinical study featuring anti-TNF- α agents, several meta-analyses have suggested that their efficacies for rheumatoid arthritis are sim-

ilar to one another (Alonso-Ruiz *et al.*, 2008; Kristensen *et al.*, 2007; Launois *et al.*, 2011).

It is difficult to directly compare the immunogenicity data of different antibodies from different studies since the patient groups, assays used, and criteria for determining immunogenicity vary among the studies (Emi Aikawa *et al.*, 2010). Given this limitation in interpreting the immunogenicity data, it is generally accepted that infliximab, a mouse-human chimeric antibody with human constant regions (~75% of the immunoglobulin sequence) and mouse variable regions (~25% of the sequence), is more immunogenic than humanized or human antibody agents such as adalimumab, golimumab, and certolizumab pegol (Yoon *et al.*, 2010). The incidence of human anti-chimeric antibody (HACA) reaction by infliximab ranges from 3% to 53% depending on the dosage and drug combination, which correlates with a reduced duration of response to treatment (Maini *et al.*, 1998). Fully human anti-TNF- α antibodies, adalimumab and golimumab, show markedly lower immunogenicity profiles, with a 5-12% HAHA (human anti-human antibody) response for adalimumab (Abbott, 2002) and 4% for golimumab (Centocor, 2009). Similarly, 8% of patients developed immunogenicity upon treatment with certolizumab pegol, a humanized antibody (Rivkin, 2009; UCB, 2008).

The affinity of these antibodies is in the picomolar range, although some variations exist in the reported values depending on the assay format; K_d values of these antibodies against soluble TNF- α are 127 pM, 44 pM, 18 pM, and 90 pM for adalimumab, infliximab, golimumab (Shealy *et al.*, 2010), and certolizumab pegol (UCB, 2008), respectively. While the affinities of these antibodies are similar, there are some differences in their antigen-binding profiles (Kim *et al.*, 2007; Kohno *et al.*, 2007). Infliximab binds trimeric soluble TNF- α at a 2:1 ratio *in vitro*, with a complex of six antibody molecules and three trimeric TNF- α being the dominant species. Adalimumab also is known to form high molecular weight complexes with the trimeric antigen. It is likely that golimumab also has a similar binding stoichiometry, as these IgG1 molecules are bivalent. As a result, various combinations of antibody: trimeric antigen interactions are possible. On the other hand, certolizumab pegol is a monovalent, pegylated Fab' and thus does not cross-link antigens to form large supramolecular complexes. Together with its lack of Fc-mediated effector function, this binding characteristic of certolizumab pegol may explain some of its distinct biological effects such as better tissue penetration, lack of ADCC and CDC, and reduced neutrophil degranulation and superoxide production (Nesbitt *et al.*, 2007; Cassinotti

et al., 2008; Palframan *et al.*, 2009; Launois *et al.*, 2011).

HER2

HER2 overexpression is found in ~30% of human breast cancers and is associated with poor disease prognosis (Hudis, 2007). Two therapeutic antibodies targeting HER2 are discussed below: trastuzumab (Herceptin) and pertuzumab (Omnitarg). Unlike TNF- α inhibitors, which function *via* the essentially same mechanism, these two antibodies bind to distinct epitopes on HER2 and have different mechanisms of action. Receptor tyrosine kinases (RTKs) such as HER2 and EGFR have a relatively large extracellular domain (ECD) consisting of multiple sub-domains, and they undergo multi-step activation processes that include ligand binding, conformational changes, and homo/heterodimerization (Schmitz and Ferguson, 2009). Antibody binding to different parts of RTKs can thus affect receptor function and signaling in different ways.

Despite being one of the most successful monoclonal antibody therapies, the mechanism of action for trastuzumab has not yet been fully elucidated. Trastuzumab is a humanized IgG1 antibody that binds HER2 with a K_d of ~5 nM (Genentech, 2002), which is considered moderate compared to many other therapeutic antibodies with a sub-nanomolar K_d . Indeed, it has been reported that anti-HER2 antibodies with moderate affinity are characterized by greater tumor accumulation and tissue penetration, whereas high-affinity antibodies tend to localize in the perivascular region of a tumor and do not penetrate well into the tumor interior (Rudnick *et al.*, 2011). This is also in contrast with antibodies targeting soluble ligands, such as anti-TNF- α agents, which typically have sub-nanomolar affinity and can bind and neutralize ligands at low serum concentration. While it is known that trastuzumab binds to domain IV of HER2-ECD, the exact molecular consequence of trastuzumab binding to HER2 is still under debate. Recent studies reported that trastuzumab inhibited HER2 homodimer-mediated cell growth (Ghosh *et al.*, 2011) as well as ligand-independent HER2-HER3 heterodimerization (Junttila *et al.*, 2009). Antibody-dependent cellular cytotoxicity (ADCC) is thought to mediate the effects of trastuzumab (Lewis *et al.*, 1993; Cooley *et al.*, 1999; Clynes *et al.*, 2000; Gennari *et al.*, 2004; Arnould *et al.*, 2006; Barok *et al.*, 2007; Valabrega *et al.*, 2007). However, the relative importance of ADCC in trastuzumab action is debatable since ~70% of patients with HER2 overexpressing metastatic breast cancer did not respond to trastuzumab monotherapy (Vogel *et al.*, 2002). Interpreting

