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Human antibodies from transgenic animals

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Laboratory mice provide a ready source of diverse, high-affinity and high-specificity monoclonal antibodies (mAbs). However, development of rodent antibodies as therapeutic agents has been impaired by the inherent immunogenicity of these molecules. One technology that has been explored to generate low immunogenicity mAbs for *in vivo* therapy involves the use of transgenic mice expressing repertoires of human antibody gene sequences. This technology has now been exploited by over a dozen different pharmaceutical and biotechnology companies toward developing new therapeutic mAbs, and currently at least 33 different drugs in clinical testing—including several in pivotal trials—contain variable regions encoded by human sequences from transgenic mice. The emerging data from these trials provide an early glimpse of the safety and efficacy issues for these molecules. Nevertheless, actual product approval, the biggest challenge so far, is required to fully validate this technology as a drug discovery tool. In the future, it may be possible to extend this technology beyond rodents and use transgenic farm animals to directly generate and produce human sequence polyclonal sera.

MAbs were among the first handful of approved therapeutic molecules generated by modern biotechnology. However, after the introduction in 1986 of muromonab-CD3 (Orthoclone OKT3; Ortho Biotech, Bridgewater, NJ, USA), a murine mAb targeting CD3 approved for treating acute organ transplant rejection, 8 years elapsed before the next therapeutic mAb was approved by the US Food and Drug Administration (FDA). One factor that contributed to this gap was the observed immunogenicity of mouse antibodies in human patients, which can lead to rapid clearance, reduced efficacy[1–3] and an increased risk of infusion reactions, which can range from relatively benign fevers and rashes to cardiopulmonary and anaphylactic-like adverse events[4]. Biotechnology and pharmaceutical companies have addressed this problem using the tools of molecular biology to generate lower immunogenicity antibody molecules. Mouse antibodies have been reengineered *in vitro* to replace framework amino acid residues with corresponding human sequences[5,6]. Libraries of human and synthetic immunoglobulin sequences have also been screened to identify novel, laboratory-derived antibodies[7].

There are now 17 therapeutic mAbs approved by the FDA (**Fig. 1**). All but three of these have been engineered to reduce immunogenicity, and include at least some human sequences. Human sequence mAbs provide a potential solution to the problem of immunogenicity associated with rodent-derived antibodies. However, early attempts to mine authentic human antibody repertoires from cancer or infectious-disease patients resulted primarily in relatively low-affinity or low-specificity IgM antibodies[8–11]. Although many of the technical difficulties associated with obtaining human mAbs directly from human B cells have been addressed by recent advances[12–14], the inherent tolerance of the human immune system to human antigens, together with the fact that human patients cannot be subjected to the types of immunization schemes used for the generation of rodent antibodies, limits our ability to access human B cell–derived antibodies to the broad spectrum of targets accessible for rodent antibodies[15].

In this review, I discuss the current status of drugs derived from an alternative technology for generating low immunogenicity therapeutic mAbs: transgenic mice comprising human immunoglobulin repertoires. Because the primary motivation for developing transgenic mouse platforms was the immunogenicity of rodent antibodies, this review focuses on immunogenicity data for human sequence antibodies in the clinic. The application of transgenic technology toward the generation of polyclonal antibody–based drugs is also discussed.

Transgenic mice with human immunoglobulin genes

Twenty years ago, Alt *et al.*[16] 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". In 1989, Bruggemann *et al.*[17] reported the expression of a repertoire of human heavy chains and the generation of a transgene-encoded immune response in mice[17]. This report and the invention of methods for introducing specific modifications into the mouse germ line[18–20] fueled the race to generate a mouse that comprised diverse human heavy- and light-chain repertoires capable of contributing to a true secondary immune response of high-affinity human mAbs, in the background of disrupted mouse heavy- and κ light-chain genes.

