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REVIEW

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# Phage display-derived human antibodies in clinical development and therapy

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**ABSTRACT**

Over the last 3 decades, monoclonal antibodies have become the most important class of therapeutic biologicals on the market. Development of therapeutic antibodies was accelerated by recombinant DNA technologies, which allowed the humanization of murine monoclonal antibodies to make them more similar to those of the human body and suitable for a broad range of chronic diseases like cancer and autoimmune diseases. In the early 1990s *in vitro* antibody selection technologies were developed that enabled the discovery of "fully" human antibodies with potentially superior clinical efficacy and lowest immunogenicity.

Antibody phage display is the first and most widely used of the *in vitro* selection technologies. It has proven to be a robust, versatile platform technology for the discovery of human antibodies and a powerful engineering tool to improve antibody properties. As of the beginning of 2016, 6 human antibodies discovered or further developed by phage display were approved for therapy. In 2002, adalimumab (Humira®) became the first phage display-derived antibody granted a marketing approval. Humira® was also the first approved human antibody, and it is currently the best-selling antibody drug on the market. Numerous phage display-derived antibodies are currently under advanced clinical investigation, and, despite the availability of other technologies such as human antibody-producing transgenic mice, phage display has not lost its importance for the discovery and engineering of therapeutic antibodies.

Here, we provide a comprehensive overview about phage display-derived antibodies that are approved for therapy or in clinical development. A selection of these antibodies is described in more detail to demonstrate different aspects of the phage display technology and its development over the last 25 years.

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## Antibody phage display

Monoclonal antibodies represent the most important class of recombinant protein therapeutics on the market. As of May 2016, over 50 antibodies and antibody conjugates have been approved by the US. Food and Drug Administration (FDA) or European Medicines Agency (EMA),[1] and about 500 antibodies are under clinical investigation. Most therapeutic antibodies are approved for cancer and autoimmune diseases and the annual sales revenues of all therapeutic antibodies exceeded 75 billion US$ in 2013.[2] The first approved monoclonal antibody, muromonab-CD (Orthoclone OKT3®), which blocks CD3-mediated activation of T cells to prevent organ rejection after transplantation,[3] was produced by hybridoma technology. However, a significant percentage of patients who were administered this murine antibody developed anti-drug antibodies (ADA) and were sensitized to OKT3 therapy.[4] In the late 1980s, recombinant DNA technology was used to substitute murine antibody sequences with human antibody sequences to reduce immunogenicity.[5,6] First, only murine constant immunoglobulin G (IgG) domains were replaced by human counterparts resulting in chimeric antibodies like rituximab (Rituxan®),[7] which lowered the risk of immunogenicity. However, the murine variable regions were still prone to generate antiidiotypic antibodies.[8] Therefore, it was also important to substitute the murine framework regions in the variable antibody domains with the closest human framework sequences[9], which results in humanized antibodies like daclizumab (Zynbrita® and formerly Zenapax®)[10,11] or bevacizumab (Avastin®).[12] Humanization, however, did not eliminate the possibility of an immune response[13,14] because the success and degree of humanization is dependent on the individual antibody, which often requires back mutations[15] and can involve a tremendous amount of antibody engineering effort. Moreover, the complementarity-determining regions (CDRs) mediating most of the interaction with the antigen are still from non-human origin, and, therefore, pose some risk for ADA responses.[5,13,14] Therefore, "fully" human antibodies were considered to be the optimal solution for therapy because they are indistinguishable from those in the human body and had the lowest risk of immunogenicity.[5]

Discovery of human antibodies required new technologies because immunization and hybridoma technology could not be transferred to human donors. Therefore, the isolation of human B cells[16] was limited to such indications, where human donors have developed a natural antibody response to the target antigen like after natural infections. Starting in the 1990s, however, transgenic mice or rats, endowed with the

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human antibody gene repertoire or parts of it, offered access to human antibodies by *in vivo* immunization and hybridoma technology.[17-22] These technologies were developed by various companies, e.g., Kirin, Medarex, Regeneron, Abgenix (transgenic mice) or OMT (transgenic rats). Despite the tremendous success of this technology, the immunization of transgenic mice does not always result in a successful *in vivo* antibody response to all types of antigens. Particularly, conserved, toxic, and unstable antigens, proteins with allosteric conformational changes and transmembrane proteins are not well suited for an immunization approach and require an alternative solution for antibody discovery.

