Human Monoclonal Antibodies from Transgenic Mice

N. Lonberg

Abstract Since the 1986 regulatory approval of muromonomab-CD3, a mouse monoclonal antibody (MAb) directed against the T cell CD3ε antigen, MAbs have become an increasingly important class of therapeutic compounds in a variety of disease areas ranging from cancer and autoimmune indications to infectious and cardiac diseases. However, the pathway to the present acceptance of therapeutic MAbs within the pharmaceutical industry has not been smooth. A major hurdle for antibody therapeutics has been the inherent immunogenicity of the most readily available MAbs, those derived from rodents. A variety of technologies have been successfully employed to engineer MAbs with reduced immunogenicity. Implementation of these antibody engineering technologies involves in vitro optimization of lead molecules to generate a clinical candidate. An alternative technology, involving

---

N. Lonberg
Medarex, 521 Cottonwood Drive, Milpitas, CA 95035, USA
e-mail: nlonberg@medarex.com

Y. Chernajovsky, A. Nissim (eds.) *Therapeutic Antibodies. Handbook of Experimental Pharmacology 181.*

the engineering of strains of mice to produce human instead of mouse antibodies, has been emerging and evolving for the past two decades. Now, with the 2006 US regulatory approval of panitumumab, a fully human antibody directed against the epidermal growth factor receptor, transgenic mice expressing human antibody repertoires join chimerization, CDR grafting, and phage display technologies, as a commercially validated antibody drug discovery platform. With dozens of additional transgenic mouse-derived human MAbs now in clinical development, this new drug discovery platform appears to be firmly established within the pharmaceutical industry.

1 Immunogenicity of Therapeutic Antibodies: Problem and Solutions

The discovery of hybridoma methods in 1975 for isolating high specificity and high affinity rodent monoclonal antibodies (MAbs) opened the door to a new class of therapeutic compounds with potential applicability across a wide range of disease indications (Kohler and Milstein 1975). This promise appeared to be fulfilled with the 1986 US regulatory approval of muromonab-CD3 for the treatment of kidney transplant rejection (Goldstein et al. 1985). However, despite the fact that muromonomab-CD3 acts as a potent immunosuppressive drug, it turned out to be an intrinsically immunogenic molecule. Because rodent antibodies are foreign proteins, the human immune system mounts its own antibody response to them, leading to rapid clearance, reduced efficacy (Goldstein et al. 1985; Pendley et al. 2003; Kuus-Reichel et al. 1994), and an increased risk of infusion reactions (Baert et al. 2003). A potential solution to the problem of immunogenicity, fully human MAbs, did not at the time appear to be practical because of the limited availability of target specific human antibodies (Larrick and Bourla 1986; James and Bell 1987; Houghton 1983; Olsson et al. 1984). Although very large panels of rodent MAbs could be easily assembled and screened for optimal binding to the intended target and low cross-reactivity to related molecules, analogous technologies for generating and isolating human MAbs with the full range of specificities and affinities afforded by rodent hybridoma methods did not exist. The smaller pools of available reactive human antibodies might have been a factor in the selection of early human MAb clinical candidates such as HA-1A, which entered clinical testing for treatment of sepsis in the late 1980s and gained European regulatory approval in 1991 (Brun-Buisson 1994). This polyreactive authentic human MAb bound to its intended target, lipid A, through relatively nonspecific hydrophobic interactions of heavy chain V region framework residues (Helmhorst et al. 1998; Bieber et al. 1995). The 1992 clinical, and US regulatory, failure of HA-1A (Spalding 1992; Edgington 1992; McCloskey et al. 1994), together with the observed immunogenicity of muromonomab-CD3, contributed to a considerable cooling of enthusiasm for antibody-based drugs within the pharmaceutical industry. However, 8 years after the approval of muromonomab-CD3, a second MAb-based drug, the engineered chimeric antibody fragment abciximab (Simoons et al. 1994), gained approval. This was followed by the approval of 18 additional MAb-based drugs in the last 10 years. As a class of drug compounds, MAbs appear to have been rescued by the use of technologies for reengineering rodent antibodies in vitro to replace framework amino acid residues with corresponding human sequences (Morrison et al. 1984; Jones et al. 1986). Additional technologies were also developed to directly isolate synthetic MAbs from libraries of human and synthetic immunoglobulin sequences (McCafferty 1990). Although these existing antibody engineering technologies appear to have been very successful in generating therapeutic products with acceptable safety and efficacy, there may still be room for improvement. Although some of the products generated by antibody engineering have not elicited patient immune responses, most of the approved MAb products, including examples from chimerization, CDR grafting and phage display, have been found to be immunogenic (Pendley et al. 2003).

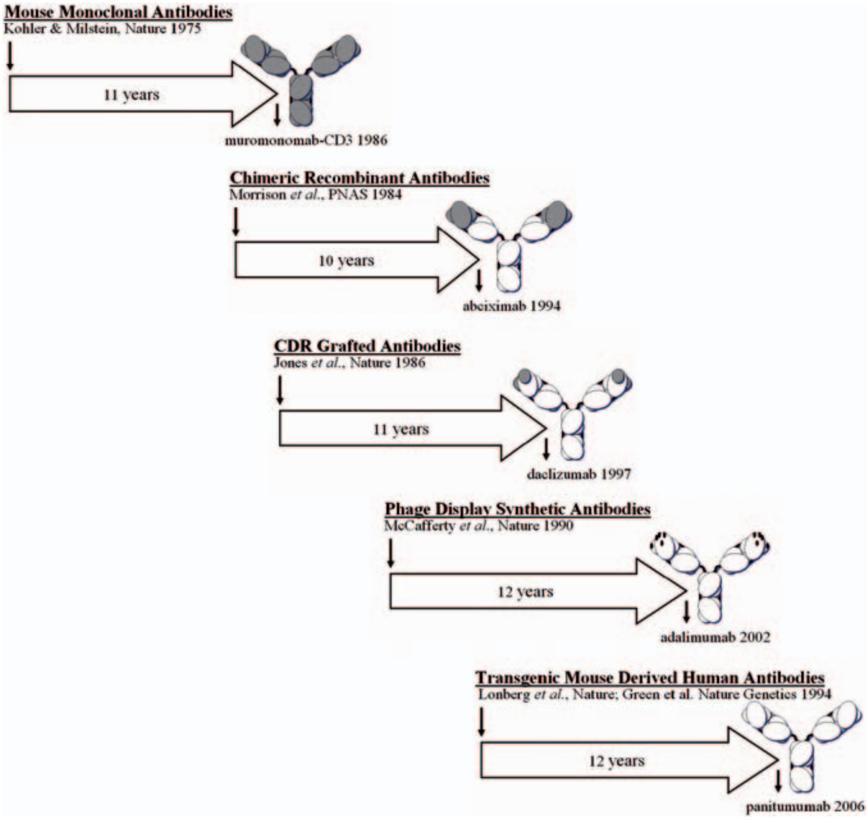

Fig. 1 Evolution of therapeutic antibody technology and progress to the clinic. FDA-approved MAbs have emerged between 10 and 12 years after the date that the new technologies on which they were based were reported in the scientific literature (Morrison et al. 1984; Jones et al. 1986; McCafferty et al. 1990; Kohler and Milstein 1975; Lonberg et al. 1994; Green et al. 1994)

Transgenic mouse strains comprising human immunoglobulin repertoires represent an alternative platform technology for discovering low immunogenicity therapeutic MAbs (Fig. 1). In contrast to antibody engineering technologies, which involve the downstream modification and optimization of individual protein molecules, transgenic technology is used for the upstream genetic engineering of strains of mice that are then used as drug discovery tools to directly generate human sequence antibodies that can be moved into the clinic without further optimization. Twelve years after their appearance in the scientific literature (Lonberg et al. 1994, Green et al. 1994), immunoglobulin transgenic mice have now been validated as drug discovery platforms by the regulatory approval of their first product, panitumumab (Gibson et al. 2006). In this review, I discuss the development of the technology and drugs derived from it.

2 Development of Techniques for Manipulation of the Mouse Genome

2.1 Pronuclear Microinjection

Fundamental basic research in mouse embryology and molecular biology by a large number of laboratories (Nagy et al. 2003) led to the development, in the early 1980s, of a set of tools for the manipulation of the mouse genome (Fig. 2). The generation of genetically engineered mice by direct microinjection of cloned DNA sequences into the pronuclei of single-cell half-day embryos was reported by several groups in 1981 (Gordon and Ruddle 1981; Costantini and Lacy 1981; Brinster et al. 1981; Harbers et al. 1981; Wagner et al. 1981a, 1981b). The microinjected DNA constructs, which are inserted into mouse chromosomes and are propagated through the germline, could include transcriptional regulatory sequences to direct expression to restricted differentiated cell types, including B cell expression of antibody genes (Brinster et al. 1983). This first report of an expressed immunoglobulin gene in transgenic mice involved a very small transgene; however, despite the fact that very fine glass needles are employed for pronuclear microinjection, the sheer forces experienced by the injected DNA do not prevent the use of this technique for introducing much larger (>100 kb) transgenes into the mouse germline. (Costantini and Lacy 1981; Taylor et al. 1992; Schedl et al. 1993; Lonberg and Huszar 1995; Fishwild et al. 1996).