In 1994, two papers, one from my laboratory[21] and the other from Green *et al.*[22] at Cell Genesys (Foster City, CA, USA), 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)[21,22]. Although both papers report the use of homologous recombination in mouse embryonic stem (ES) cells to engineer similar disruptions of the endogenous mouse loci, different technologies were used to construct and deliver the human sequence transgenes. My group[21] used pronuclear microinjection to introduce reconstructed minilocus transgenes—the heavy chain containing 3 heavy-chain variable (V_H),

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16 diversity (D) and all 6 heavy-chain joining (J_H) regions together with μ and γ1 constant-region gene segments. In the transgenic strains, this construct underwent VDJ joining, together with somatic mutation and correlated class switching[23]. The light-chain transgene included four Vκ, all five Jκ and the κ constant region (Cκ). In contrast, Green *et al.*[22] 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 Vκ and all five Jκ segments, together with Cκ. Neither my group nor that of Green *et al.*[21] inactivated the endogenous λ-light-chain locus, which in typical laboratory mouse strains contributes to only ~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[22] and IgG[21] mAbs recognizing the target antigens against which the mice had been immunized.

The successful isolation of human mAbs specifically directed against a targeted antigen was surprising, given that each of these two mouse strains contained only a fraction of the natural human V repertoire. This raises the question of why mammals have apparently been selected for such large germline V repertoires. Large germline repertoires might be required for the expression of diverse antibody sequences at the six complementarity-determining regions (CDRs), which provide direct contact with targeted antigens. However, the expressed antibody repertoire is actually a product of three sources of diversity: combinatorial, junctional and somatic, only one of which—combinatorial—is provided by the germ line (**Fig. 2**). The ability to generate antibodies to a variety of targets, using minilocus transgenes having a limited subset of the intact human combinatorial diversity, may reflect the relative importance of these three sources of diversity. 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[24–26]. 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.

Xu and Davis[27] reported that a mouse having only a single human V_H gene and three mouse Vλ genes could mount a specific antibody response 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 authors failed to obtain a response to the T-independent antigen, dextran B512, and 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.

This notion is consistent with observed correlations between immunoglobulin gene polymorphisms and response to pathogens. For example, in humans the protective antibody response to *Haemophilus influenzae* type b polysaccharide is dominated by a single Vκ and a single V_H gene segment[28]. Certain isolated human populations, or individuals having specific immunoglobulin gene haplotypes, have higher susceptibility to *H. influenzae*[29,30] than most. The Navajo population, which has a five- to tenfold higher incidence of infection, also carries a defective allele of the light-chain V gene segment responsible for the dominant nonsomatically mutated antibody response to the type b capsular polysaccharide[28,31,32]. Nevertheless, type b polysaccharide vaccines have been developed that are 97% effective in this population[33], and neutralizing antibodies can be isolated from vaccinated individuals that do not use the dominant heavy- and light-chain V gene segments[34].