*In vitro* selection technologies like antibody phage display do not depend on the *in vivo* immune response, and can be used to discover antibodies to almost every type of antigen and to a broader range of epitopes, which may be suppressed by the immune system. Phage display is the first and most widely used *in vitro* antibody selection technology. The approach is based on the groundbreaking work of George P. Smith on filamentous *E. coli* phage M13 and the fusion of peptides to the phage envelope proteins, which allows the phenotypic *in vitro* selection of the corresponding peptide encoding gene fragment packaged in the same phage particle.[23] The selection process was called "panning" because of the resemblance to the method used to find gold.[24]

Immediately after the discovery of smaller recombinant antibody formats like single-chain variable fragments (scFv)[25] and their secretory periplasmic expression in *E. coli*,[26] antibody phage display technology was independently developed in 3 different places: at the Deutsches Krebsforschungszentrum (DKFZ) in Heidelberg, Germany,[27] at the MRC Laboratory of Molecular Biology in Cambridge, United Kingdom[28,29] and at the Scripps Research Institute in La Jolla, USA.[30,31] All major phage display systems were based on antibody::pIII fusion proteins, but they differed in the location of the antibody gene expression cassette. The integration of the antibody::pIII encoding gene into the phage genome[29] was not as successful as the more flexible phagemid system,[27] which uncouples antibody::pIII expression from the expression of the phage proteins and from phage replication. The phagemid bears a bacterial origin of replication and antibiotic selection; in addition to the antibody::pIII expression cassette, it behaves more like a normal plasmid in the absence of other phage proteins. Thus, it can also be used for standard genetic manipulation and cloning procedures. For packaging into the phage particles, the phagemid contains an additional morphogenetic signal for replication of single-stranded DNA copies and packaging into phage particles during assembly, which requires the co-expression of the other M13 phage proteins provided by the helper phage.[27] This dual antibody surface display and expression system allows the switch between oligovalent and monovalent antibody display by using different helper phage, for example using Hyperphage for oligoclonal display.[32,33] After phage packaging, the antibody fragments are displayed as pIII fusion protein on the surface of M13 phage and the corresponding antibody gene fragment is packaged in the same phage particle. The combination of antibody genotype and phenotype in the same phage particle allows for the *in vitro* selection of antibody genes due to the encoded phenotypic antibody properties like antigen specificity, affinity, or stability. The physical linkage of antibody and phage pIII protein is usually achieved by genetic fusion of antibody and pIII, but there are also other approaches using conjugation via free cysteines[34] or dimerization motifs[35] or protein A-derived ZZ domains.[36] Small recombinant antibody fragments are commonly used for phage display, e.g., scFv[37-39] or fragment antigen-binding (Fab).[40,41] The smallest antibody fragments are human (single) domain (VH) antibodies (dAbs) or the variable domains of the naturally occurring camel heavy chain antibodies (VHH).[42-44] An antibody (scFv) phage and a corresponding phagemid are illustrated in Fig. 1. More detailed overviews are given in different reviews.[41,45,46] Full IgGs have even been displayed on phage,[36] but the *E. coli*'s secretion and folding apparatus cannot process complex antibody formats without a very strong bias. The analysis of a new advanced universal human scFv antibody library has not revealed such bias from the *E. coli* system regarding the frequency of V gene families and amino acid distribution in light and heavy chain CDR3s[47]. This analysis involved the comparison of more than 800 antibodies after selection to a variety of different antigens with the parental naïve library.

## The antibody libraries are "gold mines" for therapeutic antibodies

Antibody libraries are huge collections ($>10^{10}$) of antibody genes encoding antibodies with unknown properties. They are the essential source for antibody discovery by phage display and other *in vitro* selection technologies, and their design is critical to success. Two major types of antibody libraries are distinguished: 1) immune libraries, and 2) universal libraries.

Immune libraries are constructed from blood cell samples from immunized donors, which, in the case of human antibodies, is limited to donors who received vaccination, or patients who have suffered an infection or disease. In medical research, immune libraries are mostly used to discover antibodies against targets from infectious pathogens, e.g., antibodies neutralizing the human immunodeficiency virus (HIV) from long-term non-progressor patients,[48] West Nile virus[49] or immunized cancer patients.[50] Immune libraries can also be generated from immunized animals,[51-55] including macaques which have antibodies that are very similar to those of humans.[56] Use of transgenic animals containing human antibody gene repertoire allows the discovery of human antibody from such immune libraries.[57] Immune libraries already contain affinity-matured antibodies, because they are generated from antibody repertoires that underwent antigen driven *in vivo* selection, but these extended somatic hypermutations may increase the risk of immunogenicity.