2.2 Embryonic Stem Cells

Because microinjected transgenes integrate relatively randomly over a large number of potential sites within the mouse genome, it does not provide for easy manipulation of specific endogenous mouse genes. Microinjection could generate mice

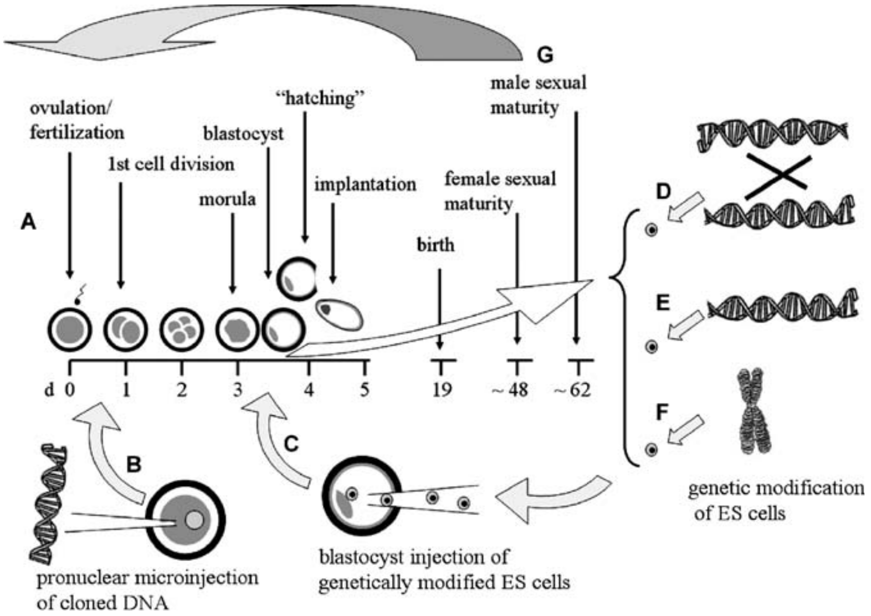

Fig. 2 Techniques developed for the manipulation of mouse embryos provide access for the modification of the germline. (**A**) Overview of mouse development. (**B**) Direct introduction of cloned DNA sequences inserted randomly into mouse chromosomes through pronuclear microinjection of half day embryos. (**C**) Embryonic Stem (ES) cells derived from 3.5-day blastocyst stage embryos can be grown in culture, genetically modified, and then reintroduced into developing blastocysts by insertion of a glass pipet into the blastocoel cavity. (**D**) Site-specific modifications of ES cell genomes can be engineered through homologous recombination followed by selection and screening for specific recombinants in culture. (**E**) Large DNA fragments can be inserted into ES cell chromosomes by transfection or yeast/bacterial cell fusion. (**F**) Entire chromosomes or chromosome fragments, which replicate without integration into endogenous mouse chromosomes, can be introduced into ES cells by microcell-mediated chromosome transfer (MMCT). (**G**) The very short (ca. 2–3 months) generation time of the mouse allows for rapid crossbreeding to combine multiple independent genetic modifications into a single animal

that expressed human genes, but the mouse ortholog was typically still active. This technical hurdle was overcome with the development of positive–negative selection vectors that allowed for the selection and screening of specifically targeted homologous recombination events in cultured cells, and with the parallel development of embryonic stem (ES) cell lines that could be cultured and manipulated in vitro and reintroduced into 3.5-day old blastocyst stage embryos to populate the germline of the resulting chimeric mice. The combination of these two technologies led to the generation of strains of engineered mice comprising specifically targeted modifications of their germlines (Mansour et al. 1988; Zijlstra et al. 1989; Schwartzberg et al. 1989). The most commonly introduced specific modification leads to the inactivation of an endogenous gene and the creation of what are commonly referred to as gene knockout mouse strains. Gene knockout technology has proved to be of enormous value for basic research, and applied to the endogenous mouse immunoglobulin loci, important for the development of transgenic mouse platforms for human antibody drug discovery.

In addition to applications for modifying endogenous mouse genes, ES cells have also proved useful as an alternative to pronuclear microinjection for the introduction of large DNA clones such as YAC clones (Strauss et al. 1993; Choi et al. 1993; Jakobovits et al. 1993; Davies et al. 1993). Very large human chromosome fragments have also been introduced into the mouse germline using ES cell technology. In this approach, called microcell-mediated chromosome transfer (MMCT), human fibroblast-derived microcells are fused with mouse ES cells resulting in pluripotent cell lines having a single human chromosome or chromosome fragment – including a centromere and both telomeres – that replicates and assorts during cell division without insertion into an endogenous mouse chromosome (Tomizuka et al. 1997).

3 Transgenic Mice with Human Immunoglobulin Genes

3.1 Expression of Human Antibody Repertoires

It was quickly recognized that the new tools developed for manipulating the mouse germline might be practically applied toward the generation of human immunoglobulin expressing transgenic mice. In 1985, Alt et al. (1985) suggested that transgenic technology could be useful for generating new human sequence MAbs starting from unrearranged, germline-configuration transgenes. The authors concluded that although this was "conceptually outlandish," it might "be realized in the not-too-distant future." A year later, Yamamura et al. (1986) reported the cell type specific expression of a human immunoglobulin gamma heavy chain transgene. This was followed by reports of expression and rearrangement of germline configuration (unrearranged) chicken and rabbit light chain transgenes in transgenic mice (Bucchini et al. 1987, Goodhardt et al. 1987), a milestone that was recognized at the time as contributing toward the development of a transgenic platform for discovering human MAbs. Buttin (1987) commented that "recent progress in this field invites us to believe that the creation of transgenic mice with B cells secreting a wide spectrum of [human] antibodies is no longer out of reach." In 1989, Bruggemann et al. (1989) reported the expression of a repertoire of human IgM heavy chains and the generation of a transgene-encoded immune response in mice. Three years later, Taylor et al. (1992) reported mice comprising germline configuration human heavy- and κ light-chain transgenes that produced a repertoire of human IgM and IgG antibodies. This group showed in a later paper (Taylor et al. 1994) that the IgG antibodies were a product of class switching, and that they comprised somatic mutations consistent with functional affinity maturation. These reports, and many others from a number of different laboratories, demonstrated that human gene sequences could direct cell type specific expression of human immunoglobulins in mice, and that those exogenous gene sequences could undergo the normal rearrangements and modifications required for generating primary and secondary antibody repertoires. However, human immunoglobulin transgenic mice with intact functional endogenous immunoglobulin loci also express mouse antibodies and chimeric mouse–human antibodies. Creation of a more useful platform for human antibody drug discovery, a mouse with disrupted endogenous immunoglobulin loci, requires combining methods for introducing human immunoglobulin transgenes with the methods described earlier for generating gene knockout mice.

3.2 Transgenic Mouse Platforms for Therapeutic MAb Drug Discovery

In 1994, two articles, one from my laboratory (Lonberg et al. 1994) and the other from Green et al. (1994), reported the generation of mice with four different germline modifications: two targeted disruptions (the endogenous mouse heavy- and κ light-chain genes) and two introduced human transgenes (encoding the heavy chain and κ light chain). Although both articles report the use of homologous recombination in mouse ES cells to engineer similar disruptions of the endogenous mouse loci, different technologies were used to construct and deliver the human sequence transgenes. Lonberg et al. (1994) used pronuclear microinjection to introduce reconstructed minilocus transgenes – the heavy chain containing 3 heavy-chain variable (V_H), 16 diversity (D), and all 6 heavy-chain joining (J_H) regions together with μ and $\gamma 1$ constant-region gene segments. In the transgenic strains, this construct underwent VDJ joining, together with somatic mutation and correlated class switching (Taylor et al. 1994). The light-chain transgene included four $V\kappa$, all five $J\kappa$ and the κ constant region ($C\kappa$). In contrast, Green et al. (1994) used fusion of yeast protoplasts to deliver yeast artificial chromosome (YAC)-based minilocus transgenes. In this case, the heavy chain included $5\,V_H$, all $25\,D$ and all 6 J_H gene segments together with μ and δ constant-region gene segments. This construct underwent VDJ joining and expressed both IgM and IgD. The light-chain YAC construct included two functional $V\kappa$ and all five $J\kappa$ segments, together with $C\kappa$. Neither group inactivated the endogenous λ-light-chain locus, which in typical laboratory mouse strains contributes to only $\sim$5% of the B cell repertoire. Functional λ-light-chain expression leads to a subpopulation of B cells producing hybrid B cell receptors and secreted antibodies that have human heavy- and mouse λ-light chains. However, the presence of this subpopulation did not prevent the isolation of hybridoma cell lines secreting fully human monoclonal IgM (Green et al. 1994) and IgG (Lonberg et al. 1994) MAbs recognizing the target antigens against which the mice had been immunized.

The ability of these engineered mouse strains, each comprising only a fraction of the natural human primary V gene segment repertoire, to generate antibodies to a variety of targets may reflect the relative importance of combinatorial diversity (encoded in the germline library of V, D, and J gene segments) and junctional and somatic diversity (a product of the assembly and maturation of antibody genes).

Although naive B cell CDR1 and CDR2 sequences are completely encoded by the germ line, junctional diversity, which is intact in minilocus transgenes, creates much of the heavy-chain CDR3 repertoire. CDR3 sequences appear to be critical for antigen recognition by unmutated B cell receptors and may be largely responsible for the primary repertoire (Ignatovitch et al. 1997; Davis 2004; Tomlinson et al. 1996). Primary repertoire B cells having low affinity for the immunogen can then enter into the T cell-mediated process of affinity maturation, which has been shown to generate high-affinity antibodies from a very limited V-gene repertoire. An extreme example of this is offered by a report of an engineered mouse strain having only a single functional human V_H gene and three mouse $V\lambda$ genes (Xu and Davis 2000). These animals demonstrated a specific antibody responses to a variety of T-dependent antigens. High affinity, somatically mutated MAbs were characterized, including a very high, 25 pM, affinity MAb against hen egg-white lysozyme. However, the minimal V-repertoire mice did not respond to the T-independent antigen, dextran B512, and the authors suggested that responses to carbohydrate antigens might drive evolutionary selection for large primary repertoires. Germline-encoded recognition of such antigens may be important for developing a rapid primary protective response to pathogens, a feature that would be selected for in the wild, but less important for isolating high-affinity antibodies from laboratory mice using hyperimmunization protocols that trigger T cell-dependent affinity maturation.

In addition to affecting the response to T-independent antigens and the kinetics of overall immune reactions, repertoire size may have an impact on B cell development and the size of different B cell compartments. Fishwild et al. (1996) compared mice having different numbers of light-chain V gene segments and found that the introduction of larger repertoires encoded by a κ light-chain YAC clone comprising approximately half the Vκ repertoire led to increased population of the peripheral and bone marrow B cell compartments relative to transgenic strains comprising only four Vκ genes. The relative number of mature and immature B cells in these compartments also appeared more normal in mice with larger V gene repertoires. Mendez et al. (1997) generated transgenic mice having nearly complete heavy-chain V repertoires and approximately half the κ-light-chain V repertoire, and compared them with the minilocus mice of Green et al. (1994). This paper, and a later analysis of the same mouse strains by Green and Jakobovits (1998), showed that V-region repertoire size had a profound effect on multiple checkpoints in B cell development, with larger repertoires capable of restoring B cell compartments to near normal levels. Despite the fact that human immunoglobulin transgenic mice express B cell receptors that are essentially hybrids of mouse and human components (e.g., human immunoglobulin, mouse Igα, Igβ, and other signaling molecules), their B cells develop and mature into what appear to be all of the normal B cell subtypes. Furthermore, the immunoglobulin transgenes undergo V(D)J joining, random nucleotide (N-region) addition, class switching, and somatic mutation to generate high-affinity MAbs to a variety of different antigens. The process of affinity maturation in these animals even recapitulates the normal pattern of somatic mutation hotspots observed in authentic human secondary repertoire antibodies (Harding and Lonberg 1995).