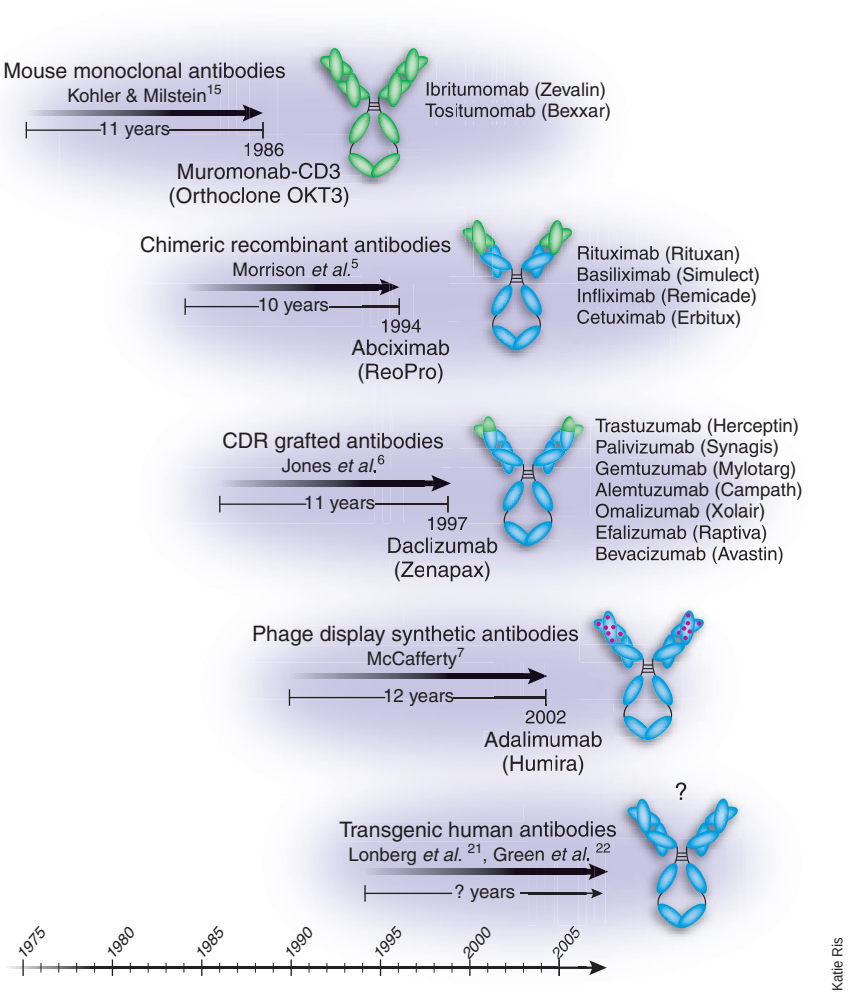

Figure 1 Evolution of therapeutic antibody technology and progress to the clinic[5–7,15,21,22]. In general, FDA-approved mAbs have emerged between 10 and 12 years after the date that the new technologies on which they were based were reported in the scientific literature.

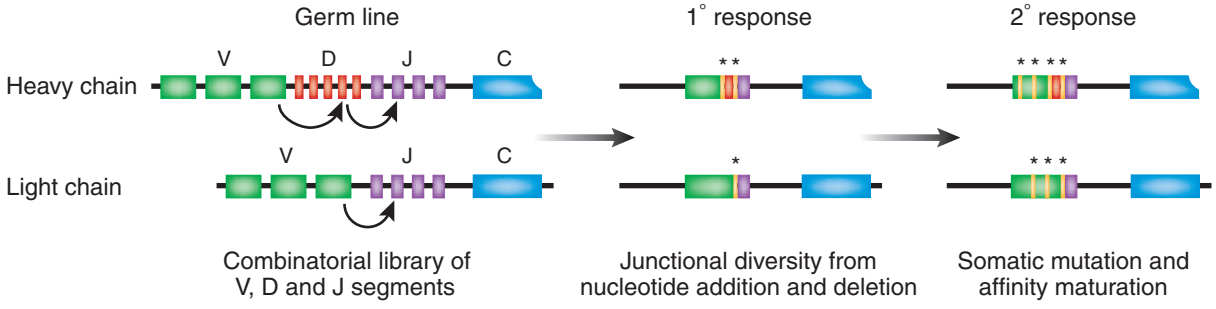

Figure 2 Three sources of diversity contribute to antibody repertoires: combinatorial, junctional and somatic. Antibody repertoire diversity is derived from the combinatorial diversity obtained by random combination of germline V, D and J segments, from the junctional diversity obtained by random addition (N-region addition) and deletion of nucleotides at the joints between these segments, and by somatic mutation of the entire variable region during T-cell-dependent secondary immune responses. Red bars below asterisks indicate nongermline-encoded amino acid residues.

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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.*[35] 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.*[36] 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.*[22] This paper, and a later analysis of the same mouse strains by Green and Jakobovits[37], 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.

There have now been multiple reports in the literature of transgenic mice that express immunoglobulin repertoires comprising human heavy- and light-chain sequences in a background having 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 one particular technological innovation: microcell-mediated chromosome transfer. In this approach, 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.

Using this technique, Tomizuka *et al.*[38] 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

