

Fully human antibodies from transgenic mouse and phage display platforms

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Over the past two decades, technologies have emerged for generating monoclonal antibodies (MAbs) derived from human immunoglobulin gene sequences. These fully human MAbs provide an alternative to re-engineered, or de-immunized, rodent MAbs as a source of low immunogenicity therapeutic antibodies. There are now two marketed fully human therapeutic MAbs, adalimumab and panitumumab, and several dozen more in various stages of human clinical testing. Most of the drugs, including adalimumab and panitumumab, were generated using either phage display or transgenic mouse platforms. The reported clinical experience with fully human MAbs demonstrates that these two platforms are, and should continue to be, a significant source of active and well tolerated experimental therapeutics. While this body of reported clinical data does not yet provide a clear distinction between the platforms, the available descriptions of the drug discovery processes used to identify the clinical candidates highlight one difference. It appears that lead optimization is more commonly applied to phage display derived leads than transgenic mouse derived leads.

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Introduction

Köhler and Milstein's invention of the hybridoma method for generating antigen specific mouse monoclonal antibodies (MAbs) opened the door for the development of antibody-based drugs. However, because rodent MAbs comprise foreign protein sequences, most of the early drugs that entered clinical development elicited immune responses from human patients [1]. Advances in molecular biology, involving the manipulation of gene sequences *in vitro*, and the expression of these manipulated sequences in bacterial, fungal, and mammalian cell culture systems, provided methods for re-engineering rodent

antibodies to partially replace the rodent antibody sequences with functionally equivalent human amino acid sequences, thus reducing the overall immunogenicity without destroying the recognition properties of the original antibody. These methods for re-engineered rodent MAbs proved to be very successful. For example, an engineered chimeric Fab fragment comprising mouse variable and human constant region sequences was immunogenic in only 1% of human patients compared with 17% for the original mouse Fab [2]. In 1994, this Fab fragment, abciximab, became the first, of over a dozen, marketed engineered antibody products [3]. Shortly after the introduction of re-engineered MAbs, new technologies were developed that allowed for the discovery of novel MAbs derived directly from human immunoglobulin gene sequences. These new technologies can be binned into two distinct categories: *in vitro* assembled recombinant human antibody libraries and transgenic mice comprising human germline configuration immunoglobulin gene sequences. The first of these categories (reviewed by Hoogenboom [4]) includes libraries of recombinant antibodies cloned into bacteriophage (phage) or yeast such that the antibody variable regions are displayed on the surface, providing a 'phenotype' that can be used to select for, and identify, the 'genotype' of the recombinant antibody clone. The impact of the host is highlighted by a comparison of the selected repertoire obtained from a single library using either phage or yeast display [5]. Antibody variable region sequences selected from the phage library represented only a subset of the clones obtained from the yeast library, and those variable region sequences not represented in the phage library were found to be poorly expressed in *E. coli*. Proteins such as antibodies, or antibody fragments, that are derived from eukaryotic organisms are often difficult to express within a prokaryotic cell, and this could be an important limitation for phage display. In addition to phage and yeast display, methods have been developed for directly selecting antibody sequences *in vitro* through the isolation of ternary complexes of mRNA, ribosome, and nascent peptide [4]. While all of these display technologies show great promise, yeast display and ribosome display are more recent, and have not had time to have an appreciable impact on existing clinical stage drug pipelines. This review will therefore focus on drugs derived from the more mature phage display methodologies. The second category of technologies for direct discovery of human sequence antibodies, transgenic mice (reviewed by Lonberg [6]), has emerged more recently than phage display, nevertheless, it has made a considerable impact on clinical stage pipelines, providing over 50 different

drugs now in human clinical trials ([supplemental material](#)).

Phage display platforms

In 1985, Smith [7] discovered that foreign DNA sequences could be cloned into filamentous bacteriophage such that the cloned sequences are expressed on the surface of the phage particles as fusion proteins. These phage particles could then be enriched for specific sequences on the basis of the binding properties of the displayed fusion proteins. This discovery was combined with PCR amplification methods developed in the late 1980s for cloning expressed immunoglobulin variable region cDNA repertoires to create diverse libraries of phage displayed antibody variable regions that could be used to rapidly access target-specific MAbs without generating a library of hybridoma clones [8]. The repertoire expressed by these phage display libraries reflects the natural B cell repertoire of the individual animal from which the cDNA was derived [9]. Thus, depending on whether or not the donor animal or human subject was previously exposed to the target antigen, these libraries can be used to harvest the primary or secondary humoral immune response. Synthetic and semisynthetic phage display libraries, where at least some of the CDR diversity is generated through the introduction of random synthetic sequences, are also used [10*,11]. For non-immune and synthetic libraries, which serve as an *in vitro* analog of the primary immune response, additional methods have been developed for mimicking the process of affinity maturation that characterizes the natural secondary immune response ([Figure 1 \[4,12*\]](#)).