the effects of ADCC is further complicated by the fact that many cancer patients have impaired immune function due to the advanced stage of the disease or previous cancer therapies (Valabrega *et al.*, 2007). A recent study suggested that the varying responses of patients with HER2-positive cancer to trastuzumab may be partly due to a difference in Fc γ R1IIa genotype, suggesting an important role for ADCC in the clinical effects of trastuzumab (Musolino *et al.*, 2008). Downregulation of HER2 *via* internalization and degradation by trastuzumab and consequent reduction in receptor dimerization and signaling has also been reported as a possible mechanism of action of trastuzumab (zum Buschenfelde *et al.*, 2002). Trastuzumab blocks shedding of HER2, which is thought to be related to its antitumor activity (Molina *et al.*, 2001), and inhibits angiogenesis (Izumi *et al.*, 2002; Klos *et al.*, 2003). Another proposed mechanism for trastuzumab is inhibition of the PI3K/Akt pathway (Nagata *et al.*, 2004). Trastuzumab binding to HER2 inhibits binding of Src to the cytoplasmic domain of HER2, thus reducing Src activity, which in turn reduces the tyrosine phosphorylation of PTEN. Dephosphorylated PTEN is subsequently recruited to the plasma membrane and activated, which in turn dephosphorylates and inactivates Akt, leading to apoptosis and inhibition of proliferation. Finally, inhibition of Akt by trastuzumab allows p27kip1 to enter the nucleus and inhibit cdk2, resulting in cell cycle arrest at G1 phase (Kute *et al.*, 2004).

Another humanized anti-HER2 antibody, pertuzumab, binds to an epitope in domain II of HER2-ECD with a K_d of ~ 2.2 nM and inhibits the ligand-dependent heterodimerization of HER2 with other ErbB receptors such as EGFR and HER3 (Persson *et al.*, 2005). HER2 is the preferred dimerization partner of HER3, which has impaired tyrosine kinase activity, and the PI3K/Akt pathway activated by the HER2/HER3 heterodimer is one of the most important HER2-related cancer cell survival/proliferation mechanisms (Hynes and MacDonald, 2009). Structural analysis of pertuzumab-HER2 binding has shown that the antibody sterically interferes with receptor dimerization and subsequent signaling (Franklin *et al.*, 2004). By blocking HER2 heterodimerization, pertuzumab shows biological effects against cancers that do not overexpress HER2 (Agus *et al.*, 2002). Unlike trastuzumab, which is substantially less effective when tested as the F(ab')₂ fragment (Spiridon *et al.*, 2004) or in mice lacking Fc receptor (Clynes *et al.*, 2000), the Fab fragment of pertuzumab is as effective as the IgG1 form in inhibiting the growth of HER2 + tumors (Agus *et al.*, 2002), implying that pertuzumab may be less dependent on ADCC than trastuzumab. On

the other hand, pertuzumab does not inhibit the ligand-independent activation of HER2 as trastuzumab does. With different epitopes and mechanisms of action, the two antibodies synergistically inhibit the survival of BT474 human breast cancer cells *in vitro* (Nahta *et al.*, 2004).

The different mechanisms of action and clinical effects of the two anti-HER2 antibodies suggests the possibility of developing antibodies against novel HER2 neutralizing epitopes that function differently from trastuzumab and pertuzumab. Recently, bacterial cell display-based screening of HER2 peptides revealed several new epitopes that can be targeted by antibodies to inhibit cell growth and proliferation (Rockberg *et al.*, 2008, 2009). While it remains unclear whether or not antibodies against those epitopes really are different from trastuzumab or pertuzumab in terms of mechanism of action, the multistep activation mechanisms of RTKs such as HER2 and EGFR can be exploited to develop neutralizing antibodies with unique biochemical and clinical properties.

EGFR

Epidermal growth factor receptor (EGFR) is overexpressed in various cancers, including colorectal cancer, head-and-neck cancer, and non-small cell lung carcinoma (Nicholson *et al.*, 2001). Although EGFR is expressed in many normal tissues, targeting of EGFR with neutralizing antibodies has proven to be effective in extending the survival of certain cancer patients (Cunningham *et al.*, 2004; Cohenuram and Saif, 2007). Two anti-EGFR antibodies, cetuximab (Erbix) and panitumumab (Vectibix), have been approved for the treatment of colorectal and head-and-neck cancers. Both of them bind to the same domain (domain III of EGFR ECD) and inhibit receptor activation and signaling (Li *et al.*, 2005; Freeman *et al.*, 2008). Nimotuzumab is another anti-EGFR antibody that has been approved in Europe and several other countries and is in phase II trials in the U.S. Several other anti-EGFR antibodies such as zalutumumab and necitumumab are also in various stages of development.

Cetuximab is a mouse-human chimeric IgG1 with high affinity ($K_d \sim 200$ pM) (Goldstein *et al.*, 1995) and has been approved for the treatment of metastatic colorectal cancer (mCRC) and squamous cell carcinoma of the head and neck (SCCHN) as a monotherapy or in combination with irinotecan (for mCRC) or radiotherapy (for SCCHN). Upon binding to EGFR, cetuximab directly blocks ligand binding and locks the receptor in an autoinhibitory conformation, thereby preventing the receptor from assuming the extended con-

formation required for dimerization (Li *et al.*, 2005). As a result, downstream signaling pathways such as the JAK/STAT, PI3K/Akt, and MAPK pathways are blocked, which inhibits cancer cell survival and proliferation (Baselga, 2001; Meira *et al.*, 2009). Cetuximab also causes cell cycle arrest similarly to trastuzumab by increasing the expression of p27kip1, which then inhibits cdk2 in the nucleus (Kiyota *et al.*, 2002; Wu *et al.*, 1996). It also reduces angiogenesis (Perrotte *et al.*, 1999; Petit *et al.*, 1997) and metastasis (Huang *et al.*, 2002) by decreasing the levels of angiogenic factors and matrix metalloproteinases. Antibody-dependent cellular cytotoxicity (Kawaguchi *et al.*, 2007; Kurai *et al.*, 2007), induction of apoptosis by increased expression of the proapoptotic protein Bax (Mandal *et al.*, 1998), and sensitization of cancer cells to chemotherapeutic agents (Mahtani and Macdonald, 2008) or radiation (Gebbia *et al.*, 2007) are other mechanisms of action that have been proposed for cetuximab.