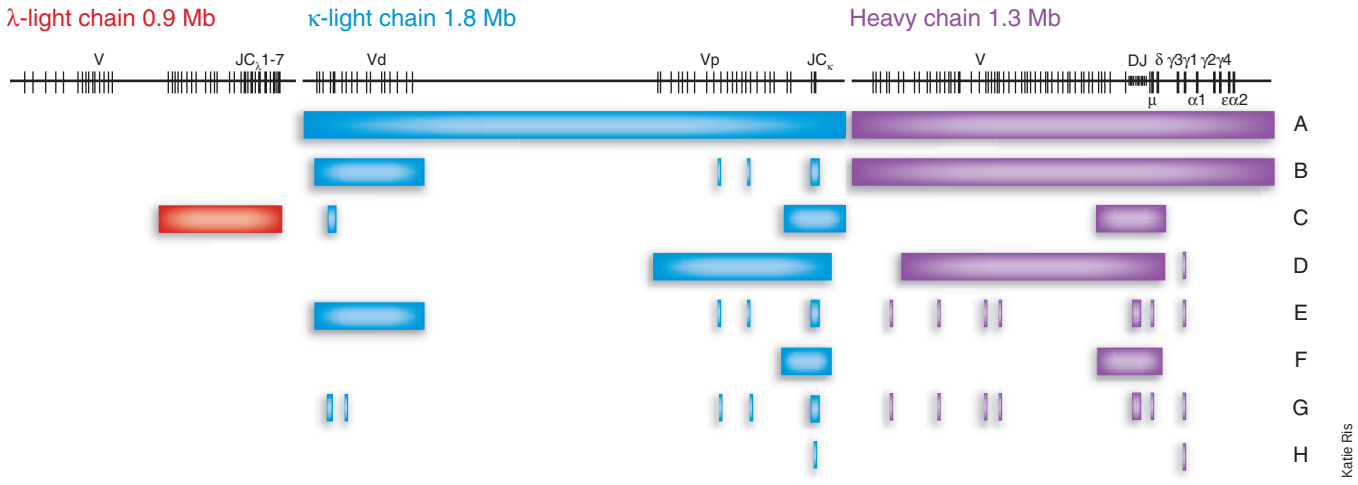

Figure 3 Human immunoglobulin sequences introduced in the germ line of mice comprising endogenous Ig heavy-chain and κ-light-chain gene inactivations (A^{39}, B^{55}, C^{42}, D^{36}, E^{35}, F^{22}, G^{21}, H^{43}). The germline configuration of the human immunoglobulin λ-light chain, κ-light chain and 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.

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background that included disruptions of the mouse heavy- and κ-light-chain loci[39]. 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.

This structure 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[40]. Bruggemann and colleagues[41] 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[42].

A conceptually analogous transgenic system that generates chimeric antibodies, rather than fully human sequence antibodies, was developed by Rajewsky and colleagues in 1994 (ref. 43). Mice generated using this approach contain relatively precise replacements of the mouse κ and γ1 constant-region gene segments with the corresponding human gene sequences[43]. 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.

In addition to the strains referred to in **Figure 3**, a review of the patent literature and of other public disclosures by companies pursuing commercial applications of human mAbs reveals that the published scientific literature represents only a subset of the transgenic mouse strains that have been created for generating human antibodies. Further modifications of the genome might also appear in future mouse strains containing human immunoglobulin genes. These include inactivation of the mouse λ-light-chain locus[44] and inactivation of the entire heavy-chain constant region[45] to eliminate trans-switching, which can otherwise lead to a population of B cells expressing 'reverse-chimeric' mAbs comprising human V regions and mouse constant regions[23]. In addition, because reverse-chimeric antibodies are also a useful source of affinity-selected human V genes, mice that have human V, D and J regions inserted directly upstream of the natural mouse constant regions could be engineered to produce only these reverse-chimeric mAbs.

The lesson from the analysis of B-cell subpopulations and of human immunoglobulin expression, and sequence analysis in various transgenic mice is that despite the fact that they express B-cell receptors that are essentially hybrids of mouse and human components (e.g., human immunoglobulin, mouse Igα, Igβ and other signaling molecules), 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 (even recapitulating the normal human pattern of somatic mutation hotspots[46]) to generate high-affinity mAbs to a variety of different antigens. Hybrid receptors might not function as efficiently as authentic mouse receptors, which might then contribute to altered humoral immune responses and differences in the spectrum of mAbs derived from transgenic mice compared with wild-type animals (or differences in the efficiency of the process of hybridoma generation).

The closest the scientific literature comes to addressing the issue of how human immunoglobulin transgenic mice compare to wild-type mice as a tool for generating mAbs is provided by the variety and properties of the derived mAbs themselves. In the 11 years since the first of these platforms was reported, a variety of different human sequence mAbs derived from transgenic mice have been described in the scientific literature. These have included mAbs against small molecules[47,48], pathogen-encoded protein[49–52] and polysaccharide antigens[53,54], human-secreted[36,55–62] and cell-surface[35,36,57,63–83] protein antigens, and human tumor–associated glycosylation variants[84]. A review of the overall binding characteristics of the IgG mAbs derived from these diverse transgenic platforms provides a perhaps surprising result: they generally fall into a relatively narrow range of affinities. Most have binding affinities in the 0.1–10 nM range, with examples of 10-pM range affinities coming from both minilocus V_H[56] and large-repertoire V_H[71] comprising strains. This is likely the result of screening assays that select for high affinity and specificity. It may also—in part—be due to the natural constraints on affinity maturation operating *in vivo*. The physical limit of large-molecule diffusion places a ceiling on antigen-antibody association rates, and germinal center B-cell selection may not be able to distinguish antigen dissociation rates longer than the half-life of the cell-surface B-cell receptor complex[85,86].