Transgenic mouse platforms

In 1994, two papers reported the generation of genetically engineered mice that expressed fully human antibody repertoires that could be accessed by conventional hybridoma technology ([Figure 1 \[13,14\]](#)). These engineered animals comprised targeted disruptions of the endogenous mouse heavy and κ light chain genes together with introduced transgenes comprising unrearranged human heavy chain and κ light chain gene segments. The unrearranged gene segments included in these original transgenes represent only a small fraction of the complete human heavy and κ light chain repertoire. Nevertheless, IgM κ [14] and IgG κ [13] MAbs specifically recognizing the immunogens were isolated. The observation that a limited germline repertoire can be used by the mouse immune system to construct high specificity and high affinity antibodies to a variety of antigens probably reflects 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) [15]. 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 Ig α , Ig β , and other signaling molecules), their B cells develop and mature into what appear to be all of the normal B cell subtypes. Following these initial reports of transgenic mice comprising relatively limited germline repertoires, a variety of additional mouse strains, created using larger human immunoglobulin transgenes, have appeared in the literature [6].

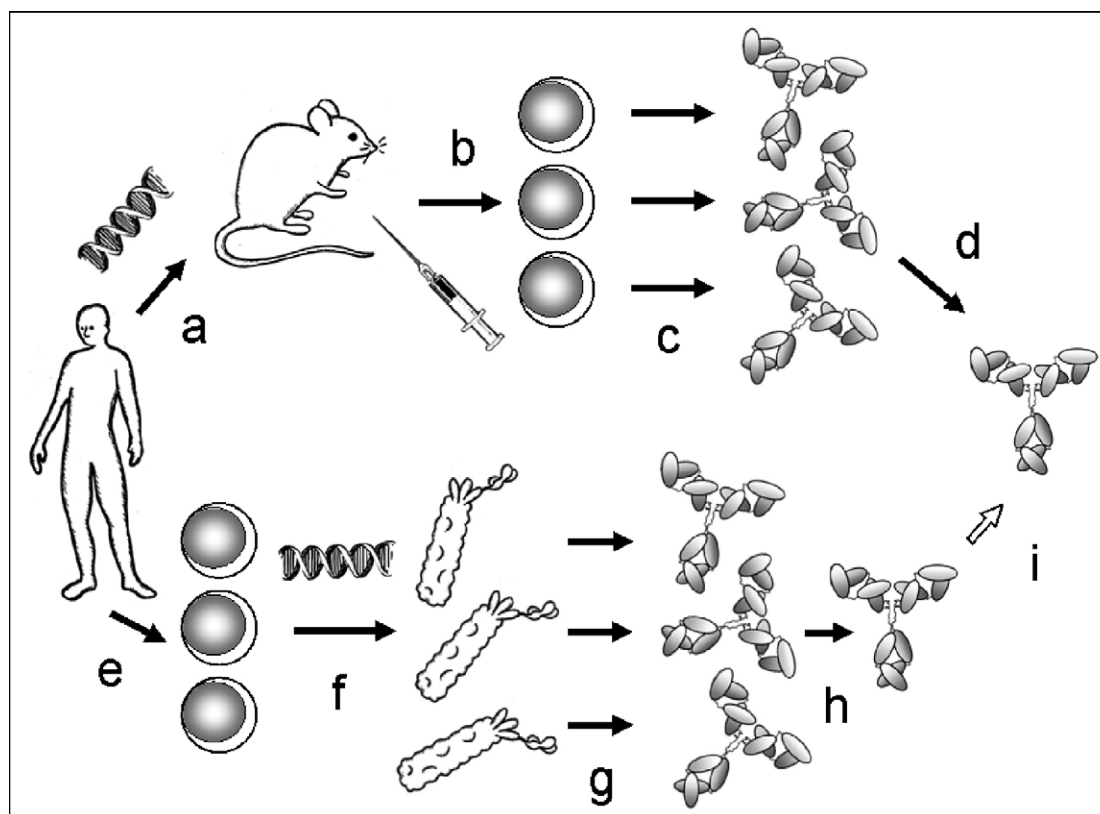
Fully human MAbs in the clinic

Laboratory experience with phage display and transgenic mouse platforms, and with the antibodies derived from them, can only support *a priori* arguments for their usefulness as a source of well tolerated and effective human therapeutics. Now that actual clinical data on phage display and transgenic mouse derived MAbs are beginning to appear in the literature, it is possible to begin an *a posteriori* analysis. Some of these data are outlined below, with an emphasis on immunogenicity, pharmacokinetics (PK), and overall tolerability.