Panitumumab is a fully human IgG2 antibody made by XenoMouse transgenic technology (Jakobovits *et al.*, 2007). It binds to domain III of EGFR with an affinity higher than that of cetuximab ($K_d \sim 50$ pM) (Kim and Grothey, 2008), and it functions by blocking ligand binding to EGFR (Freeman *et al.*, 2008; Schmitz and Ferguson, 2009). Similar to cetuximab, the suggested mechanisms of action for panitumumab include decreased EGFR signaling, cell cycle arrest, reduced angiogenesis, and receptor downregulation by internalization (Messersmith and Hidalgo, 2007; Yang *et al.*, 1999). As it has a fully human immunoglobulin sequence, panitumumab has lower immunogenicity compared to cetuximab (Yoon *et al.*, 2010). Since it is an IgG2, panitumumab is also expected to have much weaker effector functions than cetuximab and be less likely to damage normal EGFR-expressing cells, although a recent study indicated that panitumumab is effective in triggering ADCC by neutrophils and monocytes (Schneider-Merck *et al.*, 2010).

Nimotuzumab is a humanized IgG1 antibody with a much lower affinity than both cetuximab and panitumumab. Probably as a result of this, it shows antitumor activity without the severe skin rashes commonly associated with other anti-EGFR antibodies (Ramakrishnan *et al.*, 2009). Due to its lower affinity, nimotuzumab can form stable bivalent attachments to EGFR-overexpressing cells, but not to cells with a normal level of EGFR expression, minimizing skin toxicity. The low-affinity strategy has also been employed in the development of adecatumumab, which targets a pan-epithelial antigen EpCAM. These antibodies exemplify the fine balance between affinity, toxicity, and distribution

required when developing therapeutic antibodies targeting a cell surface antigen.

For anti-EGFR antibodies, KRAS mutation is a predictive biomarker of resistance to antibody treatment (Heinemann *et al.*, 2009; Lièvre *et al.*, 2006). KRAS is a small G protein that is critically involved in the Ras/MAPK pathway downstream of EGFR. Mutation of KRAS activates this signaling pathway even in the presence of EGFR-neutralizing antibodies. On the other hand, the EGFR expression level (as determined by IHC) is not correlated with the response to anti-EGFR antibody therapy (Cunningham *et al.*, 2004; Saltz *et al.*, 2004), and some patients with EGFR-negative tumors have been reported to respond to anti-EGFR therapy (Chung *et al.*, 2005). The reason for this apparent paradox is not yet clear, although it is generally undisputed that cetuximab and panitumumab exert clinical efficacy exclusively by binding to EGFR. Whatever the reason, unlike KRAS mutation status, the EGFR expression level is not considered to be a predictive biomarker when cancer patients are subjected to anti-EGFR antibody therapy. Novel or alternative therapeutic options are being investigated in patients with KRAS mutation for whom cetuximab or panitumumab treatment offers little or no clinical benefit (Dunn *et al.*, 2011; Lee *et al.*, 2011).

CD20

CD20 is a putative tetra-transmembrane cell surface antigen expressed only on B cell precursors and mature B cells. Its function has not yet been clearly elucidated, but studies have suggested that CD20 is involved in B cell growth and differentiation and probably functions as a calcium channel (Beers *et al.*, 2010). The selective depletion of CD20+ cells does not result in excessive toxicity (McLaughlin *et al.*, 1998) since pre-existing plasma cells as well as hematopoietic stem cells lack CD20, and also normal B cells are replenished by stem cell differentiation. This makes CD20 an ideal target for treatment of cancers of B cell lineage such as non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Currently, there are four approved antibody drugs targeting CD20. Ibritumomab tiuxetan (Zevalin) and tositumomab-I131 (Bexxar) are murine antibody radio-immunoconjugates. Rituximab (Rituxan/MabThera) is a human-mouse chimeric IgG1 antibody approved for the treatment of NHL, CLL, and rheumatoid arthritis (RA), as well as Wegener's granulomatosis and microscopic polyangiitis. Ofatumumab (Arzerra) is a fully human IgG1 monoclonal antibody for CLL. While all of these target and deplete CD20+ cells,

they differ from one another in preclinical and clinical characteristics.