Clinical development of transgenic-derived human mAbs

At least 35 fully human mAbs have entered clinical development as therapeutics. Two of these, α–interleukin (IL)-8 (ABX-IL8 for psoriasis; Abgenix, Fremont, CA, USA) and αMuc18 (ABX-MA1 for melanoma; Abgenix, Fremont, CA, USA) have since dropped out of development, whereas 33 remain in clinical trials (**Table 1**). These molecules are targeted against a variety of clinical indications in cancer, autoimmune or inflammatory diseases, and infectious diseases.

Four antibodies, directed against epidermal growth factor receptor (EGFR), receptor activator of nuclear factor-κB ligand (RANKL; also called osteoprotogerin ligand, OPGL), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and CD4, are now in phase 3 development. Panitumumab (ABX-EGF), which targets EGFR and blocks ligand binding[69,71,72,87], was discovered by researchers at Abgenix, which has partnered with Amgen (Thousand Oaks, CA, USA) to develop the treatment for EGFR-positive cancers. In preclinical mouse xenograft models, this mAb was found to be more potent than the mouse antibody m225 (ref. 71), the parent of the already marketed chimeric anti-EGFR antibody, cetuximab[88] (Erbitux; Imclone Systems, Branchburg, NJ, USA). Panitumumab is a human IgG2 antibody and, because IgG2 interacts only weakly with the Fc receptors CD16 and CD32, presumably mediates inhibition of tumor cell growth through mechanisms other than antibody-dependent, cell-mediated cytotoxicity. These could involve blockade of ligand-induced receptor signaling and/or altered signaling directed by mAb binding. Initial clinical results from phase 1 and phase 1/2 studies can be compared to the clinical experience with cetuximab[69,87]. Consistent with panitumumab's lack of mouse sequences and higher affinity, the fully human mAb is nonimmunogenic and appears to require a lower dosing schedule than the chimeric antibody. At the selected dose level, panitumumab is 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[89], 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[69].

A second fully human mAb directed against EGFR, 2F8 (Genmab, Copenhagen), is now in phase 1/2 testing for treatment of EGFR-posi-

tive cancers. Preclinical studies of 2F8 show that like panitumumab, it is also more potent than m225 in mouse xenograft models[67]. Unlike panitumumab this second mAb is an IgG1 antibody and may function by eliciting antibody-dependent, cell-mediated cytotoxicity in addition to blocking ligand binding and normal receptor functioning. It will be interesting to compare the clinical progress of this molecule to panitumumab to gain some insight into the role of Fc receptor interaction on the efficacy of these drugs.

Another mAb in late-stage development, MDX-010[77,78,90–93], which was discovered by Medarex (Princeton, NJ, USA) and is being codeveloped by Bristol-Myers Squibb (New York), is now in phase 3 trials in melanoma patients. MDX-010 targets the T-cell inhibitory receptor, cytotoxic T-lymphocyte antigen 4 (CTLA-4), leading to enhanced immune responses. Experiments with hamster mAbs directed at mouse CTLA-4 show that the resulting enhanced immune responses can mediate tumor rejection in syngeneic mouse tumor models[94]. Preclinical experiments in cynomolgus monkey models demonstrated that MDX-010 could stimulate humoral immune responses to coadministered vaccines[77]. A published phase 1/2 study of MDX-010 in combination with a peptide-based vaccine in 14 stage-IV melanoma patients resulted in two complete responses and one partial response, all lasting longer than 11 months[90]. In addition to MDX-010, a second transgenic mouse–derived, anti-CTLA-4 mAb is now in clinical testing in melanoma patients (CP-675,206; Pfizer, New York)[91].