Clinical experience with fully human antibodies targeting TNF α

In 2002, adalimumab, a phage display derived antibody that neutralizes tumor necrosis factor- α (TNF α), which is associated with a variety of inflammatory diseases became the first marketed fully human MAb product [16]. It is now approved for multiple indications, including rheumatoid arthritis, Crohn's disease, and plaque psoriasis. There are two other marketed MAbs that target TNF α : infliximab, a mouse human chimeric antibody, and certolizumab pegol, a CDR grafted Fab fragment that is covalently linked to polyethylene glycol to enhance the plasma half-life [17]. Another fully human MAb targeting TNF α , the transgenic mouse derived golimumab, is currently being tested in a variety of phase 3 trials in rheumatoid arthritis and other indications [18]. All four MAbs elicit anti-drug antibody responses, which might be exaggerated by an underlying predisposition within the treated autoimmune disease patient population (the incidence is reduced in patients receiving methotrexate). Reported frequencies of anti-drug antibody responses range from 10 to 40% for infliximab, compared with 14–87% for adalimumab, 9–12% for certolizumab pegol, and 12–16% for golimumab [17–22]. For infliximab and adalimumab, the anti-drug antibody response correlates with reduced efficacy, and with adverse events, such as infusion reactions [19,21], which could be due to the observed formation of very large drug/anti-drug antibody complexes [23]. However, adalimumab shows good activity when dosed at 40 mg s.c. every two weeks [16], and golimumab at 50 mg every four weeks [18], indicating that antibody responses to these fully human antibodies do not generally lead to rapid clearance or neutralization in a majority of patients, and are consistent with the observed two to three week half-life for both drugs [17,22].

Figure 1



Drug discovery process for phage display and transgenic mouse platforms. Human germline configuration immunoglobulin genes are used to provide the genetic information that is incorporated into the engineered mouse genome for transgenic mouse platforms (a). The resulting engineered mice are immunized with target antigens to stimulate an *in vivo* humoral immune response to generate somatically mutated, affinity matured, secondary repertoire B cells (b), which can be cloned and screened (c) using conventional methods, such as hybridoma fusion, developed for isolating rodent MAbs. Extensive *in vitro* characterization of candidate MAbs, involving biochemical, biophysical, and cell biology methods, together with *in vivo* testing in animal models, contributes to the selection (d) of a clinical lead that is then tested in human patients. In contrast, for phage display platforms, the harvested human genetic information is derived not from germline configuration immunoglobulin genes, but from the rearranged genes found in B cells of the primary and secondary repertoire (e). The rearranged genes can be used directly, or in combination with synthetic sequences that may provide additional diversity, to construct libraries of bacteriophage particles displaying surface fusion proteins comprising a diversity of antibody binding domains (f). These libraries can be selected or screened to isolate individual variable region sequences that bind specifically to the target antigen (g). The cloned variable region sequences are then used to generate whole antibodies, or antibody fragments, that can be further characterized to select a lead candidate (h). Typically this lead candidate is then optimized *in vitro* for improved affinity (i) to generate a clinical lead for testing in human patients.

Clinical experience with fully human antibodies targeting IL-12/IL-23

The cytokines IL-12 and IL-23 are heterodimeric proteins that share a common subunit, p40. IL-12 is associated with T_H1 mediated immune responses, while IL-23 is associated with T_H17 responses. The phage display derived antibody ABT-874 [24,25] and the transgenic mouse derived antibody ustekinumab [26•] are each directed against p40 and neutralize both IL-12 and IL-23. In a phase 2 psoriasis trial, 237 patients received ustekinumab for up to four weekly 90 mg subcutaneous doses [26•]. During the 52 week monitoring period, anti-drug antibodies were detected in 12 (4%) of

the treated patients. Patients given only a single subcutaneous dose, at either 45 or 90 mg, showed sustained disease-modifying responses for over six months following treatment. Together with the observed sustained clinical benefit, the approximately 20–30 day terminal half-life of ustekinumab appears to indicate that it does not elicit a strong antibody response. In a pair of placebo controlled phase 3 trials, involving a total of nearly 2000 psoriasis patients, 67% of patients met the primary efficacy endpoint in the cohorts dosed every three months subcutaneously with 45 mg of ustekinumab following a pair of 45 mg loading doses given month apart [27–29]. 59% of patients achieved long-term responses at 40

weeks. The adverse event profile was similar between the drug and placebo arms. Anti-drug antibodies were detected in 5% of patients; however, injection site reactions were rare and mild. The phage display antibody ABT-874 showed some signs of immunogenicity in early clinical trials, with anti-drug antibodies detectable in 3 of 63 patients, and 2 of those patients showing evidence of early clearance of the drug from the serum [25]. Like ustekinumab, ABT-874 also showed very good activity in a psoriasis phase 2 trial [24]. However, it is difficult to directly compare the activities or immunogenicities of the two drugs because ABT-874 was given at higher doses, and the patients were only followed for 12 weeks compared with the 32 week ustekinumab study. Pharmacokinetic and immunogenicity data were also not reported for the ABT-874 psoriasis study.