Rituximab is the first antibody drug approved for an oncological indication. It binds to the large extracellular loop of CD20 (Teeling *et al.*, 2006) with a binding affinity of approximately ~8 nM (Genentech, 1997). ADCC is thought to be the major mechanism of action for rituximab (Clynes *et al.*, 2000; Flieger *et al.*, 2000). CD20 is neither shed nor internalized upon antibody binding (Einfeld *et al.*, 1988; Press *et al.*, 1987), allowing effective cell killing by ADCC. Being a type I antibody that induces CD20 redistribution into lipid rafts (Cragg and Glennie, 2004), rituximab also strongly elicits complement-dependent cytotoxicity (CDC) by clustering multiple Fc regions (Cragg *et al.*, 2003). This antibody is also reported to induce apoptosis of CD20+ cells (Byrd *et al.*, 2002; Pedersen *et al.*, 2002), although the nature and significance of this mode of action remain controversial. Finally, rituximab sensitizes cancerous cells to radio- or chemotherapy (Demidem *et al.*, 1997; Skvortsova *et al.*, 2006) and is used not only as a single agent but also in combination with chemotherapeutic agents.

Ofatumumab is another type I anti-CD20 monoclonal antibody. It is a fully human antibody made from a transgenic mouse expressing human antibodies. Its epitope encompasses both the small and large extracellular loops of CD20 (Uchiyama *et al.*, 2010). With a binding affinity of ~5 nM, ofatumumab also has a slower off-rate compared to rituximab (Teeling *et al.*, 2004). Its distinct epitope and slow dissociation allow the antibody to bind much closer as well as longer to the cell surface, which may explain more rapid complement induced cell killing by ofatumumab than rituximab (Beum *et al.*, 2008). In fact, when tested on CEM cells transduced with human CD20, ofatumumab induced CDC better than rituximab, showing lytic activity against cells expressing as few as 4,500 CD20 molecules per cell and reaching full activity at a CD20 density of 60,000 molecules/cell. On the other hand, rituximab requires at least 30,000 CD20 molecules per cell to show lytic activity and does not achieve full lysis even at 135,000 CD20 molecules/cell (Teeling *et al.*, 2006). Studies suggest that ofatumumab also more potently induces ADCC than rituximab by binding more tightly to FcγRIIIa on NK cells (Craigien *et al.*, 2009).

Unlike rituximab and ofatumumab, tositumomab is a type II antibody that does not induce CD20 redistribution into lipid rafts (Beers *et al.*, 2010). Type II antibodies are not strong activators of CDC but instead induce a unique mode of cell death involving homotypic adhesion of B cells. The existence

of multiple functional epitopes that drive different cellular responses upon antibody binding, on an antigen with relatively small extracellular domains, illustrates the point that antibodies with novel mechanisms of action and improved efficacies can be developed against an established target antigen.

Antibodies in development: Future directions

Three therapeutic antibodies, each against a different new target, were approved in 2004: cetuximab (Erbix) targeting EGFR, bevacizumab (Avastin) targeting VEGF, and natalizumab (Tysabri) targeting VLA-4. Each of these antibodies recorded > 1 billion USD in global sales in 2009. No new antigen with blockbuster potential was targeted by an approved antibody until 2010, when denosumab (Prolia/Xgeva) targeting RANKL and tocilizumab (Actemra) targeting IL-6 were approved. These were soon followed in 2011 by belimumab (Benlysta) against BLYS and ipilimumab (Yervoy) against CTLA-4. It is likely that the therapeutic potential of more new targets, such as beta amyloid and VEGFR-2, will be realized in coming years. On the other hand, efforts are continuing to develop antibodies against existing targets. Particularly, many antibodies are under development against four of the most successful antibody targets (TNF-α, HER2, EGFR, and CD20) discussed in this review, reflecting the clinical validity and commercial value of these molecules. Antibodies and antibody fragments/derivatives in clinical development targeting these four antigens are summarized in Table 1. For cell-surface antigens (HER2, EGFR, and CD20), there is an emphasis on enhancing the biological effects of antibodies by strategies such as Fc modification (including glycoengineering), antibody-drug conjugation, and simultaneous targeting of more than one target. This trend reflects the accepted view that for these well-proven oncology targets, enhanced cell killing ability of the drugs is positively correlated with therapeutic efficacy. In contrast, for the soluble ligand TNF-α, the trend is apparently focused on the development of antibody fragments and domain antibodies that are significantly smaller than immunoglobulins. With essentially identical mechanisms of action and no requirement for cytolytic or cytostatic activities, new anti-TNF-α molecules must gain a competitive advantage over existing antibodies in areas such as affinity, serum half-life, immunogenicity, tissue distribution, and development/production cost. Certolizumab pegol (a pegylated Fab'), for example, has a unique distribution profile (Palframan *et al.*, 2009) and can be produced less expensively from a bacterial host. Therefore, along with first-in-

Table 1. Antibodies, antibody fragments, and antibody derivatives in various stages of clinical development that target the antigens discussed in this review

Target	Name	Developer	Format	Stage	Notes
TNF- α	CEP-37247	Domantis/Cephalon	Domain Ab	Phase II	Single domain Ab
	ESBA-105	ESBAtech	Ab fragment	Phase II	scFv
	ATN-103	Pfizer/Ablynx	Nanobody	Phase II	Camelid V _H H
HER2	Trastuzumab-DM1	Roche	Humanized	Phase III	ADC
	Ertumaxomab	TRION/Fresenius	Trifunctional	Phase II	HER2/Fc γ RI/III
	MM-111	Merrimack	Bifunctional	Phase II	HER2/3
	Zemab	Novartis	Ab fragment	Phase I	ADC
EGFR	Necitumumab	Eli Lilly	Human	Phase III	
	Zalutumumab	Genmab	Human	Phase III	
	SYM004	Symphogen	2-Ab mixture	Phase II	Two α EGFR Abs
	RG-7160	Roche/Glycart	Humanized	Phase I	Glycoengineered
CD20	Afutumumab	Roche/Glycart	Humanized	Phase III	Glycoengineered; type II
	Ocrelizumab	Biogen Idec/Roche	Humanized	Phase III	Type II
	Pro-131921	Progen	Humanized	Phase II	Modified Fc; type I
	AME-133v	MENTRIK biotech	Humanized	Phase II	Modified Fc; type II
	Veltuzumab	Immunomedics	Humanized	Phase II	Type I
	FBT-A05	TRION/Fresenius	Trifunctional	Phase I	CD20/CD3/ Fc γ RI

class antibodies, more therapeutic antibodies that target established antigens with improved and/or unique therapeutic profiles are expected to enter the market in the near future.