Table 1 mAb drugs currently in human clinical testing that include sequences derived from human immunoglobulin transgenic mice

Target	Indication	Company (developer)	Company (technology)	Clinical trial phase
EGFR	Colorectal cancer and non-small cell lung cancer, renal cell carcinoma	Amgen/Abgenix	Abgenix	2 and 3
CTLA-4	Melanoma and various other cancers	Medarex	Medarex	2 and 3
RANKL	Osteoporosis and treatment-induced bone loss	Amgen	Abgenix	3 and 3
CD4	Lymphoma	Genmab	Medarex	3
Interleukin-15	Rheumatoid arthritis	Amgen/Genmab	Medarex	2
CD30	Lymphoma	Medarex	Medarex	2
Tumor necrosis factor-α	Inflammatory disease	Johnson & Johnson (New Brunswick, NJ, USA)	Medarex	2
Interleukin-12	Psoriasis and multiple sclerosis	Johnson & Johnson	Medarex	2
Prostate-specific membrane antigen	Prostate cancer	Medarex	Medarex	2
CTLA-4	Melanoma	Pfizer	Abgenix	2
CD20	Non-Hodgkin lymphoma	Genmab	Medarex	2
EGFR	Head and neck cancer	Genmab	Medarex	1/2
Undisclosed	Psoriasis	Genmab/Medarex	Medarex	1/2
Undisclosed	Autoimmune disease	Novartis (Basel)	Medarex	1/2
Undisclosed	Autoimmune disease	Novartis	Medarex	1/2
αv Integrins	Solid tumors	Johnson & Johnson	Medarex	1
Parathyroid hormone	Hyperparathyroidism	Abgenix	Abgenix	1
Connective tissue growth factor	Pulmonary fibrosis	Fibrogen (S. San Francisco, CA, USA)	Medarex	1
Undisclosed	Undisclosed	Pfizer	Abgenix	1
Undisclosed	Undisclosed	Amgen	Medarex	1
CD89[a]	Solid tumors	Medarex	Medarex	1
Undisclosed	Undisclosed	Pfizer	Abgenix	1
Dendritic cell mannose receptor[b]	Human gonadotropin–positive cancers	Medarex	Medarex	1
TRAIL-R2	Solid tumors	Human Genome Sciences (Rockville, MD, USA)	Kirin	1
Undisclosed	Undisclosed	Amgen	Medarex	1
Clostridium difficile toxin A	*C. difficile* infection (a common hospital-acquired infection)	MBL/Medarex	Medarex	1
Undisclosed	Undisclosed	Eli Lilly (Indianapolis, IN, USA)	Medarex	1
Undisclosed	Undisclosed	Amgen	Abgenix	1
CD40	Chronic lymphocytic leukemia	Chiron (Emeryville, CA, USA)	Abgenix	1
CXCL10	Ulcerative colitis	Medarex	Medarex	1
Platelet-derived growth factor-D	Inflammatory kidney disease	Curagen (New Haven, CT, USA)	Abgenix	1
CC chemokine receptor 5	HIV infection	Human Genome Sciences	Abgenix	1
Undisclosed	Undisclosed	Amgen	Medarex	1

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

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Also in phase 3 development, AMG-162 (ref. 57) (Amgen, Thousand Oaks, CA, USA), is being tested in patients with osteoporosis and treatment-induced bone loss (TIBL). This mAb is directed against RANKL, a molecule involved in the regulation of bone remodeling. In a single-dose, placebo-controlled study in postmenopausal women, AMG-162 was found to have dose-dependent and sustained activity in blocking bone resorption, with no reported serious drug-related adverse events.

The most recent transgenic mouse–derived human mAb to enter late-stage clinical development is zanolimumab[63,64] (HuMax-CD4; Genmab, Copenhagen), which binds to the T cell–differentiation antigen CD4. A phase 3 clinical trial in patients with $CD4^+$ cutaneous T-cell lymphomas has been announced; however, the published clinical study, in psoriasis patients, was directed toward inflammatory and autoimmune indications. The observed dose-dependent decrease in circulating $CD4^+$ cells in this study may translate to efficacy for treatment of T-cell lymphomas, where the drug is currently being developed. At least six additional transgenic mouse–derived mAbs are in phase 2 clinical trials, including two directed against targets, CD20 and tumor necrosis factor α (TNFα), for which nontransgenic mouse–derived antibodies are already on the market[65]. Others described in the literature include antibodies to IL-15 (ref. 56), prostate-specific membrane antigen (PSMA)[73] and CD30 (refs. 74,75).