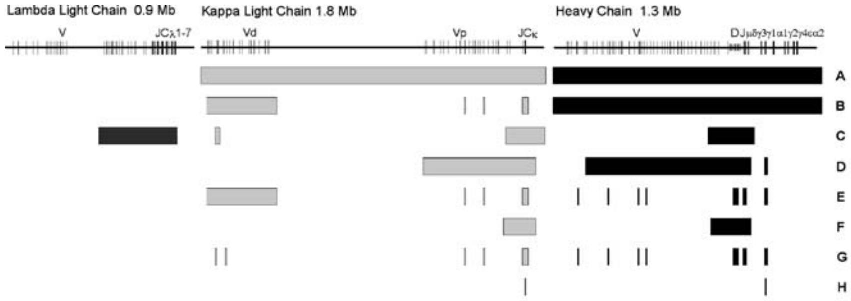

Fig. 3 Human immunoglobulin sequences introduced in the germ line of mice comprising endogenous Ig heavy-chain and κ-light-chain gene inactivations. The germline configuration of the human immunoglobulin λ-light chain, κ-light chain and heavy chain is depicted above *bars* representing those sequences used to assemble transgenes introduced into strains of mice used for generation and isolation of human sequence MAbs. **A** (Tomizuka et al. 2000), **B** (Ishida et al. 2002), **C** (Nicholson et al. 1999), **D** (Mendez et al. 1997), **E** (Fishwild et al. 1996), **F** (Green et al. 1994), **G** (Lonberg et al. 1994), **H** (Zou et al. 1994)

There have now been multiple reports in the literature of transgenic mice having immunoglobulin repertoires comprising human heavy- and light-chain sequences in the background of disrupted endogenous heavy- and κ-light-chain loci (Fig. 3). Several different technologies – including pronuclear microinjection and yeast protoplast fusion with ES cells – have been employed for engineering these mouse strains. The introduction of the largest fraction of the human germline repertoire has been facilitated by microcell-mediated chromosome transfer. Using this technique, Tomizuka et al. (1997) generated ES cell lines and chimeric mice containing fragments of human chromosomes 2 and 14, including the human κ-light-chain and heavy-chain loci, respectively. In addition, they generated chimeric mice that incorporated an apparently intact human chromosome 22, comprising the λ light chain locus. Germline transmission was obtained with the human κ-light-chain ES cell lines. In a subsequent report, germline transmission was obtained with a human heavy-chain ES cell line, and mice were created that expressed complete human heavy- and light-chain repertoires in a genetic background that included disruptions of the mouse heavy- and κ-light-chain loci (Tomizuka et al. 2000). Completely human, high-affinity (<nanomolar) MAbs were isolated from the animals. Although both chromosome fragments could be transmitted through the germ line, the κ-light chain-containing chromosome-2 fragment was found to be less mitotically stable. The observed stability of the heavy chain-containing fragment may derive from the fortuitous location of the immunoglobulin heavy-chain locus (IgH) at the very telomere of the long arm of human chromosome 14. Because of the structure of chromosome 14, a random deletion between IgH and the centromere removed most of the nonimmunoglobulin genes leaving IgH, the centromere and both telomeres functionally intact. The resulting 10- to 20-Mb fragment minimizes cross-species trisomy, which would presumably be selected against during cell division.

The observed stability of this fragment has now been exploited to create artificially constructed human chromosome fragments that include the entire human heavy-chain locus together with the entire human λ-light-chain locus (Kuroiwa et al. 2000). Bruggemann and colleagues (Popov et al. 1999) have also generated human λ-light-chain locus transgenes, using them to create transgenic mice that express partial repertoires of all three human immunoglobulin loci in the background of disrupted endogenous κ-light-chain and heavy-chain loci (Nicholson et al. 1999).

Another transgenic mouse platform, which generates chimeric antibodies rather than fully human sequence antibodies, was developed by Rajewsky and colleagues in 1994 (Zou et al. 1994). These mice comprise relatively precise replacements of the mouse κ and γ1 constant-region gene segments with the corresponding human gene sequences. The κ constant-region gene segment was replaced using homologous recombination in mouse ES cells. For the γ1 gene, only the secreted exons were replaced, and the engineering was accomplished in two steps using the Cre-*loxP* recombination system, also in mouse ES cells.

4 Transgenic Mouse-Derived Human MAbs

The scientific literature includes a large number of reports describing the characteristics of human MAbs derived from transgenic mouse platforms, and a review of this literature provides an assessment of the functionality of these platforms (Lonberg 2005). A very diverse set of antigens have been successfully targeted with transgenic derived MAbs. These include small molecules (Ball et al. 1999; Farr et al. 2002), pathogen-encoded proteins (Tzipori et al. 2004; Mukherjee et al. 2002; Mukherjee et al. 2002b; He et al. 2002; Greenough et al. 2005; Sheoran et al. 2005; Babcock et al. 2006; Coughlin et al. 2006; Vitale et al. 2006), polysaccharide antigens (Chang et al. 2002; Maitta 2004), human-secreted proteins (Mendez et al. 1997; Ishida et al. 2002; Villadsen et al. 2003; Bekker et al. 2004; Yang et al. 1999b; Huang et al. 2002; Mian et al. 2003; Ostendorf et al. 2003; Suarez et al. 2004; Parry et al. 2005; Rathanaswami et al. 2005; Burgess et al. 2006; Melnikova and Bar-Eli 2006), cell-surface proteins (Fishwild et al. 1996; Mendez et al. 1997; Fishwild et al. 1999; Yang et al. 1999, 2001; Holmes 2001; Borchmann et al. 2003; Skov et al. 2003; Teeling et al. 2004; Schuler et al. 2004; Rowinski et al. 2004; Heuck et al. 2004; Ramakrishna et al. 2004; Keler et al. 2003; Bleeker et al. 2004; Suzuki et al. 2004; Imakiire et al. 2004; Mori et al. 2004; Garambois et al. 2004; Trikha et al. 2004; Tai et al. 2005; Boll et al. 2005; Cohen et al. 2005; Kuroki et al. 2005; Sanderson et al. 2005; Tse et al. 2006; van Royen-Kerkhoff et al. 2005; Wang et al. 2005; Ma et al. 2006; Melnikova and Bar-Eli 2006; Teeling et al. 2006; Wu et al. 2006; Villadsen et al. 2007), and human tumor-associated glycosylation variants (Nozawa et al. 2004).

Most of the transgenic mouse-derived MAbs have binding affinities in the 0.1–10 nM range, the same affinity range typically seen for MAbs derived from wild-type mice (Ball et al. 1999; Yang et al. 1999, 1999b; Keler et al. 2003; Cohen et al. 2005; Burgess et al. 2006). This range is probably a function of the natural constraints on affinity maturation operating in vivo (Foote and Eisen 1995; Roost et al. 1995). However, there are also examples of transgenic-derived human MAbs with picomolar and even subpicomolar affinities. Villadsen et al. (2003) described a 10 pM affinity antibody to IL-15, Wang et al. (2005) described a 4 pM affinity MAb to the insulin-like growth factor receptor, and Rathanaswami et al. (2005) reported several anti-IL-8 MAbs in the 1–10 pM range, with one MAb having a measured affinity in the 0.5–1 pM range.

5 Transgenic Mouse-Derived Human MAbs in the Clinic

5.1 Panitumumab and Zalutumumab

The 2006 regulatory approval in the US for panitumumab was a significant milestone for transgenic mouse-derived MAbs, and marked the first commercial validation of immunoglobulin transgenic mouse drug discovery platforms. Panitumumab binds to the epidermal growth factor receptor (EGFR) with very high affinity ($K_\mathrm{d} = 5 \times 10^{-11} \mathrm{M}^{-1}$) and blocks ligand binding (Rowinski et al. 2004; Yang et al. 1999, 2001; Foon et al. 2004). In preclinical mouse xenograft models, it was found to be more potent than the lower affinity mouse antibody m225 (Yang et al. 1999), the parent of the already marketed mouse/human IgG1 chimeric anti-EGFR antibody, cetuximab (Cunningham et al. 2004).

There has been no direct comparison of the safety and efficacy of cetuximab and panitumumab in a side-by-side clinical study. In addition, the fact that cetuximab is an IgG1 antibody and panitumumab an IgG2 antibody further complicates any attempt to compare the two drugs. However, an initial survey of the available literature suggests that the fact that panitumumab is a fully human antibody derived from a transgenic mouse may differentiate it from the chimeric cetuximab (Cohenuram and Saif 2007). In early phase I and II trials, panitumumab was associated with a higher frequency of skin rashes than cetuximab; however, skin rashes (which are related to the mechanism of action of EGFR-targeted drugs, including small molecules, and in this case are not a product of drug immunogenicity) have been positively correlated with activity for cetuximab (Calvo and Rowinsky 2005), and in a renal cell carcinoma trial, involving a relatively small number of patients, skin rashes correlated with longer cancer progression–free survival for panitumumab (Rowinski et al. 2004). Later trials appear to indicate that the two molecules have similar clinical activity. In a randomized, 2-arm (231 patients in the treatment arm) phase III trial in 2nd line, chemotherapy refractory, EGFR^{+}, metastatic colorectal carcinoma patients (Gibson et al. 2006), there was an 8% objective response rate, with 28% of the patients having stable disease (compared with a 0% response rate and 10% stable disease in the control cohort). This compares to the clinical responses seen for cetuximab monotherapy in a 346 patient phase II trial in a similar set of refractory, EGFR$^+$, metastatic colorectal carcinoma patients (Lenz et al. 2006). Approximately, 12% of the cetuximab-treated patients were classified as objective responders, and 32% as stable disease. Panitumumab was dosed at 6 mg kg^{-1} every 2 weeks while the chimeric cetuximab was first given at a (roughly) 50% higher loading dose (400 mg m^{-2}), followed by a similar weekly dose of 250 mg m^{-2}. The lower dosing schedule selected for panitumumab was a reflection of the longer clearance time for the fully human antibody; however, the terminal half life (7.5 days) is still shorter than is typically found for IgG molecules. This is presumably due to the large antigen sink provided by normal tissue expression of EGFR. Gibson et al. (2006) reported that no patients had detectable levels of anti-panitumumab antibodies after treatment, and that while 5% of the patients had low-grade infusion reactions, none had grade 3 or 4 reactions. In contrast, 7.5% of the cetuximab patients experienced hypersensitivity reactions with 1.7% having grade 3 or 4 reactions, despite the fact that most of those patients had been pretreated with antihistamines to prevent infusion reactions. Lenz et al. (2006) also reported that over 4% of the cetuximab-treated patients developed human antichimeric antibodies.