Clinical experience with fully human antibodies targeting EGFR

The epidermal growth factor receptor (EGFR) is a tyrosine kinase signaling receptor that is upregulated on a variety of epithelial cancers. In 2006, panitumumab, which inhibits EGFR signaling [30–34,35,36], became the first marketed MAb derived from a transgenic mouse platform. There has been no direct comparison of the safety and efficacy of panitumumab and cetuximab, the already marketed chimeric anti-EGFR MAb [37]; however, the transgenic-derived MAb does appear to be less immunogenic than the chimeric cetuximab. None of the 231 panitumumab treated patients in a phase III study had detectable levels of anti-drug antibodies [32], compared with over 4% reported for cetuximab [33]. A separate study reported immunogenicity rates ranging from 0.3 to 4% for panitumumab, with the higher rate coming from an assay that detected lower affinity antibodies [36]. The discrepancy between these two assays highlights the difficulty of comparing immunogenicity across studies. The observed rate of infusion reactions in the panitumumab phase III, which have correlated with immunogenicity for other MAbs [19], was comparatively low, and could be the most significant point of differentiation between the two drugs. Only 5% of the panitumumab-treated patients experienced adverse events that could be classified as infusion reactions, and no patients experienced grade 3 or 4 reaction [34]. By 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 pre-treated with antihistamines to prevent infusion reactions. These reactions are at least partly attributable to the presence of variable region glycosylation comprising galactose- α -1,3-galactose [38], which is absent in human cells. This carbohydrate structure derives from the method of manufacture (engineered mouse myeloma cells), and from the fact that cetuximab has an exposed variable region glycosylation site that is a better substrate for galactose addition than the less exposed constant region glycosylation site at residue 297

that is found in all IgG MAbs, and is not a consequence of the rodent origin of the parent antibody.

Two additional fully human MAbs directed against EGFR have now entered clinical development. The transgenic mouse derived zalutumumab is in phase 3 testing and the phage display derived IMC-11F8 is in phase 2. Clinical data are not yet available for IMC-11F8, but an early stage trial for zalutumumab has been reported. In a 28 patient dose escalation, safety trial, the drug was found to be safely administrable at the highest dose of 8 mg/kg [39]. Like cetuximab, both zalutumumab and IMC-11F8 are IgG1 MAbs, and may elicit greater effector function than the IgG2 panitumumab [40]. Structural studies of each of these two antibodies have been reported [41,42]. Both inhibit signaling by locking the receptor into an inactive conformation; however, zalutumumab may have an additional mode of action because bivalent binding of two EGFR monomers appears to separate the chains so that they cannot form a functional signaling dimer.

Clinical experience with fully human antibodies targeting CD20

Expression of the CD20 antigen is restricted to B lineage lymphocytes and neoplasms derived from these cell types, making it an attractive target for both B cell lymphomas and B cell mediated autoimmune disease. The transgenic mouse derived IgG1 ofatumumab [43,44,45] and the mouse–human IgG1 chimeric MAb rituximab [46] – which is currently approved for treatment of non-Hodgkin's lymphoma (NHL) and rheumatoid arthritis – both bind CD20 but recognize distinct epitopes [45,47]. The human antibody is reported to be a more potent mediator of complement-dependent cytotoxicity *in vitro* than rituximab. This difference in potency is more pronounced at lower antigen density, and could theoretically translate into greater activity in low CD20 expressing lymphomas such as chronic lymphocytic leukemia (CLL). Results of an initial safety/efficacy trial of ofatumumab in 33 patients with relapsed or refractory CLL showed tolerability and promising clinical activity [44]. Patients received up to 6.5 g of drug over four weekly infusions (approximately double the typical dosing for rituximab). The response rate for the 26 patients in the highest dose cohort was 50%. This is comparable to the best results seen with rituximab in CLL patients; however, the small sample size and the differences in dosing make it difficult to compare the two drugs. No anti-ofatumumab antibodies were detected in treated patients.

Clinical experience with fully human antibodies targeting CD4

Zanolimumab is a transgenic mouse derived human IgG1 antibody directed against the T cell antigen CD4. In an 85 patient placebo-controlled, phase 2 trial in psoriasis, no

patients developed anti-drug antibodies. In phase 2 trials, in cutaneous T cell lymphoma patients (mycosis fungoides and Sezary syndrome), only 1 of 47 patients had measurable anti-drug antibodies [48]. The anti-zanolimumab titer was very low in this patient, and did not appear to be neutralizing, as demonstrated by continued clinical activity of the drug. Dose dependent clinical activity of zanolimumab was observed within the mycosis fungoides patient cohorts, with three out of four patients treated at 980 mg experiencing an objective clinical response.

Clinical experience with fully human antibodies targeting CTLA-4

CTLA-4 (CD152) is a negative T cell signaling molecule that binds to the two ligands CD80 and CD86. This molecule is a potential target for cancer therapy based on the CTLA-4 blockade induced upregulation of immune responses to tumor cells. There are two transgenic mouse derived MAb directed against CTLA-4, the IgG1 MAb ipilimumab [49], and the IgG2 MAb tremelimumab [50], in phase 3 clinical testing. Neither of these fully human antibodies elicits strong anti-drug antibody responses, which is particularly notable given that both drugs enhance immune responses, and induce very strong inflammatory reactions in some patients. A terminal half-life of 22 days was reported for tremelimumab [51], and one month post-dosing serum trough levels of 10 µg/ml ipilimumab were reported after five months of repeated monthly dosing at 3 mg/kg [52], indicating that neither drug is cleared by anti-drug antibodies. Clinical data in cancer patients have been reported for both ipilimumab [52–54] and tremelimumab [50,51,55]. Objective and durable antitumor responses were observed for both drugs. Rosenberg and colleagues analyzed pooled data from 139 metastatic melanoma patients in three different phase 2 trials of ipilimumab involving different dosing regimens, including some patients that received a peptide-based vaccine in addition to the drug [54]. Median survival for ipilimumab-treated patients was 15.7 months; in contrast to the historical 6.3 month median survival derived from an analysis of phase 2 trials in metastatic melanoma over the past three decades [56].