Immunogenicity of human mAbs from transgenic mice

A review of the available clinical data cited above gives us an opportunity to ask whether the transgenic mouse platforms have actually solved the problem of immunogenicity that originally motivated their development. Although transgenic mouse–derived human mAbs have yet to emerge from a phase 3 clinical study, thus providing data comparable to those available for approved products, the initial results are encouraging.

Foon *et al.*[87] did not detect any human polyclonal antibody responses to panitumumab in 88 treated patients. The observed low intrapatient variability of drug exposure and absence of significant infusion reactions, also point to very low immunogenicity for this molecule. The published study of AMG-162 in 49 patients also showed very low intrapatient variability in pharmacokinetics, with dose-dependent mean residence time increasing with dose from 12 to 46 days[57]. There was no evidence of patient immune reaction to the drug.

Since immunogenicity of a given mAb is likely to be related not only to inherent properties of the molecule, but also to the immune status of the patient, it may be important to look at data from trials involving patients with inflammatory or autoimmune diseases. In the zanolimumab psoriasis trial discussed above, none of the 85 patients, each of whom received four doses over 1 month, developed a measurable immune response to the human mAb[63]. Perhaps the most striking data from patients with elevated immune responses come from trials with αCTLA-4 mAbs. Antibodies that block CTLA-4 specifically upregulate immune responses and elicit autoimmunity in patients[78,90–93]. Remarkably, given the potent immune-stimulatory activity of these drugs, no immune responses to the transgenic mouse–derived human mAbs have been observed in human patients, or even in monkeys. Preclinical studies showed no evidence of monkey anti-human antibody formation in cynomolgus macaques dosed five times over 140 days[77], despite the fact that the mAb upregulated the monkey humoral immune responses to coadministered vaccines. There was no sign of immune clearance, with drug titers never falling below 20 μg/ml over the course of the 5-month study.

Similar data were seen in human clinical trials. In the melanoma trial discussed above, Phan *et al*. treated 14 patients with up to four doses over 9 weeks[90]. Pharmacokinetic data for this trial, and for a second trial involving 19 patients given up to eight doses over 1 year[78], showed accumulation on re-treatment and absence of patient antibody response to the mAb.

In contrast to the lack of observed patient immune responses to human mAbs from transgenic mice, many existing therapeutic mAbs have proven to be immunogenic in the clinic. Data from large numbers of patients collected during clinical studies show that 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[1]. It is of particular interest that both adalimumab (Humira; Abbott Laboratories, Abbott Park, IL, USA), a phage display–derived antibody directed against TNFα and another phage display antibody directed against IL-12 (ref. 95) are both immunogenic in human patients, despite the fact that they are derived from human rather than mouse sequences. Both drugs were immunogenic in patients with inflammatory diseases (rheumatoid arthritis and Crohn disease) and it is possible such responses are an inevitable consequence of dosing in this population, which is prone to autoimmunity. It is also possible that new T-cell epitopes were created from the amino acid changes introduced during the process of *in vitro* affinity maturation, which is used to optimize phage display–derived antibodies. Even natural human antibodies could be recognized as foreign by the human immune system if the rearrangements and somatic mutations that formed the genes for that particular molecule had created new T-cell epitopes from these nongermline–encoded sequences (**Fig. 2**).

Nevertheless, if the currently observed lack of immunogenicity for transgenic mouse–derived mAbs is confirmed by larger studies, it could reflect the contribution of factors other than sequence-encoded, T-cell epitopes. Factors such as instability and aggregation, which contribute to the immunogenicity of protein therapeutics[96] may be selected against during *in vivo* B-cell development and affinity maturation. Analysis of V-region usage in mouse and humans shows that antibody sequences are under heavy selective pressure at all stages of B-cell development, even before light-chain rearrangement[97,98]. There is also evidence that CDR3 sequences are selected for attributes that may be more important for properties such as stability and aggregation than for simple molecular recognition. A population analysis of heavy-chain CDR3 sequences reveals a distribution of calculated hydropathicities suggesting strong selection against extremely hydrophobic or hydrophilic CDR loops[99]. Analogous selective pressures could be missing from current laboratory protocols for antibody engineering through CDR grafting and phage display. Helms and Wetzel[100] demonstrated just how sensitive V-region stability is to small changes in CDR sequence. Alteration of CDR loop sequence dramatically reduced the overall stability of the entire variable region domain.