Because panitumumab is a human IgG2 antibody and, because IgG2 antibodies are poor mediators of Fc dependent cell killing, the activity of the drug may be a function of non-Fc mediated mechanisms. These could involve blockade of ligand-induced receptor signaling and/or altered signaling directed by MAb binding. This is consistent with the observation that the MAb is active in mouse xenograft models while a sibling human IgG2 antibody that does not block ligand binding has no activity (Yang et al. 2001). However, although IgG2 molecules do not show significant binding to human FcγRIII (CD16), they do bind to the common H131 variant of FcγRIIa (CD32A, Parren et al. 1992). This variant is also associated with clinical responses to rituximab (Weng and Levy 2003). It is, therefore, a formal possibility that in human patients some of the activity of the panitumumab is mediated through FcγRIIa in H131 individuals. Gibson et al. (2006) did not report any data on the FcγRIIa allotype of the patients that responded to panitumumab; however, if a positive correlation between the H131 allotype and clinical responses were found it might indicate that some of the activity of the MAb is Fc mediated. Because IgG1 is a more potent mediator of Fc-dependent activity, it might then follow that a human IgG1 variant of panitumumab could have improved activity. This theory could be tested in the near future as clinical data becomes available for a second EGFR binding MAb, zalutumumab, derived from transgenic mice (Bleeker et al. 2004; Lammerts et al. 2006). Zalutumumab is now in phase 3 testing for treatment of EGFR-positive squamous cell cancer of the head and neck. Preclinical studies of 2F8 show that like panitumumab, it is also more potent than m225 in mouse xenograft models (Bleeker et al. 2004). However, unlike panitumumab it is an IgG1 antibody and may function by eliciting Fc-mediated effector cell activity in addition to blocking ligand binding and normal receptor functioning. A comparison of the clinical activity of these two molecules may provide some insight into the role of Fc-receptor interaction on the efficacy and safety of these drugs.

5.2 MAbs in Phase 3 Clinical Testing

There are at least eight transgenic mouse-derived human MAbs in Phase 3 clinical trials (Table 1). These include zalutumumab, the EGFR binding MAb discussed earlier, two different antibodies directed against CTLA-4, and one each directed against CD20 and CD4 for treatment of cancer, two neutralizing MAbs directed against TNFα and the common subunit of IL-12 and IL-23 for inflammatory indications, and an antibody directed against RANKL for bone loss.

5.2.1 Denosomab

Denosomab is an antibody directed against RANKL, a TNF family member that stimulates the maturation and activation of osteoclasts, which mediate bone resorption. The drug is now in phase 3 clinical trials for treatment of bone loss in postmenopausal women and in cancer patients with treatment induced bone loss or skeletal disease caused by bone metastases. A single subcutaneous dose escalation, phase 1 study in osteoporotic patients showed dose-dependent and sustained activity (up to 6 months) in blocking bone resorption, with no reported serious drug-related adverse events (Bekker et al. 2004). Denosomab was found to have dose-dependent pharmacokinetics (PK), with a terminal half life of 32 days at the highest 3 $\mathrm{mg\,kg^{-1}}$ dose. A second single dose trial in patients with multiple myeloma or bone metastases from breast cancer showed decreased bone metabolism that persisted for the 84-day study follow-up period, and a mean half life of 46 days after a single 3 $\mathrm{mg\,kg^{-1}}$ dose (Body et al. 2006). These studies measured bone metabolism using urine concentrations of peptide products of collagen catabolism as an indirect measure. Another trial, in postmenopausal women, looked at 3-month and 6-month repeat dosing, and also directly measured bone density (McClung et al. 2006). Consistent with the reduction in bone metabolism seen in the single dose studies, bone density was found to be increased for the 12 months of the study, even for patients given only 60 mg of drug every 6 months. The adverse event profile for the treatment group was not significantly different from that of the placebo group, and only 2 of the 314 treated patients showed transient levels of anti-denosomab antibodies in single blood samples, which were not confirmed in later blood samples. The low incidence of measurable antidrug antibodies, the safety profile, and the very long half-life and sustained drug activity are all consistent with an antibody that is relatively nonimmunogenic. Because infrequent dosing may be very important for patient compliance for a parenterally delivered protein-based therapeutic that is directed at chronic indications such as osteoporosis, low immunogenicity could be a critical feature for the success of this product.

Table 1 Disclosed targets for transgenic mouse derived antibody drugs tested in human subjects

Target	Drug	Indication	Company (developer)	Company (technology)	Highest Development Stage
EGFR	panitumumab	Colorectal cancer and non-small cell lung cancer, renal cell carcinoma	Amgen	Abgenix	Launched
CD20	ofatumumab	Non-Hodgkin lymphoma	Genmab	Medarex	Phase 3
CD4	zanolimumab	Lymphoma	Genmab	Medarex	Phase 3
CTLA-4	ipilimumab	Melanoma and various other cancers	Medarex	Medarex	Phase 3
CTLA-4	CP-675206	Melanoma	Pfizer	Abgenix	Phase 3
EGFR	zalutumumab	Head and neck cancer	Genmab	Medarex	Phase 3
IL-12/IL-23 p40	CNTO 1275	Psoriasis and multiple sclerosis	Johnson & Johnson	Medarex	Phase 3
RANKL	denosomab	Osteoporosis and treatment-induced bone loss	Amgen	Abgenix	Phase 3
TNFα	golimumab	Inflammatory disease	Johnson & Johnson	Medarex	Phase 3
CD30	MDX-060	Lymphoma	Medarex	Medarex	Phase 2
Clostridium difficile toxins A and B	MDX-066/ MDX-1388[a]	Hospital acquired *C. difficile* associated diarrhea	MBL/Medarex	Medarex	Phase 2
IGF-1R	CP-751,871	Cancer	Pfizer	Abgenix	Phase 2
IL-15	AMG 714	Rheumatoid arthritis	Amgen/Genmab	Medarex	Phase 2
IGF-1R	CP-751,871	Cancer	Pfizer	Abgenix	Phase 2
IL-15	AMG 714	Rheumatoid arthritis	Amgen/Genmab	Medarex	Phase 2
PSMA	MDX-070	Prostate cancer	Medarex	Medarex	Phase 2

αv Integrins	CNTO 95	Solid tumors	Johnson & Johnson	Medarex	Phase 2
CTGF	FG-3019	Diabetic nephropathy and pulmonary fibrosis	Fibrogen	Medarex	Phase 1b
PDGF-D	CR002	Inflammatory kidney disease	Curagen	Abgenix	Phase 1b
CD89	MDX – 214^{b}	Solid tumors	Medarex	Medarex	Phase 1/2
Alpha Interferons	MDX-1103/MEDI-545	Lupus	Medimmune/Medarex	Medarex	Phase 1
Anthrax protective antigen	MDX-1303	*B. Anthracis* infection	Pharmathene/Medarex	Medarex	Phase 1
CCR5	CCR5 mAb	HIV Infection	Human Genome Sciences	Abgenix	Phase 1
CD30	MDX – 1401^{c}	Lymphoma	Medarex	Medarex	Phase 1
CD3ε	NI-0401	Autoimmune disease	NovImmune	Medarex	Phase 1
CD40	CP-870,893	Cancer	Pfizer	Abgenix	Phase 1
CD40	CHIR-12.12	Chronic lymphocytic leukemia	Novartis/Xoma	Abgenix	Phase 1
CDw137	BMS-66513	Cancer	Bristol-Myers Squibb	Medarex	Phase 1
CXCL10	MDX-1100	Ulcerative colitis	Medarex	Medarex	Phase 1
Dendritic cell mannose receptor	MDX – 1307^{d}	Human gonadotropin–positive cancers	Celldex	Medarex	Phase 1
HGF/SF	AMG 102	Solid Tumors	Amgen	Abgenix	Phase 1
IL-8	ABX-IL8	Psoriasis	Abgenix	Abgenix	Phase 1
Melanoma antigen glycoprotein NMB	CR011 – vcMMAE^{e}	Melanoma	Curagen	Abgenix	Phase 1

Table 1 (continued)

Target	Drug	Indication	Company (developer)	Company (technology)	Highest Development Stage
Muc18	ABX-MA1	Melanoma	Abgenix	Abgenix	Phase 1
Parathyroid hormone	ABX-PTH	Hyperparathyroidism	Amgen	Abgenix	Phase 1
PD-1	MDX-1106/ONO-4538	Cancer	Ono Pharmaceuticals/Medarex	Medarex	Phase 1
PDGFRα	IMC-3G3	Cancer	ImClone	Medarex	Phase 1
PSCA	AGS-PSCA/MK-4721	Prostate cancer	Agensys/Merck	Abgenix	Phase 1
TRAIL-R2	HGS-TR2J	Solid tumors	Human Genome Sciences	Kirin	Phase 1

[a] Combination of two different monoclonal antibodies directed against each of the two toxins