Concluding remarks

Multiple therapeutic antibodies against one soluble ligand (TNF- α) and three cell surface proteins (HER2, EGFR, and CD20) were discussed in this review. While TNF- α is targeted by as many as four antibodies plus etanercept (a soluble receptor-Fc fusion protein), their mechanisms of action are essentially the same, i.e., blockade of ligand binding to the cell surface receptor. In contrast, antibodies against a cell surface antigen tend to have unique mechanisms of action or different *in vitro* and *in vivo* characteristics, as evidenced by trastuzumab/pertuzumab and rituximab/ofatumumab. This may be explained by the fact that an antibody can influence cell survival and proliferation in many different ways by directly binding to the cell surface. While targeting a soluble ligand may sometimes be advantageous in such aspects as toxicity, specificity, and delivery, targeting a membrane receptor is much more likely to yield antibodies that are mechanistically and clinically distinct from existing molecules. It is thus expected that scientific and technological advancements in the field will continue to allow generation of novel agents that can overcome or supplement the limitations of existing drugs.

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References

- Abbott. Humira full prescribing information. 2002, <http://www.rxabbott.com/pdf/humira.pdf>
- Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, Lofgren JA, Tindell C, Evans DP, Maiese K, Scher HI, Sliwkowski MX. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell* 2002;2:127-37
- Alonso-Ruiz A, Pijoan JI, Ansuategui E, Urkaregi A, Calabozo M, Quintana A. Tumor necrosis factor alpha drugs in rheumatoid arthritis: systematic review and metaanalysis of efficacy and safety. *BMC Musculoskelet Disord* 2008;9:52
- Arnould L, Gelly M, Penault-Llorca F, Benoit L, Bonnetain F, Migeon C, Cabaret V, Fermeaux V, Bertheau P, Garnier J, Jeannin JF, Coudert B. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? *Br J Cancer* 2006;94:259-67
- Barok M, Isola J, Palyi-Krek Z, Nagy P, Juhasz I, Vereb G, Kauraniemi P, Kapanen A, Tanner M, Szollosi J. Trastuzumab causes antibody-dependent cellular cytotoxicity-mediated growth inhibition of submacroscopic JIMT-1 breast cancer xenografts despite intrinsic drug resistance. *Mol Cancer Ther* 2007;6:2065-72
- Baselga J. The EGFR as a target for anticancer therapy - focus on cetuximab. *Eur J Cancer* 2001;37:S16-22