Clinical experience with fully human antibodies targeting TRAIL-R1/TRAIL-R2

Tumor necrosis factor related apoptosis inducing ligand (TRAIL) induces apoptosis by binding to the receptors TRAIL-R1 and TRAIL-R2. Two phage display derived antibodies, mapatumumab, which targets TRAIL-R1, and lexatumumab, which targets TRAIL-R2, are now in phase 2 clinical trials. Both of these drugs have agonist activity and demonstrate tumor cell killing *in vitro* and in preclinical animal models [57,58]. Results from phase 1 and 2 studies have been reported for mapatumumab [59,60]. In a phase 1 trial in 49 advanced solid tumor patients, there were no detectable anti-drug antibodies elicited, and the *in vivo* half-life was two to three weeks,

as expected for a non-immunogenic human IgG1 antibody. Hepatic toxicity was seen in 2 out of 11 patients treated every two weeks at the highest dose of 10 mg/kg; however, this toxicity was not seen at 10 mg/kg dosed every three weeks in 32 non-small cell lung cancer patients in the multi-dose phase 2 study, and it is not clear if it was drug related. The phase 2 study confirmed that the drug was not immunogenic, with no anti-drug antibodies detected in any of the patients. In a phase 1 study in 37 patients with advanced solid tumors, lexatumumab was also found to be well tolerated and non-immunogenic [61]. The reported *in vivo* half-life was two to three weeks. Two of six patients treated at the highest dose developed grade 4 hepatic toxicity, but no dose-limiting toxicities were seen at 10 mg/kg. A third TRAIL receptor targeted fully human MAb, HGS-TR2J, which is derived from transgenic mice and binds to TRAIL-R2 [62], has entered clinical development; however, no clinical data have been published.

Clinical experience with fully human antibodies targeting EpCAM

EpCAM (CD136) is a widely expressed epithelial cell surface glycoprotein that is upregulated on human adenocarcinomas and squamous cell carcinoma. Several different MABs directed against EpCAM have been tested in human patients, and a mouse MAB, edrecolomab, was previously marketed in Germany [63]. Normal epithelial cell expression of EpCAM appears to lead to dose-limiting pancreatitis for high affinity MABs. To avoid toxicity, a low affinity ($K_D \sim 200$ nM) MAB, adecatumumab, was selected from a phage display library as a clinical candidate [64]. It was hypothesized that because of avidity from intact bivalent antibody binding, the drug would retain activity against tumor cells having a high density of cell surface EpCAM but would not be as destructive toward lower expressing normal tissue. In a dose escalating phase I study in 20 prostate cancer patients, it was well tolerated at up to 262 mg/m², with no dose-limiting toxicities [65]. None of the patients developed detectable anti-drug antibodies, consistent with the calculated 15 day *in vivo* half-life. Pharmacodynamic activity was observed (as measured by transient increases in serum TNFα); however, no PSA declines or other measures of potential clinical activity were observed.

Clinical experience with fully human antibodies targeting RANKL

Receptor activator of nuclear factor-κB ligand (RANKL) is a TNF family member that stimulates the maturation and activation of osteoclasts, which mediate bone resorption. The transgenic mouse derived, human IgG2 antibody, denosumab blocks RANKL activity to inhibit bone resorption. A recent review listed 21 different clinical trials for the drug, including 10 ongoing phase 3 studies for treatment of bone loss in postmenopausal women, and

in cancer patients with treatment induced bone loss or skeletal disease caused by bone metastases [66]. A single subcutaneous administration, dose escalation, phase 1 study in osteoporotic patients showed dose dependent and sustained activity (up to six months) in blocking bone resorption, with no reported serious drug-related adverse events [67]. Denosumab was found to have a terminal half-life of 32 days at the highest 3 mg/kg dose. In a randomized, multi-dose, dose ranging, trial in 255 metastatic breast cancer patients with bone disease, denosumab was tested directly against approved bisphosphonate drugs [68]. With very low and infrequent dosing (60 mg subcutaneous every three months) the human MAb had biological activity comparable to bisphosphonate. No anti-denosumab antibodies were detected. Efficacy and safety was evaluated in a large two year phase 2 study of 412 postmenopausal women with low bone mineral density [69,70]. Overall adverse event profiles were similar between the placebo, denosumab, or bisphosphonate arms, and no neutralizing antibodies were detected. In a phase 3 trial [71] enrolling 332 women with less severe osteoporosis, patients were randomized to receive either 60 mg denosumab s.c. or placebo every six months. The mean increase in bone mineral density at 24 months was 6.5% for the treatment arm compared with a decrease of 0.6% in the placebo arm. Two patients in the denosumab arm and three patients in the placebo arm had measurable levels of anti-drug antibodies; however, none were neutralizing, and there was no evidence of an effect on safety or PK. Overall incidence of adverse events was similar in both arms; however, in the denosumab-treated cohort there were eight patients who required hospitalization for treatment of infections, compared with 1 patient in the placebo arm. Because RANKL is expressed on activated T and B cells, and dendritic cells, and because mice homozygous for RANKL gene deletion are born without lymph nodes, it is theoretically possible that denosumab could effect immune function; however, mouse and rat animal models involving continuous blockade of RANKL show no evidence of immune suppression [72]. Additional human studies involving larger numbers of patients will be required to determine if infection is an actual concern.