Universal libraries generated from natural naïve human antibody gene repertoires are relatively close to the human antibody germ line and have low risk of immunogenicity. These also called "single-pot" libraries contain a very broad repertoire of antigen specificities theoretically covering every possible antigen.[45,58] Naïve antibody libraries are constructed from rearranged V genes from B cells of non-immunized donors, i.e., the IgM repertoire. These antibodies have already undergone *in vivo* selection for functional B cell receptors, including a selection for low immunogenicity and low toxicity. Nevertheless, antibodies to most if not all human antigens with relatively

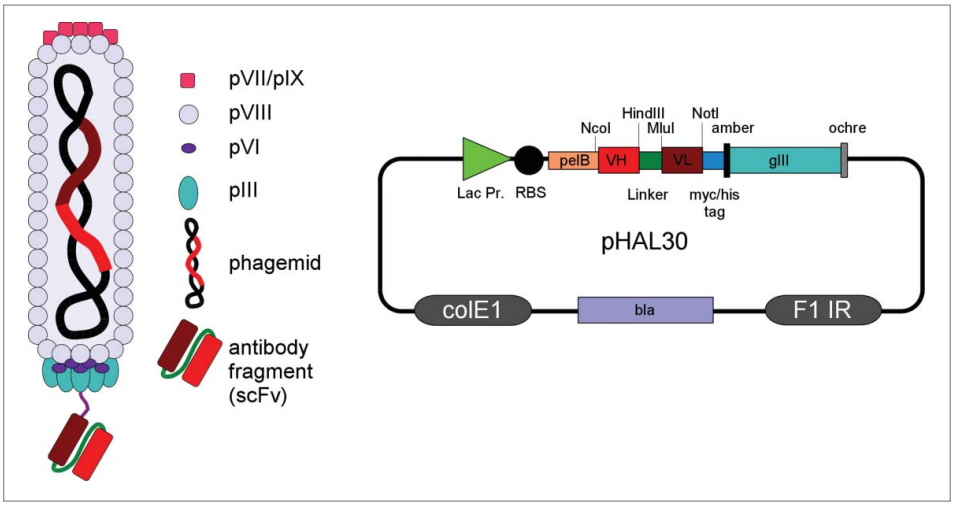


**Figure 1.** Schema of an antibody (scFv) phage and phage display vector (phagemid) pHAL30 [47]. Abbreviations: bla = $\beta$-lactamase, ampicillin resistance; ColE1 = bacterial origin of DNA replication; F1 IR = intergenic region of phage f1, phagemid packaging signal; gIII = phage gene encoding pIII; Lac Pro = promoter of lacZ; pIII, pVI, pVII, pVIII, pVIX = phage protein III, VI, VII, VIII, VIX; pelB = *Erwinia carotovora* pectate lyase B leader peptide; scFv = single chain fragment variable; VH = variable domain heavy chain; VL= variable domain light chain.

high affinities (low nanomolar to picomolar range) from naïve antibody libraries have been described.[59] Affinity engineering, a well-established technology, can also be done by phage display.[60] Examples of naïve universal libraries are the human Fab library constructed by de Haard and colleagues at Dyax (now Shire),[40] the scFv libraries from Cambridge Antibody Technology (CAT),[39,61] scFv and Fab libraries from XOMA[59] or the HAL scFv libraries.[37,47]