[b] Human antigen-binding fragment (Fab) fused to epidermal growth factor

[c] Nonfucosylated Fc variant of MDX-060

[d] Human Fab fused to βhCG

[e] Antibody-drug conjugate with the small molecule microtubule inhibitor MMAE

5.2.2 Ipilimumab and CP-675206

The two transgenic mouse-derived human antibodies directed against CTLA-4, ipilimumab, and CP-675206, also do not appear to elicit strong patient antidrug antibody responses, despite the fact that the mechanism of action for these drugs results in a very potent up-modulation of patient immune responses. CTLA-4 is a negative T cell signaling molecule that binds to the two ligands CD80 and CD86, both of which are also recognized by the positive T cell signaling molecule CD28 (Korman et al. 2006). Ipilimumab (Keler et al. 2003) is a human IgG1 antibody, while CP-675206 (Ribas et al. 2005) is an IgG2 antibody. Both molecules bind to human CTLA-4 so as to block ligand binding and antagonize CTLA-4 signaling, resulting in the activation of certain T cell responses. Experiments with hamster MAbs that block mouse CTLA-4 show that the resulting enhanced immune responses can mediate tumor rejection in syngeneic mouse tumor models (Leach et al. 1996). Preclinical experiments in cynomolgus monkey models demonstrated that ipilimumab could stimulate humoral immune responses to coadministered vaccines (Keler et al. 2003). Clinical data in cancer patients has been reported for both ipilimumab (Phan et al. 2003; Hodi et al. 2003; Ribas et al. 2004; Attia et al. 2005; Blansfield et al. 2005; Maker et al. 2005, 2005b, 2006 Sanderson et al. 2005; Beck et al. 2006; Thompson et al. 2006) and CP-675206 (Ribas et al. 2004, 2005, Reuben et al. 2006). Objective and durable antitumor responses were observed for both drugs. Rosenberg and colleagues conducted a trial in patients with metastatic melanoma who were treated with ipilimumab at $3\,\mathrm{mg\,kg^{-1}}$ every 3 weeks for up to six cycles or were given a loading dose of ipilimumab at $3\,\mathrm{mg\,kg^{-1}}$ followed by $1\,\mathrm{mg\,kg^{-1}}$ every 3 weeks for up to six cycles. All patients were administered a subcutaneous gp100 peptide vaccine (Attia et al. 2005). The overall objective response rate for the 56 patients in the combined cohorts was 13%, with ongoing complete and partial responses reported at 25, 26, 30, 31, and 34 months. A follow-up paper by this group included additional metastatic melanoma patients treated with and without the vaccine, some receiving ipilimumab doses as high as $9\,\mathrm{mg\,kg^{-1}}$, together with 61 renal cell carcinoma patients treated with ipilimumab at up to $3\,\mathrm{mg\,kg^{-1}}$ (Beck et al. 2006). The overall objective response rate for the 198 patients in this report was 14%. This group also combined ipilimumab and high dose IL-2 in metastatic melanoma patients and reported a 22% objective response rate in patients administered ipilimumab at $3\,\mathrm{mg\,kg^{-1}}$. In a phase 1 single dose, monotherapy, dose escalation trial of CP-675206 in metastatic melanoma, with patients receiving doses as high as $15\,\mathrm{mg\,kg^{-1}}$, the authors reported a 10% objective response rate (Ribas et al. 2005), although one of the four responders had also received ipilimumab (Ribas et al. 2004). The serious adverse events reported for both ipilimumab and CP-675206 comprise a spectrum of immune-related inflammatory responses including rash, enterocolitis, and hypophysitis (Jaber et al. 2006; Blansfield et al. 2005; Ribas et al. 2005; Beck et al. 2006). However, because the mechanism of action of CTLA-4 blocking MAbs involves the activation of immune responses, these have been considered as target-related toxicities, and have in fact correlated with clinical responses (Beck et al. 2006, Reuben et al. 2006). Beck et al.

(2006) reported 36% and 35% objective response rates for melanoma and renal cell cancer patients having enterocolitis, with response rates of only 11% and 2% for patients without enterocolitis. The inflammatory adverse events have been reported to respond to medical management, which may include corticosteroids. Interestingly, corticosteroid treatment does not appear to abrogate objective tumor responses (Attia et al. 2005; Beck et al. 2006). Despite the observed up-regulation of immune responses in patients treated with these two MAbs, the drugs themselves do not appear to be readily recognized and cleared by the human immune system. A terminal half-life of 22 days was reported for CP-675206 (Ribas 2005), and 1-month post-dosing serum trough levels of 10 μg ml^{-1} ipilimumab were reported after 5 months of repeated monthly dosing at 3 mg kg^{-1} (Sanderson et al. 2005). Sanderson et al. (2005) also reported that these repeatedly dosed patients did not develop a measurable antibody response to ipilimumab. These data are consistent with the data from preclinical studies that showed no evidence of monkey anti-human antibody formation in cynomolgus macaques dosed five times over 140 days (Keler et al. 2003), despite the fact that the MAb upregulated the monkey humoral immune responses to coadministered vaccines. There was no sign of immune clearance by monkey anti-human antibodies, with drug titers never falling below 20 μg ml^{-1} over the course of the 5-month study.

5.2.3 CNTO 1275 and Golimumab

Another transgenic-derived human MAb in phase 3 development is CNTO 1275, which is directed against the common p40 subunit shared by IL-12 and IL-23. Results have been reported from a phase 1 trial in multiple sclerosis (Kasper et al. 2006) and from phase 1 and 2 trials in psoriasis (Kauffman et al. 2004, Toichi et al. 2006, Krueger et al. 2007). In the phase 1 psoriasis trial, the drug showed sustained activity over 16 weeks of follow-up with a single i.v. administration, with 67% of the patients achieving at least a 75% improvement (assessed by the Psoriasis Area and Severity Index). There were no treatment-related serious adverse events, and no infusion reactions. Antidrug antibodies were detected in 1 of 18 patients; however, presence of drug in the serum because of the very long terminal half life, 19–27 days, precluded accurate assessment in most of the patients. A similar 20–31 day terminal half-life was observed in the multiple sclerosis trial where the drug was given by subcutaneous administration. One of the 16 treated patients developed a detectable antidrug response; however, as with the psoriasis trial, the persistence of the drug in the serum made it difficult to accurately measure antidrug antibodies. In the phase 2 psoriasis trial, 237 patients received the drug for up to four weekly 90 mg subcutaneous doses. Antidrug antibodies were detected once or more in the 52 weeks of monitoring in 12 (4%) of the treated patients. However, the measured antibody response did not correlate with injection site reactions, which occurred at the same 2% frequency in both placebo and drug-treated cohorts. Patients given only a single subcutaneous dose, at either 45 or 90 mg, showed sustained disease-modifying responses for over 6 months following treatment. Together with the observed sustained clinical benefit, the approximately 20–30 day terminal half-life of CNTO 1275 appears to indicate that it does not elicit a strong drug-clearing antibody response. As further clinical data are reported, it will be interesting to compare the immunogenicity, PK, safety, and efficacy of the transgenic mouse-derived CNTO 1275 to the phage display derived ABT-874, which is also directed against the common p40 subunit of IL12 and IL-23 (Mannon et al. 2004, Fuss et al. 2006). The phage display antibody also showed some signs of immunogenicity, with antidrug antibodies detectable in 3 of 63 patients, and 2 of those patients showing evidence of early clearance of the drug from the serum (Mannon et al. 2004); however, because patients received up to 7 weekly doses of the drug, it is difficult to compare the data to that reported for CNTO 1275. The terminal half life of ABT-874 was not reported.

Data should also soon be available to compare a second pair of antibodies directed against a shared target but derived from the competing transgenic mouse and phage display technology platforms. Both golimumab, a human sequence antibody from transgenic mice is now in phase 3 clinical testing in rheumatoid arthritis, and adalimumab, a phage display derived antibody currently approved for the same indication, are high affinity TNFα blocking MAbs (Weinblatt et al. 2003). Both are also IgG1 molecules formulated for subcutaneous administration. This comparison may be of particular interest because adalimumab has been reported to elicit antidrug antibodies at a high frequency, despite the fact that it was genetically engineered from a lead molecule originally isolated from a phage display library constructed from human immunoglobulin sequences. The formation of these antidrug antibodies correlated with adverse events and reduced efficacy in a study of 15 rheumatoid arthritis patients (Bender et al. 2007). Another approved TNFα blocking antibody, infliximab, is a chimeric mouse–human antibody that also elicits a strong antidrug antibody response, which correlates with infusion reactions and reduced efficacy (Baert et al. 2003). Data from a third MAb derived from an alternative technology may provide some insight into the relative importance of factors such as drug target, patient population, and intrinsic immunogenicity on the efficacy and safety of antibody-based therapeutics.

5.2.4 Zanolimumab

Zanolimumab is a transgenic mouse-derived human antibody directed against the T cell antigen CD4 (Fishwild et al. 1999). Results from an 85 patient, placebo-controlled, phase 2 trial in psoriasis have been reported (Skov et al. 2003), and the drug is now in phase 3 clinical testing in cutaneous T cell lymphoma. In the published psoriasis study, there was an observed dose-dependent decrease in circulating CD4$^+$ cells, particularly in the CD45RO$^+$ memory T cell population. This may translate to efficacy in the cancer setting where the drug is currently being developed (Villadsen et al. 2007). The drug was well tolerated with one likely drug-related serious adverse event, a rash appearing after the second dose at 160 mg. No patients developed antidrug antibodies. In the published preclinical study (Fishwild et al. 1999), the antibody was found to be nonimmunogenic in chimpanzees; however, it did induce a blocking antibody response in a majority of the dosed cynomolgus monkeys (demonstrating that primate models may, in some cases, overestimate immunogenicity).

5.2.5 Ofatumumab

Ofatumumab (Teeling et al. 2004), a transgenic mouse-derived antibody directed at the B cell surface antigen CD20, is also in phase 3 clinical development. Although this antibody shares the same target as the mouse–human chimeric MAb rituximab, which is currently approved for treatment of non-Hodgkin's lymphoma (NHL) and rheumatoid arthritis (Coiffier et al. 1998, Cohen et al. 2006), it recognizes a distinct epitope and may, as a result, have a different mechanism of action (Teeling et al. 2006). Rituximab appears to recognize only one of the two extracellular loops of CD20, while the ofatumumab epitope comprises residues from both loops and the antibody is a more potent mediator of complement-dependent cytotoxicity in vitro than rituximab. This difference in potency is more pronounced at lower antigen density, and may translate into greater activity in low CD20 expressing lymphomas such as chronic lymphocytic leukemia (CLL), where the drug is currently being tested in phase 3 clinical trials. Because of the potential difference in mechanism of action and activity between ofatumumab and rituximab, a comparison of their relative safety and efficacy profiles may not be as useful for evaluating the potential of human vs. chimeric antibodies; however, the process for selecting ofatumumab as the lead clinical candidate (Teeling et al. 2004, Teeling et al. 2006) does highlight an important advantage of the transgenic mouse platform over other antibody drug discovery platforms. Unlike antibody engineering technologies for making low immunogenicity MAbs, where an early lead candidate is then modified or optimized in vitro to reduce immunogenicity, with the transgenic mouse platforms, the process of lead optimization is bypassed, making it possible to test each potential lead candidate in a series of increasingly sophisticated in vitro and in vivo assays in essentially the same molecular form as it will eventually be used in humans. Resources that would otherwise be devoted to optimization of a small number of lead hits can be devoted to better characterization of a larger number of lead candidates comprising a wider variety of functional properties.