- Beers SA, Chan CH, French RR, Cragg MS, Glennie MJ. CD20 as a target for therapeutic type I and II monoclonal antibodies. *Semin Hematol* 2010;47:107-14
- Beum PV, Lindorfer MA, Beurskens F, Stukenberg PT, Lokhorst HM, Pawluczukowycz AW, Parren PW, van de Winkel JG, Taylor RP. Complement activation on B lymphocytes opsonized with rituximab or ofatumumab produces substantial changes in membrane structure preceding cell lysis. *J Immunol* 2008;181:822-32
- Byrd JC, Kitada S, Flinn IW, Aron JL, Pearson M, Lucas D, Reed JC. 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;99:1038-43
- Carter PJ, Senter PD. Antibody-drug conjugates for cancer therapy. *Cancer J* 2008;14:154-69
- Cassinotti A, Ardizzone S, Porro GB. Certolizumab pegol: an evidence-based review of its place in the treatment of Crohn's disease. *Core Evid* 2008;2:209-29
- Centocor. Simponi full prescribing information. 2009, <https://www.simponi.com/sites/default/files/pdf/prescribing-information.pdf>
- Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tse A, Hamilton A, Pan D, Schrag D, Schwartz L, Klimstra DS, Fridman D, Kelsen DP, Saltz LB. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol* 2005;23:1803-10
- Clark M. Antibody humanization: a case of the 'Emperor's new clothes'? *Immunol Today* 2000;21:397-402
- Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. *Nat Med* 2000;6:443-6
- Cohenuram M, Saif MW. Panitumumab the first fully human monoclonal antibody: from the bench to the clinic. *Anticancer Drugs* 2007;18:7-15
- Cooley S, Burns LJ, Repka T, Miller JS. Natural killer cell cytotoxicity of breast cancer targets is enhanced by two distinct mechanisms of antibody-dependent cellular cytotoxicity against LFA-3 and HER2/neu. *Exp Hematol* 1999;27:1533-41
- Cragg MS, Morgan SM, Chan HTC, Morgan BP, Filatov AV, Johnson PW, French RR, Glennie MJ. Complement-mediated lysis by anti-CD20 mAb correlates with segregation into lipid rafts. *Blood* 2003;101:1045-52
- Cragg MS, Glennie MJ. Antibody specificity controls *in vivo* effector mechanisms of anti-CD20 reagents. *Blood* 2004;103:2738-43
- Craigien JL, Mackus WJM, Engleberts P, Miller SR, Speller S, Chamberlain LC, Davis BG, McHugh SM, Bullmore E, Cox CJ, Wetten S, Perdock G, Bakker JM, van de Winkel JGJ, Parren PWHI. Ofatumumab, a human mab targeting a membrane-proximal small-loop epitope on CD20, induces potent NK cell-mediated ADCC. *Blood (ASH annual meeting abstracts)* 2009;114:1725
- Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;351:337-45
- Demidem A, Lam T, Alas S, Hariharan K, Hanna N, Bonavida B. Chimeric anti-CD20 (IDEC-C2B8) monoclonal antibody sensitizes a B cell lymphoma cell line to cell killing by cytotoxic drugs. *Cancer Biother Radiopharm* 1997;12:177-86
- Dunn EF, Iida M, Myers RA, Campbell DA, Hintz KA, Armstrong EA, Li C, Wheeler DL. Dasatinib sensitizes KRAS mutant colorectal tumors to cetuximab. *Oncogene* 2011;30:561-74
- Einfeld DA, Brown JP, Valentine MA, Clark EA, Ledbetter JA. Molecular cloning of the human B cell CD20 receptor predicts a hydrophobic protein with multiple transmembrane domains. *Embo J* 1988;7:711-7
- Emi Aikawa N, de Carvalho JF, Artur Almeida Silva C, Bonfa E. Immunogenicity of anti-TNF-alpha agents in autoimmune diseases. *Clin Rev Allergy Immunol* 2010;38:82-9
- Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996;14:397-440
- Flieger D, Renoth S, Beier I, Sauerbruch T, Schmidt-Wolf I. Mechanism of cytotoxicity induced by chimeric mouse human monoclonal antibody IDEC-C2B8 in CD20-expressing lymphoma cell lines. *Cell Immunol* 2000;204:55-63
- Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell* 2004;5:317-28
- Freeman D, Sun J, Bass R, Jung K, Ogbagabriel S, Elliott G, Radinsky R. Panitumumab and cetuximab epitope mapping and *in vitro* activity. *J Clin Oncol* 2008;26:(20 suppl) abstr 14536
- Gebbia V, Giuliani F, Valori VM, Agueli R, Coiucci G, Maiello E. Cetuximab in squamous cell head and neck carcinomas. *Ann Oncol* 2007;18:5-7
- Genentech. Rituxan full prescribing information. 1997, <http://www.gene.com/gene/products/information/pdf/rituxan-prescribing.pdf>
- Genentech. U.S. BLA Supplement: HERCEPTIN. 2002, <http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalsApplications/TherapeuticBiologicApplications/ucm092753.pdf>
- Gennari R, Menard S, Fagnoni F, Ponchio L, Scelsi M, Tagliabue E, Castiglioni F, Villani L, Magalotti C, Gibelli N, Oliviero B, Ballardini B, Da Prada G, Zambelli A, Costa A. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. *Clin Cancer Res* 2004;10:5650-5
- Ghosh R, Narasanna A, Wang SE, Liu S, Chakrabarty A, Balko JM, Gonzalez-Angulo AM, Mills GB, Penuel E, Winslow J, Sperinde J, Dua R, Pidaparathi S, Mukherjee A, Leitzel K, Kostler WJ, Lipton A, Bates M, Arteaga CL. Trastuzumab has preferential activity against breast cancers driven by HER2 homodimers. *Cancer Res* 2011;71:1871-82

- Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res* 1995;1:1311-8
- Heinemann V, Stintzing S, Kirchner T, Boeck S, Jung A. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. *Cancer Treat Rev* 2009;35:262-71
- Huang SM, Li J, Harari PM. Molecular inhibition of angiogenesis and metastatic potential in human squamous cell carcinomas after epidermal growth factor receptor blockade. *Mol Cancer Ther* 2002;1:507-14
- Hudis CA. Trastuzumab - mechanism of action and use in clinical practice. *N Engl J Med* 2007;357:39-51
- Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol* 2009;21:177-84
- Izumi Y, Xu L, di Tomaso E, Fukumura D, Jain RK. Tumour biology: Herceptin acts as an anti-angiogenic cocktail. *Nature* 2002;416:279-80
- Jakobovits A, Amado RG, Yang X, Roskos L, Schwab G. From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice. *Nat Biotechnol* 2007;25:1134-43
- Junttila TT, Akita RW, Parsons K, Fields C, Lewis Phillips GD, Friedman LS, Sampath D, Sliwkowski MX. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. *Cancer Cell* 2009;15:429-40
- Kaneko E, Niwa R. Optimizing therapeutic antibody function: progress with Fc domain engineering. *BioDrugs* 2011;25:1-11
- Kawaguchi Y, Kono K, Mimura K, Sugai H, Akaike H, Fujii H. Cetuximab induce antibody-dependent cellular cytotoxicity against EGFR-expressing esophageal squamous cell carcinoma. *Int J Cancer* 2007;120:781-7
- Kim GP, Grothey A. Targeting colorectal cancer with human anti-EGFR monoclonal antibodies: focus on panitumumab. *Biologics* 2008;2:223-8
- Kim MS, Lee SH, Song MY, Yoo TH, Lee BK, Kim YS. Comparative analyses of complex formation and binding sites between human tumor necrosis factor- α and its three antagonists elucidate their different neutralizing mechanisms. *J Mol Biol* 2007;374:1374-88
- Kiyota A, Shintani S, Mihara M, Nakahara Y, Ueyama Y, Matsumura T, Tachikawa T, Wong DT. Anti-epidermal growth factor receptor monoclonal antibody 225 upregulates p27(KIP1) and p15(INK4B) and induces G1 arrest in oral squamous carcinoma cell lines. *Oncology* 2002;63:92-8
- Klos KS, Zhou X, Lee S, Zhang L, Yang W, Nagata Y, Yu D. Combined trastuzumab and paclitaxel treatment better inhibits ErbB-2-mediated angiogenesis in breast carcinoma through a more effective inhibition of Akt than either treatment alone. *Cancer* 2003;98:1377-85
- Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495-7
- Kohn T, Tam LT, Stevens SR, Louie JS. Binding characteristics of tumor necrosis factor receptor-Fc fusion proteins vs anti-tumor necrosis factor mAbs. *J Invest Dermatol Symp Proc* 2007;12:5-8
- Kristensen LE, Christensen R, Bliddal H, Geborek P, Danneskiold-Samsøe B, Saxne T. The number needed to treat for adalimumab, etanercept, and infliximab based on ACR50 response in three randomized controlled trials on established rheumatoid arthritis: a systematic literature review. *Scand J Rheumatol* 2007;36:411-7
- Kurai J, Chikumi H, Hashimoto K, Yamaguchi K, Yamasaki A, Sako T, Touge H, Makino H, Takata M, Miyata M, Nakamoto M, Burioka N, Shimizu E. Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines. *Clin Cancer Res* 2007;13:1552-61
- Kute T, Lack CM, Willingham M, Bishwokama B, Williams H, Barrett K, Mitchell T, Vaughn JP. Development of herceptin resistance in breast cancer cells. *Cytometry A* 2004;57:86-93
- Launois R, Avouac B, Berenbaum F, Blin O, Bru I, Fautrel B, Joubert JM, Sibilia J, Combe B. Comparison of certolizumab pegol with other anticytokine agents for treatment of rheumatoid arthritis: A multiple-treatment Bayesian metaanalysis. *J Rheumatol* 2011;38:835-45
- Lee J, Lee I, Han B, Park JO, Jang J, Park C, Kang WK. Effect of simvastatin on cetuximab resistance in human colorectal cancer with KRAS mutations. *J Natl Cancer Inst* 2011;103:674-88
- Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, Shepard HM. Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. *Cancer Immunol Immunother* 1993;37:255-63
- Li S, Schmitz KR, Jeffrey PD, Wiltzius JJ, Kussie P, Ferguson KM. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. *Cancer Cell* 2005;7:301-11
- Lièvre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, Cote JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;66:3992-5
- Mahtani RL, Macdonald JS. Synergy between cetuximab and chemotherapy in tumors of the gastrointestinal tract. *Oncologist* 2008;13:39-50
- Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor α monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1998;41:1552-63
- Mandal M, Adam L, Mendelsohn J, Kumar R. Nuclear targeting of Bax during apoptosis in human colorectal cancer cells. *Oncogene* 1998;17:999-1007
- McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. 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

Meira DD, Nobrega I, de Almeida VH, Mororo JS, Cardoso AM, Silva RL, Albano RM, Ferreira CG. Different antiproliferative effects of matuzumab and cetuximab in A431 cells are associated with persistent activity of the MAPK pathway. *Eur J Cancer* 2009;45:1265-73

Messersmith WA, Hidalgo M. Panitumumab, a monoclonal anti epidermal growth factor receptor antibody in colorectal cancer: another one or the one? *Clin Cancer Res* 2007;13:4664-6

Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res* 2001;61:4744-9

Müller D, Kontermann RE. Bispecific antibodies for cancer immunotherapy: Current perspectives. *BioDrugs* 2010;24:89-98

Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, Laccabue D, Zerbini A, Camisa R, Bisagni G, Neri TM, Ardizoni A. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol* 2008;26:1789-96

Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 2004;6:117-27

Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res* 2004;64:2343-6

Nesbitt A, Fossati G, Bergin M, Stephens P, Stephens S, Foulkes R, Brown D, Robinson M, Bourne T. Mechanism of action of certolizumab pegol (CDP870): *in vitro* comparison with other anti-tumor necrosis factor alpha agents. *Inflamm Bowel Dis* 2007;13:1323-32

Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001;37 (Suppl 4):S9-15

Palfreman R, Airey M, Moore A, Vugler A, Nesbitt A. Use of biofluorescence imaging to compare the distribution of certolizumab pegol, adalimumab, and infliximab in the inflamed paws of mice with collagen-induced arthritis. *J Immunol Methods* 2009;348:36-41

Pedersen IM, Buhl AM, Klausen P, Geisler CH, Jurlander J. The chimeric anti-CD20 antibody rituximab induces apoptosis in B-cell chronic lymphocytic leukemia cells through a p38 mitogen activated protein-kinase-dependent mechanism. *Blood* 2002;99:1314-9

Perrotte P, Matsumoto T, Inoue K, Kuniyasu H, Eve BY, Hicklin DJ, Radinsky R, Dinney CP. Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin Cancer Res* 1999;5:257-65

Persson M, Tolmachev V, Andersson K, Gedda L, Sandstrom M, Carlsson J. [(177)Lu]pertuzumab: experimental studies on targeting of HER-2 positive tumour cells. *Eur J Nucl Med Mol Imaging* 2005;32:1457-62