In addition to naïve universal libraries, which are derived completely from natural human IgM repertoire, there are also synthetic or semi-synthetic universal libraries consisting of only synthetic sequences or a mixture of natural and synthetic antibody sequences, respectively. Semi-synthetic libraries are constructed either from unrearranged V genes from pre-B cells[62] or by using one antibody framework[63] in which one (at least HCDR3) or several CDRs is endowed with randomized sequences. Semi-synthetic libraries like the Tomlinson I and J libraries consist of the stable VH IGHV3-23 framework and the $V_\kappa$ IGKV1-39 framework with randomized positions in CDR2 and CDR3.[64] Another strategy for semi-synthetic libraries is the amplification of all 6 CDRs of the natural antibody repertoire to create a pool of diverse CDRs, which are cloned into one defined antibody framework by overlap extension polymerase chain reaction (PCR) like the nCoDeR library of BioInvent.[65] A combination of naïve and synthetic repertoires was used for the Dyax FAB310 library. Here, entire light chains from autoimmune patients were combined with an Fd with the human IGHV3-23 framework endowed with synthetic CDR1 and CDR2 as well as naïve CDR3 regions from autoimmune patients.[41] Fully synthetic libraries developed by MorphoSys employing 7 human VH and 7 VL frameworks endowed with synthetic CDR cassettes generated by trinucleotide synthesis (human combinatorial antibody libraries, HuCAL). Recently, MorphoSys' Ylanthia library was generated based on 36 fixed variable VH and VL chain pairs, which cover a broad range of canonical CDR structures.[34,66-68] The theoretical size of universal libraries is now greater than $10^{10}$ independent clones.[38,47,59,61,68,69]

A particular type of antibody library is generated during guided phage display selection of human VH and VL repertoires using the counter chain of a murine monoclonal antibody as template. This strategy has been used for humanization, but often also for the discovery of new "fully" human antibodies with properties similar to the murine antibody template, like for adalimumab, the first approved phage display-derived therapeutic antibody.[70]

## *In vitro* selection of antibody panning nuggets

The process of *in vitro* selection of antibodies from libraries is usually referred to as "panning," because it resembles the procedure used to extract gold flakes or nuggets from a vast excess of silt by exploiting different physical properties. The situation in antibody phage display is quite similar because the appropriate antibody candidates are very rare in the library. As briefly discussed in the previous section, immune libraries contain a smaller antibody repertoire and often a higher percentage of antigen-specific antibodies, which may be one specific binder in $10^3$–$10^6$ non-antigen specific antibodies. In universal libraries the ratio can be one binder in $10^7$–$10^9$. Particularly for universal libraries, the *In vitro* selection process is extremely critical to successfully isolate antigen-specific antibodies. *In vitro* selection requires the immobilization of the target antigen to a solid surface, such as magnetic beads,[71] column matrices[27] or plastic surfaces with high protein binding capacity as polystyrene tubes or plates.[72] The latter is the most widely used method. Panning in solution, which involves the use of biotinylated antigens followed by a "pull-down" with streptavidin beads,[73] can also be done. The vast excess of non-binding antibody phage must be removed by stringent washing. Subsequently, the bound antibody phage will be eluted and reamplified by infection of *E. coli* and packaging with helper phage to produce a new antibody phage sublibrary, which will be used for another panning round until a significant enrichment of antigen specific antibody phage is achieved. Usually 2-3 panning rounds are required to enrich antibodies even from the

largest available libraries. The optimal selection strategy depends on many parameters, including the target antigen, the optimal antigen immobilization strategy, the quality of the library (particularly the frequency of antigen specific antibodies therein) and the binding and washing conditions. The tight control of the *in vitro* conditions allows the pre-design of antibody properties during the selection including epitope specificity,[73,74] and even conformation specificity[75,76] and interspecies cross-reactivity.[77] Screening for monoclonal antibodies can be performed either by antibody phage using enyzme-linked immunosorbent assay (phage ELISA) or by soluble expression of antibody fragments in *E. coli* and immunoassays, including ELISA or flow cytometry.[78] The antibody fragment can be directly used or converted into other antibody formats for therapeutic development, e.g., IgG, antibody-drug conjugates.[41,79-81] Fig. 2 shows a schema of the antibody phage display panning procedure.