5.3 MAbs in Phase 1 and 2 Clinical Testing

The available published scientific literature does not include the same level of detailed data on drug tolerability, PK, and efficacy for molecules that have not yet entered phase 3 clinical testing; however, published abstracts from scientific meetings and discussions in review articles does provide some information. A transgenic mouse-derived anti-CD30 MAb, MDX-060 (Borchmann et al. 2003; Heuck et al. 2004; Boll et al. 2005), has been tested in Hodgkin's lymphoma and anaplastic large cell lymphoma patients (Borchmann et al. 2004; Borchmann et al. 2005; Klimm et al. 2005). Fifty-six patients were reported to have been treated with up to 15 $\mathrm{mg\,kg^{-1}}$ every week for 4 weeks without significant infusion reactions. The preliminary results were interpreted to indicate that the drug was well tolerated and had clinical activity. Preclinical results have also been published for additional transgenic-derived MAbs now in clinical testing. These include antibodies directed against IL-15 (Villadsen et al. 2003), PSMA (Holmes 2001), *Clostridium difficile* toxins A and B (Babcock et al. 2006), CD40 (Tai et al. 2005), anthrax protective antigen (Vitale et al. 2006), hepatocyte growth factor (Burgess et al. 2006), melanoma antigen glycoprotein NMB (Tse et al. 2006), insulin-like growth factor receptor (Cohen et al. 2005), and αv integrins (Trikha et al. 2004; Martin et al. 2005).

6 Conclusions

Transgenic mice that express human antibody repertoires have proven to be useful for generating high-affinity human sequence MAbs against a wide variety of potential drug targets. The clinical experience with a variety of transgenic mouse derived fully human antibodies in human patients shows promising efficacy and safety profiles for several of these molecules. Furthermore, the overall experience to date is that the technology has succeeded in delivering human MAbs that demonstrate relatively low immunogenicity and have relatively long in vivo half lives. Twelve years after the first publications describing transgenic mice having disrupted endogenous immunoglobulin loci and expressing human heavy and light chain repertoires, the US regulatory approval of panitumumab provides commercial validation for this drug discovery platform. In addition, the variety and very large number of different clinical and preclinical programs involving human MAbs from transgenic mice suggest that the technology will continue to contribute new therapeutic drugs.

Acknowledgements I thank Michelle Temple for assistance with the manuscript.

References

Alt FW, Blackwell TK, Yancopoulos GD (1985) Immunoglobulin genes in transgenic mice. Trends Genet 1:231–236

Attia P, Phan GQ, Maker AV et al. (2005) Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 23:6043–6053

Babcock GJ, Broering TJ, Hernandez HJ et al. (2006) Human monoclonal antibodies directed against toxins A and B prevent Clostridium difficile-induced mortality in hamsters. Infect Immun 74:6339–6347

Baert F, Noman M, Vermeire S et al. (2003) Influence of immunogenicity on the long-term efficacy of imfliximab in Crohn's disease. N Engl J Med 348:601–608

Ball WJ, Kasturi R, Dey P et al. (1999) Isolation and characterization of human monoclonal antibodies to digoxin. J Immunol 163:2291–2298

Beck KE, Blansfield JA, Tran KQ et al. (2006) Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 24:2283–2289

Bekker PJ, Holloway D, Rasmussen S et al. (2004) A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. J Bone Miner Res 19:1059–1066

Bender NK, Heilig CE, Dröll B et al. (2007) Immunogenicity, efficacy and adverse events of adalimumab in RA patients. Rheumatol Int 27:269–274

Bieber MM, Bhat NM, Teng NN (1995) Anti-endotoxin human monoclonal antibody A6H4C5 (HA-1A) utilizes the VH4.21 gene. Clin Infect Dis 21(Suppl 2):S186–S189

Blansfield JA, Beck KE, Tran K et al. (2005) Cytotoxic T-lymphocyte-associated antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. J Immunother 28:593–598

Bleeker WK, Lammerts van Bueren JJ, van Ojik HH et al. (2004) Dual mode of action of a human anti-epidermal growth factor receptor monoclonal antibody for cancer therapy. J Immunol 173:4699–4707

Body JJ, Facon T, Coleman RE et al. (2006) A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. Clin Cancer Res 12:1221–1228

Boll B, Hansen H, Heuck F et al. (2005) The fully human anti-CD30 antibody 5F11 activates NF-{kappa}B and sensitizes lymphoma cells to bortezomib-induced apoptosis. Blood 106:1839–1842

Borchmann P, Treml JF, Hansen H et al. (2003) The human anti-CD30 antibody 5F11 shows in vitro and in vivo activity against malignant lymphoma. Blood 102:3737–3742

Borchmann P, Schnell R, Schulz H et al. (2004) Monoclonal antibody-based immunotherapy of Hodgkin's lymphoma. Curr Opin Investig Drugs 5:1262–1267

Borchmann P, Schnell R, Engert A (2005) Immunotherapy of Hodgkin's lymphoma. Eur J Haematol 75(Suppl 66):159–165

Brinster RL, Chen HY, Trumbauer M et al. (1981) Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. Cell 27:223–231

Brinster RL, Ritchie KA, Hammer RE et al. (1983) Expression of a microinjected immunoglobulin gene in the spleen of transgenic mice. Nature 306:332–336

Bruggemann M, Caskey HM, Teale C et al. (1989) A repertoire of monoclonal antibodies with human heavy chains from transgenic mice. Proc Natl Acad Sci USA 86:6709–6713

Brun-Buisson C (1994) The HA-1A saga: the scientific and ethical dilemma of innovative and costly therapies. Intensive Care Med 20:314–316

Bucchini D, Reynaud CA, Ripoche MA et al. (1987) Rearrangement of a chicken immunoglobulin gene occurs in the lymphoid lineage of transgenic mice. Nature 326:409–411

Burgess T, Coxon A, Meyer S et al. (2006) Fully human monoclonal antibodies to hepatocyte growth factor with therapeutic potential against hepatocyte growth factor/c-Met-dependent human tumors. Cancer Res 66:1721–1729

Buttin G (1987) Exogenous Ig gene rearrangement in transgenic mice: a new strategy for human monoclonal antibody production? Trends Genet 3:205–206

Calvo E, Rowinsky EK (2005) Clinical experience with monoclonal antibodies to epidermal growth factor receptor. Curr Oncol Rep 7:96–103

Chang Q, Zhong Z, Lees A et al. (2002) Structure-function relationships for human antibodies to pneumococcal capsular polysaccharide from transgenic mice with human immunoglobulin Loci. Infect Immun 70:4977–4986

Choi TK, Hollenbach PW, Pearson BE et al. (1993) Transgenic mice containing a human heavy chain immunoglobulin gene fragment cloned in a yeast artificial chromosome. Nat Genet 4:117–123

Cohen BD, Baker DA, Soderstrom C et al. (2005) Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751,871. Clin Cancer Res 11:2063–2073

Cohen SB, Emery P, Greenwald MW et al. (2006) Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum 54:2793–806

Cohenuram M, Saif MW (2007) Panitumumab the first fully human monoclonal antibody: from the bench to the clinic. Anticancer Drugs 18:7–15

Coiffier B, Haioun C, Ketterer N et al. (1998) Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood 92:1927–32

Costantini F, Lacy E (1981) Introduction of a rabbit beta-globin gene into the mouse germ line. Nature 294:92–94

Coughlin M, Lou G, Martinez O et al. (2006) Generation and characterization of human monoclonal neutralizing antibodies with distinct binding and sequence features against SARS coronavirus using XenoMouse((R)). Virology 361:93–102

Cunningham D, Humblet Y, Siena S et al. (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351:337–345

Davies NP, Rosewell IR, Richardson JC et al. (1993) Creation of mice expressing human antibody light chains by introduction of a yeast artificial chromosome containing the core region of the human immunoglobulin kappa locus. Nat Bio 11:911–914

Davis MM (2004) The evolutionary and structural ‘logic’ of antigen receptor diversity. Semin Immunol 16:239–243

Edgington SM (1992) What went wrong with Centoxin? Nat Bio 10:617–619

Farr CD, Tabet MR, Ball WJ et al. (2002) Three-dimensional quantitative structure-activity relationship analysis of ligand binding to human sequence antidigoxin monoclonal antibodies using comparative molecular field analysis. J Med Chem 45:3257–3270

Fishwild D, O’Donnell SL, Bengoechea T et al. (1996) High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice. Nat Bio 14:845–851

Fishwild D, Hudson DV, Deshpande U et al. (1999) Differential effects of administration of a human anti-CD4 monoclonal antibody, HM6G, in nonhuman primates. Clin Immunol 92:138–152

Foon KA, Yang XD, Weiner LM et al. (2004) Preclinical and clinical evaluations of ABX-EGF, a fully human anti-epidermal growth factor receptor antibody. Int J Radiat Oncol Biol Phys 58:984–990

Foote J, Eisen H (1995) Kinetic and affinity limits on antibodies produced during immune responses. Proc Natl Acad Sci USA 92:1254–1256

Fuss IJ, Becker C, Yang Z et al. (2006) Both IL-12p70 and IL-23 are synthesizedduring active Crohn’s disease and are down-regulated by treatment with anti-IL-12 p40 monoclonal antibody. Inflamm Bowel Dis 12:9–15

Garambois V, Glaussel F, Foulquier E et al. (2004) Fully human IgG and IgM antibodies directed against the carcinoembryonic antigen (CEA) Gold 4 epitope and designed for radioimmunotherapy (RIT) of colorectal cancers. BMC Cancer 4:75

Gibson TB, Ranganathan A, Grothey A (2006) Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. Clin Colorectal Cancer 6:29–31