Concluding remarks

At least 66 different therapeutic drugs, derived from either phage display or transgenic mouse platforms, have entered human clinical testing. The reported binding affinities for these MAbs range over five orders of magnitude; however, most are subnanomolar, and nearly half of the MAbs from each platform have affinities in the 0.1–1 nanomolar range, with similar representation of each platform on either side of that range. Thus, the published literature does not appear to distinguish fully the human antibody drug discovery technologies based on binding affinity. Drugs derived from either platform are in general well tolerated in human patients, with good PK and

immunogenicity profiles. It is difficult to draw conclusions about the relative merits of these platforms based on reported immunogenicity because of differences in the sensitivity of the assays used [36] and because of differences between individual drugs with regard to target Fc receptor and complement binding, patient population, and route, frequency, and magnitude of dosing; all factors that could affect immunogenicity. A recent review of the literature concluded that there is a clear immunogenicity advantage for chimeric antibodies over mouse antibodies, and a strong suggestion that CDR grafting confers a further advantage [1]. The reported incidence of anti-drug antibodies in treated patients ranged from 0 to 34% for CDR grafted MAbs, with approximately 40% of the drugs having no detectable immunogenicity. The experience to date is more limited for fully human antibodies; however, the existing data fall into a similar range. As more fully human antibodies become approved, it may be possible to directly compare the immunogenicity of antibodies derived from different platforms. It will be particularly interesting to compare the fully human phage display derived adalimumab and ABT-874 to the transgenic mouse derived golimumab and ustekinumab, which comprise matched pairs of drugs targeting TNF α and IL-12/IL-23 p40. This analysis may address the potential contribution to immunogenicity from the introduction of novel sequences during lead optimization of two phage displayed derived MAbs (discussed below).

The most striking apparent difference between the phage display and transgenic mouse platforms relates to the processes employed for discovery of the drugs now in clinical development (Figure 1). While the descriptions available from the published literature are often incomplete, the transgenic mouse derived antibodies appear to have typically been moved from lead selection directly to clinical development without undergoing lead optimization steps. The drug discovery process is at least partially described in the literature for 25 transgenic mouse derived MAbs (supplemental material). In these reports, it appears that the drug taken into human clinical testing comprises the variable region sequences of the parent transgenic mouse B cell. By contrast, for the 10 phage display derived MAbs for which the drug discovery process has been reported (supplemental material), 7 include descriptions of lead optimization for improved affinity. In one case where lead optimization was not employed, low affinity was actually one of the criteria for the clinical candidate [64]. The discovery of the phage display derived eotaxin neutralizing antibody bertilimumab provides a more typical example of the lead-selection-followed-by-lead-optimization strategy employed for phage display platforms [73]. The potency of the parental antibody was improved by over three orders of magnitude using a lead optimization strategy involving randomization of the heavy chain CDR3 sequence, point mutagenesis, and shuffling of the parental light chain with a

diverse library of light chain sequences. Lead optimization by heavy chain CDR3 sequence randomization was also employed in the absence of light chain shuffling for the discovery of belimumab, resulting in an order of magnitude increase in potency as measured by neutralization of the B cell stimulatory factor BLys [74]. Light chain shuffling strategies have also been successfully employed alone to optimize affinity before clinical development [75,76]. Notably, the reported affinities for antibodies derived from the two types of platforms fall into the same range, with no overall significant difference between affinity optimized phage display derived MAb and un-optimized transgenic derived MAb. Lead optimization as employed for phage display derived antibodies may not be required for transgenic mouse derived antibodies that have already undergone affinity maturation *in vivo*. This opportunity to bypass lead optimization entirely has potential advantages for the overall drug discovery process, as it might decrease timelines, and at the same time, increase the epitope diversity of the high affinity lead candidate pool.

Conflict of interest statement

The author has a financial interest in Medarex, which in turn has a financial interest in many of the drugs discussed in this article.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.coi.2008.06.004](https://doi.org/10.1016/j.coi.2008.06.004).

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This paper shows the impact of the host organism on the selected repertoire for phage display libraries. It appears that the poor expression of certain eukaryotic-derived sequences, such as antibody variable

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This paper takes a minimalist approach to the design of a phage display library, demonstrating that a very restricted set of amino acid residues can be used to construct CDR3 sequences for specific high affinity binding species. While this approach may not be directly applicable to the discovery of human therapeutics, it does provide insight into the minimal requirements for repertoire construction.

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Fully human antibodies from transgenic mouse and phage display platforms

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Supplemental Material

Table 1. Selected phage display, and transgenic mouse, derived fully human antibodies in clinical development.