## Phage display derived antibodies in clinical development

For this review, we collected data on phage display-derived therapeutic antibodies from different sources. General information about the antibodies and about their clinical development were collected from PubMed-indexed scientific articles and reviews. Additional information was also obtained from the clinical trial database www.clinicaltrials.gov, company websites, international ImMunoGeneTics information system (http://www.imgt.org/mAb-DB/query.action, Development status: Phase M in search field), conference presentations, patents and prescribing drug information and personal communication. Data on phage display-derived human antibodies that are approved for therapy by EMA or FDA, or have been investigated in clinical studies are provided in Table 1. Antibodies from discontinued clinical studies are also included. For some antibodies in clinical development, the origin and even the target is not publicly available, and we could not include these in the table. For example, NOV-7 to -11 were probably generated by phage display by MorphoSys in collaboration with Novartis (www.morphosys.com/pipeline/clinical), but the exact source of these antibodies has not been disclosed. We did not include phage display-derived antibodies in preclinical development or antibodies discovered by other technologies. A thorough and annually updated review about therapeutic antibodies in late-stage developments can be found in the "Antibodies to watch" article series from Janice Reichert.[1,82-85] A selection of phage display-derived antibodies and other peptide/protein therapeutics are described by Nixon *et al.*[86] A selection of phage display-derived antibodies is described in more detail in the following sections.

### *Adalimumab (Humira®)*

Adalimumab was the first phage display-derived monoclonal antibody, as well as the first human antibody, approved for therapy. It binds tumor necrosis factor (TNF) with subnanomolar affinity[87] and prevents TNF from binding to its receptors. TNF, the key initiator molecule in the proinflammatory cytokine cascade, binds to the receptors TNF-R1 and -R2, which leads to cell activation, inflammation and fever, acute phase responses and sepsis, cell survival and proliferation, as well as apoptosis.[88] The cross-talk of the different TNF-signaling pathways interferes on several physiological levels, and made it impossible to develop TNF itself as therapeutic agent. However, use of TNF inhibition with antibodies or soluble receptors to suppress a broad range of inflammatory autoimmune diseases

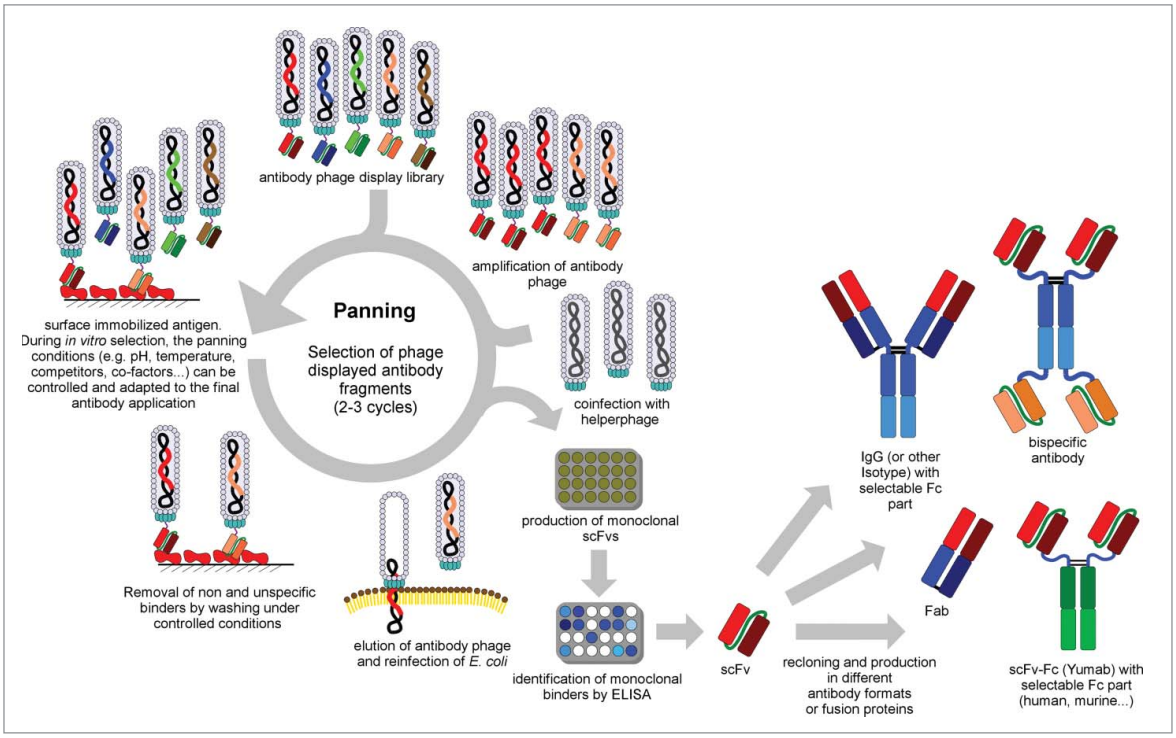


**Figure 2.** Schema of antibody (scFv) phage display selection, screening and reformating/production of other antibody formats (modified figure from reference 207).