Goldstein G et al. (1985) A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. Ortho Multicenter Transplant Study Group. N Engl J Med 6:337–342

Goodhardt M, Cavelier P, Akimenko MA et al. (1987) Rearrangement and expression of rabbit immunoglobulin kappa light chain gene in transgenic mice. Proc Natl Acad Sci USA 84:4229–4233

Gordon JW, Ruddle FH (1981) Integration and stable germ line transmission of genes injected into mouse pronuclei. Science 214:1244–1246

Green LL, Jakobovits A (1998) Regulation of B cell development by variable gene complexity in mice reconstituted with human immunoglobulin yeast artificial chromosomes. J Exp Med 188:483–495

Green LL, Hardy MC, Maynard-Currie CE et al. (1994) Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. Nat Genet 7:13–21

Greenough TC, Babcock GJ, Roberts A et al. (2005) Development and characterization of a severe acute respiratory syndrome-associated coronavirus-neutralizing human monoclonal antibody that provides effective immunoprophylaxis in mice. J Infect Dis 191:507–514

Harbers K, Jahner D, Jaenisch R (1981) Microinjection of cloned retroviral genomes into mouse zygotes: integration and expression in the animal. Nature 293:540–542

Harding FA, Lonberg N (1995) Class switching in human immunoglobulin transgenic mice. Ann NY Acad Sci 764:536–546

He Y, Honnen WJ, Krachmarov CP et al. (2002) Efficient isolation of novel human monoclonal antibodies with neutralizing activity against HIV-1 from transgenic mice expressing human Ig loci. J Immunol 169:595–605

Helmerhorst EJ, Maaskant JJ, Appelmelk MJ (1998) Anti-lipid A monoclonal antibody centoxin (HA-1A) binds to a wide variety of hydrophobic ligands. Infect Immun 66:870–873

Heuck F, Ellermann J, Borchmann P et al. (2004) Combination of the human anti-CD30 antibody 5F11 with cytostatic drugs enhances its antitumor activity against Hodgkin and anaplastic large cell lymphoma cell lines. J Immunother 27:347–353

Hodi FS, Mihm MC, Soiffer RJ et al. (2003) Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci USA 100:4712–4717

Holmes EH (2001) PSMA specific antibodies and their diagnostic and therapeutic use. Expert Opin Investig Drugs 10:511–519

Houghton AN, Brooks H, Cote RJ et al. (1983) Detection of cell surface and intracellular antigens by human monoclonal antibodies. Hybrid cell lines derived from lymphocytes of patients with malignant melanoma. J Exp Med 158:53–65

Huang S, Mills L, Mian B et al. (2002) Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. Am J Pathol 161:125–134

Ignatovitch O, Tomlinson IM, Jones PT et al. (1997) The creation of diversity in the human immunoglobulin V(lambda) repertoire. J Mol Biol 268:69–77

Imakiire T, Kuroki M, Shibaguchi H et al. (2004) Generation, immunologic characterization and antitumor effects of human monoclonal antibodies for carcinoembryonic antigen. Int J Cancer 108:564–570

Ishida I, Tomizuka K, Yoshida H et al. (2002) Production of human monoclonal and polyclonal antibodies in TransChromo animals. Cloning Stem Cells 4:91–102

Jaber SH, Cowen EW, Haworth LR et al. (2006) Skin Reactions in a Subset of Patients With Stage IV Melanoma Treated With Anti–Cytotoxic T-Lymphocyte Antigen 4 Monoclonal Antibody as a Single Agent. Arch Dermatol 142:166–172

Jakobovits A, Moore AL, Green LL et al. (1993) Germ-line transmission and expression of a human-derived yeast artificial chromosome. Nature 362:255–258

James K, Bell GT (1987) Human monoclonal antibody production. Current status and future prospects. J Immunol Methods 100:5–40

Jones PT, Dear PH, Foote J et al. (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 321:522–525

Kauffman CL, Araia N, Toichi E et al. (2004) A phase I study evaluating the safety, pharmacokinetics, and clinical response of a human IL-12 p40 antibody in subjects with plaque psoriasis. J Invest Dermatol 123:1037–1044

Kasper LH, Everitt D, Leist TP (2006) A phase I trial of an interleukin-12/23 monoclonal antibody in relapsing multiple sclerosis. Curr Med Res Opin 22:1671–1678

Keler T, Halk E, Vitale L et al. (2003) Activity and safety of CTLA-4 blockade combined with vaccines in cynomolgus macaques. J Immunol 171:6251–6259

Klimm B, Schnell R, Diehl V et al. (2005) Current treatment and immunotherapy of Hodgkin's lymphoma. Haematologica 90:1680–1692

Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497

Korman AJ, Peggs KS, Allison JP (2006) Checkpoint blockade in cancer immunotherapy. Adv Immunol 90:297–339

Krueger GG, Langley RG, Leonardi C et al. (2007) A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. N Engl J Med 356:580–592

Kuroiwa Y, Tomizuka K, Shinohara T et al. (2000) Manipulation of human minichromosomes to carry greater than megabase-sized chromosome inserts. Nat Biotechnol 18:1086–1090

Kuroki M, Yamada H, Shibaguchi H et al. (2005) Preparation of human IgG and IgM monoclonal antibodies for MK-1/Ep-CAM by using human immunoglobulin gene-transferred mouse and gene cloning of their variable regions. Anticancer Res 25:3733–3739

Kuus-Reichel K, Grauer LS, Karavodin LM et al. (1994) Will immunogenicity limit the use, efficacy, and future development of therapeutic monoclonal antibodies? Clin Diagn Lab Immunol 1:365–372

Lammerts van Bueren JJ, Bleeker WK, Bogh HO et al. (2006) Effect of target dynamics on pharmacokinetics of a novel therapeutic antibody against the epidermal growth factor receptor: implications for the mechanisms of action. Cancer Res 66:7630–7638

Larrick JW, Bourla JM (1986) Prospects for the therapeutic use of human monoclonal antibodies. J Biol Response Mod 5:379–393

Leach DR, Krummel, MF, Allison JP (1996) Enhancement of antitumor immunity by CTLA-4 blockade. Science 271:1734–1736

Lenz H-J, Van Cutsem E, Khambata-Ford S et al. (2006) Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irenotecan, oxaloplatin, and fluoropyrimidines. J Clin Onc 24:4914–4921

Lonberg N (2005) Human antibodies from transgenic animals. Nat Biotechnol 23:1117–1125

Lonberg N, Huszar D (1995) Human antibodies from transgenic mice. Int Rev Immunol 13:65–93

Lonberg N, Taylor LD, Harding FA et al. (1994) Antigen-specific human antibodies from mice comprising four distinct genetic modifications. Nature 368:856–859

Ma D, Hopf CE, Malewicz, AD et al. (2006) Potent antitumor activity of an auristatin-conjugated, fully human monoclonal antibody to prostate-specific membrane antigen. Clin Cancer Res 12:2591–2596

Maitta RW (2004) Protective and nonprotective human immunoglobulin M monoclonal antibodies to Cryptococcus neoformans glucuronoxylomannan manifest different specificities and gene use profiles. Infect Immun 72:4810–4818

Maker AV, Phan GQ, Attia P et al. (2005) Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. Ann Surg Oncol 12:1005–1016

Maker AV, Attia P, Rosenberg SA (2005b) Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. J Immunol 175: 7746–7754

Maker AV, Yang JC, Sherry RM et al. (2006) Intrapatient dose escalation of anti-CTLA-4 antibody in patients with metastatic melanoma. J Immunother 29:455–463

Mannon PJ, Fuss IJ, Mayer L et al. (2004) Anti-interleukin-12 antibody for active Crohn's disease. N Engl J Med 351:2069–2079

Mansour SL, Thomas KR, Capecchi MR (1988) Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. Nature 336:348–352

Martin PL, Jiao Q, Cornacoff J, Hall W et al. (2005) Absence of adverse effects in cynomolgus macaques treated with CNTO 95, a fully human anti-alphav integrin monoclonal antibody, despite widespread tissue binding. Clin Cancer Res 11:6959–6965

McCafferty J, Griffiths AD, Winter G et al. (1990) Phage antibodies: filamentous phage displaying antibody variable domains. Nature 348:552–554

McCloskey RV, Straube RC, Sanders et al. (1994) Treatment of septic shock with human monoclonal antibody HA-1A. A randomized, double-blind, placebo-controlled trial. CHESS Trial Study Group. Ann Intern Med 121:1–5

McClung MR, Lewiecki EM, Cohen SB et al. (2006) Denosumab in postmenopausal women with low bone mineral density. N Engl J Med 354:821–31

Melnikova VO, Bar-Eli M (2006) Bioimmunotherapy for melanoma using fully human antibodies targeting MCAM/MUC18 and IL-8. Pigment Cell Res 19:395–405

Mendez MJ, Green LL, Corvalan JRF et al. (1997) Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. Nat Genet 15:146–156

Mian BM, Dinney CPN, Bermejo CE et al. (2003) Fully human anti-interleukin 8 antibody inhibits tumor growth in orthotopic bladder cancer xenografts via down-regulation of matrix metalloproteases and nuclear factor-kappaB. Clin Cancer Res 9:3167–3175

Mori E, Thomas M, Motoki K et al. (2004) Human normal hepatocytes are susceptible to apoptosis signal mediated by both TRAIL-R1 and TRAIL-R2. Cell Death Differ 11:203–207

Morrison SL, Johnson MJ, Herzenberg LA et al. (1984) Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci USA 81:6851–6855

Mukherjee J, Chios K, Fishwild D et al. (2002) Human Stx2-specific monoclonal antibodies prevent systemic complications of Escherichia coli O157:H7 infection. Infect Immun 70:612–619

Mukherjee J, Chios K, Fishwild D et al. (2002b) Production and characterization of protective human antibodies against Shiga toxin 1. Infect Immun 70:5896–5899

Nagy A, Gerstenstein M, Vinterstein K et al. (2003) Manipulating the Mouse Embryo, A Laboratory Manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Nicholson IC, Zou X, Popov AV et al. (1999) Antibody repertoires of four- and five-feature translocus mice carrying human immunoglobulin heavy chain and kappa and lambda light chain yeast artificial chromosomes. J Immunol 163:6898–6906

Nozawa S, Aoki D, Tsukazaki K et al. (2004) HMMC-1: a humanized monoclonal antibody with therapeutic potential against Mullerian duct-related carcinomas. Clin Cancer Res 10: 7071–7078