Target	Drug	Indication	Discovery Platform	Company (developer)	Company (technology)	Highest Development Stage	Reported Affinity (nM) ^g	Reference(s)
Alpha Interferons	MDX-1103/ MEDI-545	Lupus	Transgenic Mouse	Astra Zeneca/ Medarex	Medarex	Phase 1		company information
Amyloid β	R-1450	Alzheimers	Phage Display	Roche	Morphosys	Phase 1		company information
Anthrax protective antigen	raxibacumab	<i>B. Anthracis</i> infection	Phage Display	Human Genome Sciences	CAT	Phase 2		company information
Anthrax protective antigen	Valortim™	<i>B. Anthracis</i> infection	Transgenic Mouse	Pharmathene/ Medarex	Medarex	Phase 1		[1]
BLys	belimumab	B cell mediated autoimmune diseases	Phage Display	Human Genome Sciences	CAT	Phase 3		[2,3]
CCR5	CCR5 mAb	HIV Infection	Transgenic Mouse	Human Genome Sciences	Abgenix	Phase 2		[4]
CD19	MDX-1342	Lymphoma/ RA	Transgenic Mouse	Medarex	Medarex	Phase 1		company information
CD20	ofatumumab	Non-Hodgkin lymphoma	Transgenic Mouse	Genmab	Medarex	Phase 3		[5-7]
CD30	MDX-060, MDX-1401 ^c	Lymphoma	Transgenic Mouse	Medarex	Medarex	Phase 2	0.6	[8]
CD38	HuMax-CD38	Multiple Myeloma	Transgenic Mouse	Genmab	Medarex	Phase 1		company information
CD3 ϵ	NI-0401	Autoimmune disease	Transgenic Mouse	NovImmune	Medarex	Phase 1		company information
CD4	zanolimumab	Lymphoma	Transgenic Mouse	Genmab	Medarex	Phase 3	0.1	[9-11]

CD70	MDX-1411	Renel Cell Carcinoma	Transgenic Mouse	Medarex	Medarex	Phase 1		company information
CD89	MDX-214 ^b	Solid tumors	Transgenic Mouse	Medarex	Medarex	Phase 1		company information
CDw137	BMS-66513	Cancer	Transgenic Mouse	Bristol-Myers Squibb	Medarex	Phase 1		company information
<i>Clostridium difficile</i> toxins A and B	MDX-066/ MDX-1388 ^a	Hospital acquired <i>C. difficile</i> associated diarrhea	Transgenic Mouse	Medarex/Massachusetts Biologic Laboratories	Medarex	Phase 2		[12]
CTGF	FG-3019	Diabetic nephropathy/pulmonary fibrosis/pancreatic cancer	Transgenic Mouse	Fibrogen	Medarex	Phase 1		[13,14]
CTLA-4	ipilimumab	Melanoma and various other cancers	Transgenic Mouse	Medarex	Medarex	Phase 3	3	[15-19]
CTLA-4	tremelimumab	Melanoma	Transgenic Mouse	Pfizer	Abgenix	Phase 3	0.3	[20-22]
CXCL10	MDX-1100	Ulcerative colitis	Transgenic Mouse	Medarex	Medarex	Phase 2		company information
Dendritic cell mannose receptor	MDX-1307 ^d	Human gonadotropin-positive cancers	Transgenic Mouse	Celldex	Medarex	Phase 1		[23]
EGFR	panitumumab	Colorectal cancer and non-small cell lung cancer, renal cell carcinoma	Transgenic Mouse	Amgen	Abgenix	Launched	0.05	[4,24-29]
EGFR	zalutumumab	Head and neck cancer	Transgenic Mouse	Genmab	Medarex	Phase 3	20	[30-32]
EGFR	IMC-11F8	Solid Tumors	Phage	Imclone	Dyax	Phase 2	3	[33]

			Display					
Eotaxin-1	bertilimumab	allergic conjunctivitis	Phage Display	iCO	CAT	Phase 2	0.08	[34]
EpCAM	adecatumumab	Solid Tumors	Phage Display	Micromet/ Merck Serono	Micromet	Phase 2	200	[35,36]
GM-CSF	MOR103	Rheumatoid Arthritis	Transgenic Mouse	Morphosys	Morphosys	Phase 1		Company information
HGF/SF	AMG 102	Solid Tumors	Transgenic Mouse	Amgen	Abgenix	Phase 1	0.1	[37]
HLA-DR	1D093C	Lymphoid Cancers	Phage Display	GPC Biotech	Morphosys	Phase 1 ^f		company information
IGF-1R	IMC-A12	Solid Tumors	Phage Display	ImClone	Dyax	Phase 2	0.04	[38,39]
IGF-1R	CP-751,871	Cancer	Transgenic Mouse	Pfizer	Abgenix	Phase 2	2	[40]
IGF-1R	R1507	Solid Tumors	Transgenic Mouse	Roche	Medarex	Phase 2		company information
IGF-1R	19D12	Cancer	Transgenic Mouse	Schering Plough	Medarex	Phase 2	0.004	[41]
IL-12/ IL-23 p40	ustekinumab	Psoriasis and multiple sclerosis	Transgenic Mouse	Johnson & Johnson	Medarex	Phase 3		[42-45]
IL-12/IL-23 p40	ABT-874	Psoriasis	Phage Display	Abbott	CAT	Phase 3	0.1	[46,47]
IL-13	CAT-354	Allergic rhinitis, asthma	Phage Display	Astra Zeneca	CAT	Phase 2		[48]
IL-15	AMG 714	Rheumatoid arthritis/ psoriasis	Transgenic Mouse	Amgen/ Genmab	Medarex	Phase 2		[49]