**Table 1.** Phage display-derived antibodies and antibody conjugates evaluated in clinical studies, including those approved by FDA or EMA.

| Development name | Trade name | Target | Phage display library type and format | Phage display technology | Final antibody format | Sponsoring company | Highest development phase | Indication(s) studied |
|---|---|---|---|---|---|---|---|---|
| 1D09C3 | — | HLA-DR | synthetic, scFv | MorphoSys | IgG4 | GPC Biotech AG | Phase 1 (currently terminated) | Lymphoma, lymphocytic leukemia |
| Adalimumab (D2E7) | Humira | Tumor necrosis factor (TNF) | humanization by guided selection, scFv | CAT | IgG$\kappa$ | AbbVie | FDA approved 2002 | Rheumatoid arthritis |
| Adecatumumab (MT201/HD69) | — | EpCAM (CD326) | guided selection of light chain, naïve (IgD), Fab | Micromet | IgG1 | Amgen | Phase 2 (currently terminated) | Colorectal liver metastases, Prostate cancer |
| AMG-780 | — | Angiopoietin (ANG1/2) | naïve, Fab | Dyax | IgG2 | Amgen | Phase 1 (currently terminated) | Advanced solid tumors |
| Anetumab ravatansine (MF-T/BAY 86-1903, conjugate BAY 94-9343) | — | Mesothelin | synthetic, Fab | MorphoSys | IgG1$\lambda$, maytansinoid tubulin inhibitor DM4 conjugate | Bayer | Phase 2 | Adenocarcinoma, Mesothelioma, Non-squamous NSCLC |
| Anti-MIF (Imalumab, BAX69) | — | Macrophage migration inhibitory factor (MIF) | naïve, Fab | Dyax | IgG1 | Baxter | Phase 2 | Colorectal cancer, Advanced solid tumors |
| Avelumab | — | Programmed death-ligand 1 (PD-L1) | naïve, Fab | Dyax | IgG1$\lambda$ | EMD Serono, Pfizer | Phase 3 | NSCLC, Merkel cell carcinoma |
| BAY1093884 | — | Tissue factor pathway inhibitor (TFPI) | synthetic, Fab | MorphoSys | IgG2 | Bayer | Phase 1 | Hemophilia |
| BAY1129980 | — | C4.4a | synthetic, scFv | BioInvent | IgG1 (ADC) | Bayer Healthcare | Phase I | Advanced solid tumors |
| BAY1179470 | — | Fibroblast growth factor receptor 2 (FGFR2) | synthetic, scFv | BioInvent | IgG1 | Bayer Healthcare | Phase I | Advanced solid tumors |
| BAY1213790 | — | FXIa | naïve, Fab | Dyax | IgG1 | Bayer Healthcare | Phase I | Arterial thrombosis; Venous thrombosis |
| Belimumab | Benlysta | B-lymphocyte stimulator (BLyS) | naïve, scFv | CAT | IgG1$\lambda$ | GSK | FDA approved 2011 | SLE |
| Bertilimumab (iCo-008/CT-213) | — | Eotaxin-1 (CCL-11) | naïve, scFv | CAT | IgG4$\kappa$ | IMMUNE Pharma-ceuticals | Phase 2 | Ulcerative Colitis, Bullous pemphigoid |
| BHQ880 | — | Dickkopf-1 (DKK1) | synthetic, Fab | MorphoSys | IgG1 | Novartis | Phase 2 | Multiple myeloma |
| BI-505 | — | ICAM-1 (CD54) | synthetic, scFv | BioInvent | IgG1 | BioInvent | Phase 2 | Multiple myeloma |
| BI-1206 (6G11) | — | FcgRIIB (CD32B) | synthetic, scFv | BioInvent | IgG1 | BioInvent | Phase 1/2 | Non-Hodgkin lymphoma, Chronic lymphatic leukemia |
| BI 836845 | — | Insulin-like growth factor I and II (IGF-I, IGF-II) | naïve Fab | MorphoSys | IgG1$\lambda$ | Boehringer Ingelheim | Phase 1/2 | Advanced solid tumors, NSCLC, metastatic breast cancer, castrate resistant prostate-cancer |
| Bimagrumab (BYM338) | — | Activin A receptor type IIB (ACVR2B) | synthetic, Fab | MorphoSys | IgG1$\lambda$ | Novartis | Phase 3 | Cachexia, Sporadic inclusion body myositis, |
| BPS804 (MOR05813) | — | Sclerostin | synthetic, Fab | MorphoSys | IgG2 | Mereo Biopharma | Phase 2 | Osteoporosis, Hypophosphatasia, Osteogenesis Imperfecta |
| Briakinumab (ABT874) | — | Interleukin 12/13 | naïve, scFv | CAT | IgG1$\lambda$ | AbbVie | Phase 3 (currently terminated) | Psoriasis, MS, Crohn's Disease |
| Carlumab (CNTO-888) | — | Chemokine (C-C motif) ligand 2 (CCL2) | synthetic, Fab | MorphoSys | IgG1$\kappa$ | Janssen | Phase 2 (currently terminated) | Prostate cancer, Pulmonary fibrosis |
| Cixutumumab (IMCA12) | — | Insulin-like growth factor-1 receptor (IGF-1R) | naïve, Fab | Dyax | IgG1$\lambda$ | ImClone | Phase 2 (currently terminated) | Different cancer types (also in combination with other drugs) |
| CNTO-3157 | — | Toll-Like Receptor 3 (TLR-3) | synthetic, Fab | MorphoSys | ND | Janssen | Phase 2 | Asthma |
| CNTO-6785 | — | Interleukin 17A (IL17A) | synthetic, Fab | MorphoSys | ND | Janssen | Phase 2 | Rheumatoid Arthritis, Chronic obstructive pulmonary disease |
| Darleukin (L19-IL2) | — | Extra-domain B of fibronectin | semi-synthetic, scFv | "Pini" library | scFv-IL2 fusion | Philogen | Phase 3 | Melanoma |
| Dekavil (F8-IL10) | — | Extra-domain A of fibronectin | semi-synthetic, scFv | ETH-2 | scFv-IL10 fusion | Pfizer | Phase 2 | Rheumatoid Arthritis |