Olsson L, Andreasen RB, Ost A et al. (1984) Antibody producing human-human hybridomas. II. Derivation and characterization of an antibody specific for human leukemia cells. J Exp Med 159:537–550

Ostendorf T, van Roeyen CRC, Peterson JD et al. (2003) A fully human monoclonal antibody (CR002) identifies PDGF-D as a novel mediator of mesangioproliferative glomerulonephritis. J Am Soc Nephrol 14:2237–2247

Parren PW, Warmerdam PA, Boeije LC et al. (1992) On the interaction of IgG subclasses with the low affinity Fc gamma RIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. J Clin Invest 90:1537–1546

Parry R, Schneider D Hudson D et al. (2005) Identification of a novel prostate tumor target, mindin/RG-1, for antibody-based radiotherapy of prostate cancer. Cancer Res 65:8397–8405

Pendley C, Schantz A, Wagner C (2003) Immunogenicity of therapeutic monoclonal antibodies. Curr Opin Mol Ther 5:172–179

Phan GQ, Yang JC, Sherry RM et al. (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci USA 100:8372–8377

Popov AV, Zou X, Xian J et al. (1999) A human immunoglobulin lambda locus is similarly well expressed in mice and humans. J Exp Med 189:1611–1620

Ramakrishna V, Treml JF, Vitale L et al. (2004) Mannose receptor targeting of tumor antigen pmel17 to human dendritic cells directs anti-melanoma T cell responses via multiple HLA molecules. J Immunol 172:2845–2852

Rathanaswami P, Roalstad S, Roskos L et al. (2005) Demonstration of an in vivo generated subpicomolar affinity fully human monoclonal antibody to interleukin-8. Biochem Biophys Res Commun 334:1004–1013

Reuben JM, Lee BN, Li C et al. (2006) Biologic and immunomodulatory events after CTLA-4 blockade with ticilimumab in patients with advanced malignant elanoma. Cancer 106: 2437–2444

Ribas A, Camacho LH, Lopez-Berestein G et al. (2005) Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675206. J Clin Oncol 23:8968–8977

Ribas A, Glaspy JA, Lee Y et al. (2004) Role of dendritic cell phenotype, determinant spreading, and negative costimulatory blockade in dendritic cell-based melanoma immunotherapy. J Immunother 27:354–367

Roost HP, Bachmann MF, Haag A et al. (1995) Early high-affinity neutralizing anti-viral IgG responses without further overall improvements of affinity. Proc Natl Acad Sci USA 92: 1257–1261

Rowinski EK, Schwartz GH, Gollob JA et al. (2004) Safety, pharmacokinetics, and activity of ABX-EGF, a fully human anti-epidermal growth factor receptor monoclonal antibody in patients with metastatic renal cell cancer. J Clin Oncol 22:3003–3015

Sanderson K, Scotland R, Lee P et al. (2005) Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and Montanide ISA 51 for patients with resected stages III and IV melanoma. J Clin Oncol 23:741–750

Schedl A, Larin Z, Montoliu L et al. (1993) A method for the generation of YAC transgenic mice by pronuclear microinjection. Nucleic Acids Res 21:4783–4787

Schuler W, Bigaud M, Brinkmann V et al. (2004) Efficacy and safety of ABI793, a novel human anti-human CD154 monoclonal antibody, in cynomolgus monkey renal allotransplantation. Transplantation 77:717–726

Schwartzberg PL, Goff SP, Robertson EJ (1989) Germ-line transmission of a c-abl mutation produced by targeted gene disruption in ES cells. Science 246:799–803

Sheoran AS, Chapman-Bonofiglio S, Harvey BR et al. (2005) Human antibody against shiga toxin 2 administered to piglets after the onset of diarrhea due to Escherichia coli O157:H7 prevents fatal systemic complications. Infect Immun 73:4607–4613

Simoons ML, de Boer MJ, van den Brand MJ et al. (1994) Randomized trial of a GPIIb/IIIa platelet receptor blocker in refractory unstable angina. European Cooperative Study Group. Circulation 89:596–603

Skov L, Kragbelle K, Zachariae C et al. (2003) HuMax-CD4: a fully human monoclonal anti-CD4 antibody for the treatment of psoriasis vulgaris. Arch Dermatol 139:1433–1439

Spalding BJ (1992) FDA setback flattens Centocor. Nat Bio 10:616

Strauss WM, Dausman J, Beard C et al. (1993) Germ line transmission of a yeast artificial chromosome spanning the murine alpha 1(I) collagen locus. Science 259:1904–1907

Suarez E, Yáñez R, Barrios Y, Díaz-Espada F (2004) Human monoclonal antibodies produced in transgenic BABkappa,lambda mice recognising idiotypic immunoglobulins of human lymphoma cells. Mol Immunol 41:519–526

Suzuki N, Aoki D, Tamada Y et al. (2004) HMOCC-1, a human monoclonal antibody that inhibits adhesion of ovarian cancer cells to human mesothelial cells. Gynecol Oncol 95:290–298

Tai YT, Li X, Tong X et al. (2005) Human anti-CD40 antagonist antibody triggers significant antitumor activity against human multiple myeloma. Cancer Res 65:5898–5906

Taylor LD, Carmack CE, Schramm SR et al. (1992) A transgenic mouse that expresses a diversity of human sequence heavy and light chain immunoglobulins. Nucleic Acids Res 20:6287–6295

Taylor LD, Carmack CE, Huszar D et al. (1994) Human immunoglobulin transgenes undergo rearrangement, somatic mutation and class switching in mice that lack endogenous IgM. Int Immunol 6:579–591

Teeling JL, French RR, Cragg MS et al. (2004) Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. Blood 104: 1793–1800

Teeling JL, Mackus WJ, Wiegman LJJM et al. (2006) The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol 177:362–371

Thompson RH, Allison JP, Kwon ED (2006) Anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) immunotherapy for the treatment of prostate cancer. Urol Oncol 24:442–447

Toichi E, Torres G, McCormick TS (2006) An anti-IL-12p40 antibody down-regulates type 1 cytokines, chemokines, and IL-12/IL-23 in psoriasis. J Immunol 177:4917–4926

Tomizuka K, Yoshida H, Uejima H et al. (1997) Functional expression and germline transmission of a human chromosome fragment in chimaeric mice. Nat Genet 16:133–143

Tomizuka K, Shinohara T, Yoshida H et al. (2000) Double trans-chromosomic mice: maintenance of two individual human chromosome fragments containing Ig heavy and kappa loci and expression of fully human antibodies. Proc Natl Acad Sci USA 97:722–727

Tomlinson IM, Walterb G, Jonesc PT et al. (1996) The imprint of somatic hypermutation on the repertoire of human germline V genes. J Mol Biol 256:813–817

Trikha M, Zhou Z, Nemeth JA et al. (2004) CNTO 95, a fully human monoclonal antibody that inhibits alpha v integrins, has antitumor and antiangiogenic activity in vivo. Int J Cancer 110:326–335

Tse KF, Jeffers M, Pollack VA et al. (2006) CR011, a fully human monoclonal antibody-auristatin E conjugate, for the treatment of melanoma. Clin Cancer Res 12:1373–1382

Tzipori S, Sheoran A, Akiyoshi D et al. (2004) Antibody therapy in the management of shiga toxin-induced hemolytic uremic syndrome. Clin Microbiol Rev 17:926–941

van Royen-Kerkhof A, Sanders EA, Walraven V et al. (2005) A novel human CD32 mAb blocks experimental immune haemolytic anaemia in FcgammaRIIA transgenic mice. Br J Haematol 130:130–137

Villadsen LS, Schuurman J, Beurskens F et al. (2003) Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model. J Clin Invest 112:1571–1580

Villadsen LS, Skov L, Dam TN et al. (2007) In situ depletion of CD4$^+$ T cells in human skin by Zanolimumab. Arch Dermatol Res 298:449–455

Vitale L, Blanset D, Lowy I et al. (2006) Prophylaxis and therapy of inhalational anthrax by a novel monoclonal antibody to protective antigen that mimics vaccine-induced immunity. Infect Immun 74:5840–5847

Wagner EF, Stewart TA, Mintz B (1981a) The human beta-globin gene and a functional viral thymidine kinase gene in developing mice. Proc Natl Acad Sci USA 78:5016–5020

Wagner TE, Hoppe PC, Jollick JD et al. (1981b) Microinjection of a rabbit beta-globin gene into zygotes and its subsequent expression in adult mice and their offspring. Proc Natl Acad Sci USA 78:6376–6380

Wang Y, Hailey J, Williams D et al. (2005) Inhibition of insulin-like growth factor-I receptor (IGF-IR) signaling and tumor cell growth by a fully human neutralizing anti-IGF-IR antibody. Mol Cancer Ther 4:1214–1221

Weinblatt ME, Keystone EC, Furst DE et al. (2003) Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 48:35–45

Weng WK, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 21:3940–3947

Wu Y, Zhong Z, Huber J et al. (2006) Anti-vascular endothelial growth factor receptor-1 antagonist antibody as a therapeutic agent for cancer. Clin Cancer Res 12:6573–6584

Xu JL, Davis, MM (2000) Diversity in the CDR3 region of V(H) is sufficient for most antibody specificities. Immunity 13:37–45

Yamamura K-I, Kudo A, Ebihara T et al. (1986) Cell-type-specific and regulated expression of a rearranged human gamma1 heavy-chain immunoglobulin gene in transgenic mice. Proc Natl Acad Sci USA 83:2152–2156

Yang X-D, Jia X-C, Corvalan JR et al. (1999) Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. Cancer Res 59:1236–1243

Yang X-D, Corvalen JR, Wang P et al. (1999b) Fully human anti-interleukin-8 monoclonal antibodies: potential therapeutics for the treatment of inflammatory disease states. J Leukoc Biol 66:401–410

Yang X-D, Jia X-C, Corvalan JR et al. (2001) Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. Crit Rev Oncol Hematol 38:17–23

Zijlstra M, Li E, Sajjadi F et al. (1989) Germ-line transmission of a disrupted beta 2-microglobulin gene produced by homologous recombination in embryonic stem cells. Nature 342:435–438

Zou YR, Muller W, Gu H et al. (1994) Cre-loxP-mediated gene replacement: a mouse strain producing humanized antibodies. Curr Biol 4:1099–1103