Petit AM, Rak J, Hung MC, Rockwell P, Goldstein N, Fendly B, Kerbel RS. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells *in vitro* and *in vivo*: angiogenic implications for signal transduction therapy of solid tumors. *Am J Pathol* 1997;151:1523-30

Press OW, Appelbaum F, Ledbetter JA, Martin PJ, Zarling J, Kidd P, Thomas ED. Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. *Blood* 1987;69:584-91

Ramakrishnan MS, Eswaraiah A, Crombet T, Piedra P, Saurez G, Iyer H, Arvind AS. Nimotuzumab, a promising therapeutic monoclonal for treatment of tumors of epithelial origin. *MAbs* 2009;1:41-8

Ritchlin C, Haas-Smith SA, Hicks D, Cappuccio J, Osterland CK, Looney RJ. Patterns of cytokine production in psoriatic synovium. *J Rheumatol* 1998;25:1544-52

Rivkin A. Certolizumab pegol for the management of Crohn's disease in adults. *Clin Ther* 2009;31:1158-76

Rockberg J, Lofblom J, Hjelm B, Uhlen M, Stahl S. Epitope mapping of antibodies using bacterial surface display. *Nature Methods* 2008;5:1039-45

Rockberg J, Schwenk JM, Uhlen M. Discovery of epitopes for targeting the human epidermal growth factor receptor 2 (HER2) with antibodies. *Mol Oncol* 2009;3:238-47

Rudnick SI, Lou J, Shaller CC, Tang Y, Klein-Szanto AJ, Weiner LM, Marks JD, Adams GP. Influence of affinity and antigen internalization on the uptake and penetration of Anti-HER2 antibodies in solid tumors. *Cancer Res* 2011;71:2250-9

Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004;22:1201-8

Schmitz KR, Ferguson KM. Interaction of antibodies with ErbB receptor extracellular regions. *Exp Cell Res* 2009;315:659-70

Schneider-Merck T, Lammerts van Bueren JJ, Berger S, Rossen K, van Berkel PH, Derer S, Beyer T, Lohse S, Bleeker WK, Peipp M, Parren PW, van de Winkel JG, Valerius T, Dechant M. Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage. *J Immunol* 2010;184:512-20

Shealy D, Cai A, Staquet K, Baker A, Lacy ER, Johns L, Vafa O, Gunn G 3rd, Tam S, Sague S, Wang D, Brigham-Burke M, Dalmonte P, Emmell E, Pikounis B, Bugelski PJ, Zhou H, Scallan B, Giles-Komar J. Characterization of golimumab, a human monoclonal antibody specific for human tumor necrosis factor alpha. *MAbs* 2010;2

Skvortsova I, Skvortsov S, Popper BA, Haidenberger A, Saurer M, Gunkel AR, Zwierzina H, Lukas P. Rituximab enhances radiation-triggered apoptosis in non-Hodgkin's lymphoma cells *via* caspase-dependent and - independent mechanisms. *J Radiat Res (Tokyo)* 2006;47:183-96

Spiridon CI, Guinn S, Vitetta ES. A comparison of the *in vitro* and *in vivo* activities of IgG and F(ab')₂ fragments of a mixture of three monoclonal anti-Her-2 antibodies. *Clin Cancer Res* 2004;10:3542-51

Teeling JL, French RR, Cragg MS, van den Brakel J, Pluyter M, Huang H, Chan C, Parren PW, Hack CE, Dechant M, Valerius T, van de Winkel JG, Glennie MJ. Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. *Blood* 2004;104:1793-800

Teeling JL, Mackus WJ, Wiegman LJ, Van den Brakel JH, Beers SA, French RR, van Meerten T, Ebeling S, Vink T, Slootstra JW, Parren PW, Glennie MJ, Van de Winkel JG. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. *J Immunol* 2006;177:362-71

UCB. Cimzia full prescribing information. 2008, http://www.ucb.com/_up/ucb_com_products/documents/Cimzia_12_21_10.pdf

Uchiyama S, Suzuki Y, Otake K, Yokoyama M, Ohta M, Aikawa S, Komatsu M, Sawada T, Kagami Y, Morishima Y, Fukui K. Development of novel humanized anti-CD20 antibodies based on affinity constant and epitope. *Cancer Sci* 2010;101:201-9

Valabrega G, Montemurro F, Aglietta M. Trastuzumab:

mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. *Ann Oncol* 2007;18:977-84

Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002;20:719-26

Williams RO, Paleolog E, Feldmann M. Cytokine inhibitors in rheumatoid arthritis and other autoimmune diseases. *Curr Opin Pharmacol* 2007;7:412-7

Wu X, Rubin M, Fan Z, DeBlasio T, Soos T, Koff A, Mendelsohn J. Involvement of p27KIP1 in G1 arrest mediated by an anti-epidermal growth factor receptor monoclonal antibody. *Oncogene* 1996;12:1397-403

Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG, Jakobovits A. Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. *Cancer Res* 1999;59:1236-43

Yoon S, Kim YS, Shim H, Chung J. Current perspectives on therapeutic antibodies. *Biotech Bioproc E* 2010;15:709-15

zum Buschenfelde CM, Hermann C, Schmidt B, Peschel C, Bernhard H. Antihuman epidermal growth factor receptor 2 (HER2) monoclonal antibody trastuzumab enhances cytolytic activity of class I-restricted HER2-specific T lymphocytes against HER2-overexpressing tumor cells. *Cancer Res* 2002;62:2244-7