(*Continued*)

**Table 1.** (*Continued*)

| Development name | Trade name | Target | Phage display library type and format | Phage display technology | Final antibody format | Sponsoring company | Highest development phase | Indication(s) studied |
|---|---|---|---|---|---|---|---|---|
| Drozitumab (Apomab, PRO95780) | — | TRAIL-R2 | ND, scFv | Genentech | IgG1$\lambda$3 | Janssen | Phase 1, Phase 1b (currently terminated) | Advanced/metastatic solid malignancy or NHL, metastatic colorectal cancer. |
| Elgemtumab (LJM716, NOV6) | — | ERRB3 (HER3) | synthetic, Fab | MorphoSys | IgG1$\kappa$ | Novartis | Phase 1/2 | Esophageal squamous cell carcinoma, breast cancer, gastric cancer, neoplasm |
| Fibromun (L19-TNF) | — | Extra-domain B of fibronectin | semi-synthetic, scFv | "Pini" library | scFv-TNF fusion | Philogen | Phase 2 | Melanoma, soft tissue sarcoma |
| Fresolimumab (GC1008) | — | Transforming growth factor $\beta$ (TGF$\beta$) | naïve, scFv | CAT | IgG4$\kappa$ | Sanofi | Phase 2 | Scleroderma, metastatic breast cancer, NSCLC, fibrosis, focal segmental glomerulosclerosis |
| Foravirumab (CL184, CR4098) | — | Rabies virus, glycoprotein | immune, scFv | Crucell | IgG1$\kappa$ | Sanofi | Phase 2 | Rabies |
| Ganitumab (AMG479) | — | Insulin-like growth factor receptor (IGF-1R) | naïve, Fab | Dyax | IgG1$\kappa$ | Amgen | Phase 3 (currently Phase 2; Phase 3 study for pancreatic cancer terminated) | Pancreatic cancer, Metastatic Ewing Sarcoma, metastatic colorectal cancer |
| Gantenerumab (R1450) | — | Amyloid fibrils | synthetic, Fab | MorphoSys | IgG1$\kappa$ | Roche | Phase 3 | Alzheimer |
| Guselkumab (CNTO1959) | — | P19 subuntit of Interleukin 23 | synthetic, Fab | MorphoSys | IgG1$\lambda$ | Janssen | Phase 3 | Psoriasis |
| IMC-3C5 (LY3022856) | — | Vascular endothelial growth factor receptor-3 (VEGFR-3) | naïve, Fab | Dyax | IgG1 | Eli Lilly | Phase 1 (currently terminated) | Solid tumors |
| Istiratumab (MM-141) | | Bispecific antibody recognizing insulin-like growth factor 1 receptor (IGF-1R) and ErbB3 | anti-ErbB3: semi-synthetic Fab | anti ErbB3: Dyax | Bispecific IgG2-scFv | Merrimack | Phase 2 | Metastatic pancreatic cancer |
| Lanadelumab (DX-2930) | — | Kalikrein | naïve, Fab | Dyax | IgG1 | Dyax | Phase 3 | Hereditary angioedema |
| Lexatumumab (HGSETR2) | — | TRAIL receptor 2 (TRAIL-R2) | naïve, scFv | CAT | IgG1$\lambda$ | HGS | Phase 1 (currently terminated) | Solid tumors |