IL-1beta	ACZ885	Muckle Wells Syndrome	Transgenic Mouse	Novartis	Medarex	Phase 3		company information
IL-8	ABX-IL8	Psoriasis	Transgenic Mouse	Abgenix	Abgenix	Phase 1 ⁱ	0.05	[50,51]
IL-8	HuMax-IL8	Palmoplantar Pustulosis	Transgenic Mouse	Genmab	Medarex	Phase 1	0.4	[52]
Melanoma antigen glycoprotein NMB	CR011-vcMMAE ^e	Melanoma	Transgenic Mouse	Curagen	Abgenix	Phase 2	50	[53]
Muc18	ABX-MA1	Melanoma	Transgenic Mouse	Abgenix	Abgenix	Phase 1 ⁱ		[50]
myostatin	stamulumab	muscle waisting diseases	Phage Display	Wyeth	CAT	Phase 2		[54]
OX40L	LC001	Asthma	Transgenic Mouse	Roche/Genentech	Medarex	Phase 1		[55]
Parathyroid hormone	ABX-PTH	Hyperparathyroidism	Transgenic Mouse	Amgen	Abgenix	Phase 1 ⁱ		company information
PD-1	MDX-1106/ ONO-4538	Cancer/ HCV	Transgenic Mouse	Ono Pharmaceuticals/ Medarex	Medarex	Phase 1		[56]
PDGF-D	CR002	Inflammatory kidney disease	Transgenic Mouse	Curagen	Abgenix	Phase 1	0.2	[57]
PDGFR α	IMC-3G3	Cancer	Transgenic Mouse	ImClone	Medarex	Phase 1		[58]
PSCA	AGS-PSCA/ MK-4721	Prostate Cancer	Transgenic Mouse	Agensys/ Merck	Abgenix	Phase 1		[4]
PSMA	MDX-070	Prostate cancer	Transgenic Mouse	Medarex	Medarex	Phase 2 ⁱ		[59]

PSMA		Prostate Cancer	Transgenic Mouse	Progenics	Abgenix	Phase 1		[60,61]
RANKL	denosumab	Osteoporosis and treatment-induced bone loss	Transgenic Mouse	Amgen	Abgenix	Phase 3		[62-68]
TGF- β 1	metelimumab	fibrotic disease	Phage Display	Astra Zeneca	CAT	Phase 2 ⁱ		[69]
TGF- β 1	GC-1008	fibrotic disease	Phage Display	Genzyme	CAT	Phase 1		company information
TNF α	adalimumab	Inflammatory disease	Phage Display	Abbott	CAT	Launched	0.1 ^h	[70-75]
TNF α	golimumab	Inflammatory disease	Transgenic Mouse	Johnson & Johnson	Medarex	Phase 3		[76,77]
TRAIL-R1	mapatumumab	Solid Tumors	Phage Display	Human Genome Sciences	CAT	Phase 2		[78-81]
TRAIL-R2	lexatumumab	Solid Tumors	Phage Display	Human Genome Sciences	CAT	Phase 2		[79,82]
TRAIL-R2	AMG 655	Solid tumors	Transgenic Mouse	Amgen	Abgenix	Phase 1		[4]
TRAIL-R2	HGS-TR2J	Solid tumors	Transgenic Mouse	Human Genome Sciences	Kirin	Phase 1	0.07	[83]
VAP-1	VAP-1 Mab	Inflammatory disease	Transgenic Mouse	BioTie Therapeutics/ Roche	Medarex	Phase 1		company information
VEGFR-1	IMC-18F1	Cancer	Transgenic Mouse	ImClone	Medarex	Phase 1	0.05	[84]
VEGFR-2	IMC-1121B	Solid tumors	Phage Display	ImClone	Dyax	Phase 3	0.1	[85,86]

α v Integrins	CNTO 95	Solid tumors	Transgenic Mouse	Johnson & Johnson	Medarex	Phase 2	0.2	[87,88]
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^a Combination of two different monoclonal antibodies directed against each of two toxins.

^b Human antigen-binding fragment (Fab) fused to epidermal growth factor.

^c Nonfucosylated glycovariant of MDX-060.

^d Human Fab fused to β hCG.

^e Antibody-drug conjugate with small molecule microtubule inhibitor MMAE.

^f Clinical development interrupted or terminated.

^g Reported affinity values rounded to a single significant digit.

^h Three different affinity values for adalimumab, ranging from 0.1 to 1 nM, have been reported in the literature [73-75].

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