In addition to providing a platform for the discovery of low immunogenicity therapeutic mAbs, human immunoglobulin–producing transgenic mice may be advantageous over other technologies simply because of innate differences in the drug discovery processes dictated by the different systems. *In vitro* antibody engineering technologies for making low immunogenicity mAbs necessitate a process analogous to that used for small-molecule drug discovery: lead discovery followed by a potentially lengthy period of lead optimization. However, with transgenic mice it is possible to bypass the lead optimization step entirely because B-cell development and affinity maturation can generate *in vivo* optimized antibodies. This allows a process where each potential candidate is tested before lead selection 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.

With transgenic mouse platforms, selection decisions are based on data from preoptimized leads; however, for lead optimization–based

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processes, selection decisions must rely on data from un-optimized leads, data that are not always relevant to the properties of the final optimized compound. In addition, because clonal antibody–secreting cell lines are directly generated by the hybridoma fusion methods typically used for generating drug candidates from transgenic animals, the process is particularly well suited for screening protocols that involve a variety of different cell-free, *in vitro* cell-based and/or *in vivo* assays. At each screening point, hybridoma cell lines can be grown at the scale required to rapidly generate the required quantity of antibody. In contrast, to screen for optimized derivatives of laboratory-engineered mAbs, new recombinant cell lines must be generated for each candidate molecule that needs to tested *in vitro* or *in vivo* as a whole antibody. Similarly, it is possible to use the original hybridoma cell lines to directly manufacture transgenic mouse–derived mAbs for human clinical trials, thus avoiding the time-consuming process of generating a recombinant cell line before moving into the clinic. Several different mAbs produced directly from their parent hybridomas have entered the clinic; however, although this may be desirable in those particular cases where a rapid go/no-go development decision can be made from initial clinical data, more typically, when a lead candidate is chosen, companies have opted to take immediate advantage of technological advances in protein production from recombinant cell lines to minimize manufacturing costs. For this reason, after a clinical lead has been selected, the development and manufacturing path for transgenic mouse–derived antibodies is similar to that followed for therapeutic mAbs derived from other technologies.

Polyclonal antibodies from large animals

The therapeutic mAbs on the market today are in many ways a significant improvement over the polyclonal serum therapies originally pioneered by Kitasato, Behring and Ehrlich over a century ago for treating diphtheria and tetanus. MAbs have proven to be a source of well-characterized, low-immunogenicity and highly efficacious drugs; however, there still is a place for polyclonal human serum–derived[101] and even animal serum–derived antibodies[102] in the clinic. Just as the natural human immune system uses polyclonal rather than mAbs in responding to pathogens, polyclonal antibodies may also be preferable for passive immunotherapy in some cases. Advantages of polyclonal antibodies include their potential increased potency in immune complex formation, their utility in combating infectious diseases caused by diverse strains of pathogens or that require neutralization of multiple epitopes for successful treatment, and their potential for neutralizing snake and insect venoms that comprise multiple toxic components.

Nonrodent transgenic animals, such as cows, chickens and rabbits, could be exploited in the biological production of human polyclonal antibodies. Some work leading toward this goal has already appeared in the scientific literature. Kuroiwa *et al.*[103] generated transgenic calves with introduced artificial chromosomes comprising the entire human germline heavy-chain and λ-light-chain loci. The calves expressed correctly rearranged human heavy- and light-chain antibody transcripts. The same group also used sequential gene targeting in fibroblast cells, together with cloning by nuclear transfer, to generate homozygous heavy-chain knockout mutant calves[104]. The combination of these technologies, together with light-chain knockouts, could generate a new transgenic platform for producing animal-derived, human-sequence polyclonal antibodies. The potential advantages of this system include the ability to hyperimmunize the animals against specific pathogens or human disease–associated proteins, as well as improved lot consistency and reduced risk of human pathogen contamination. The potential increased risk of bovine spongiform encephalitis transmission has also been addressed by targeting the bovine prion protein[104].

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 initial data emerging from the 33 transgenic-derived human mAbs in clinical testing provide multiple examples of apparently active drugs that are also well tolerated. Nevertheless, actual patient benefit for each of these mAb-based drugs will need to be demonstrated in pivotal trials. If one of these molecules does demonstrate patient benefit, it could be the first novel biologic actually first generated within a transgenic mouse. Future applications of this technology include the potential for creating large transgenic farm animals that can be directly used for production of therapeutic human-sequence polyclonal antibodies.

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The author declares competing financial interests (see the *Nature Biotechnology* website for details).

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