| Mapatumumab (HGSETR1) | — | TNF-related apoptosis inducing ligand receptor 1 (TRAIL-1) | naïve, scFv | CAT | IgG1 | HGS/GSK | Phase 2 | Hepatocellular carcinoma, multiple myeloma, cervical cancer, NSCLC |
| Mavrilimumab (CAM3001) | — | Granulocyte macrophage-colony stimulating factor receptor (GM-CSF) | naïve, scFv | CAT | IgG4$\lambda$2 | Astra Zeneca | Phase 2 | Rheumatoid arthritis |
| MEDI4212 | — | IgE | naïve, scFv | CAT | IgG1 | MedImmune | Phase 1 | Asthma |
| MEDI9447 | — | CD73 | naïve scFv | CAT | IgG | MedImmune | Phase 1 | Solid tumors |
| MM-302 (C6.5) | — | HER2/neu (ErbB2) | naïve scFv | "Schier" library | scFv, targeted liposomal doxorubicin | Merrimack | Phase 2/3 | Breast cancer |
| MOR103 (MOR04357, GSK3196165) | — | Granulocyte macrophage-colony stimulating factor receptor (GM-CSF) | synthetic, Fab | MorphoSys | IgG1 | GSK | Phase 2 | Rheumatoid arthritis, MS |
| MOR202 | — | CD38 | synthetic, Fab | MorphoSys | IgG1 | MorphoSys | Phase 1/2a | Multiple myeloma |
| Namilumab (MT203) | — | Granulocyte macrophage-colony stimulating factor receptor (GM-CSF) | humanization by guided selection, naïve (rat CDR3 in human VH), scFv | Micromet | IgG1$\kappa$ | Takeda | Phase 2 | Rheumatoid arthritis, Psoriasis |
| Necitumumab (IMC11F8) | Portrazza | Epidermal Growth Factor Receptor (EGFR) | naïve, Fab | Dyax | IgG1$\kappa$ | ImClone/ Lilly | FDA approved 2015 | Squamous NSCLC |

(*Continued*)

**Table 1.** (*Continued*)

| Development name | Trade name | Target | Phage display library type and format | Phage display technology | Final antibody format | Sponsoring company | Highest development phase | Indication(s) studied |
|---|---|---|---|---|---|---|---|---|
| NI-0501 | — | Interferon-gamma | naïve, scFv | CAT | IgG1 | Novimmune/ Serono | Phase 2/3 | Hemophagocytic lymphohistiocytosis |
| Opicinumab (BIIB033) | — | LINGO1 | naïve, Fab | Dyax | IgG1$\kappa$ | Biogen | Phase 2 | Acute optic neuritis, MS |
| Orticumab (BI-204/ MLDL1278A) | — | oxLDL | synthetic, scFv | BioInvent | IgG1$\lambda$ | Genentech | Phase 2 (currently terminated) | Atherosclerotic vascular disease |
| Radretumab (L19-$^{131}$I, L19SIP) | — | extra-domain B of fibronectin | semin-synthetic, scFv | "Pini" library | scFv-$C_H4$-Iodine-131 fusion | Philogen | Phase 2 | Hodgkin Lymphoma |
| Ramucirumab (IMC1121B) | Cyramza | Vascular endothelial growth factor receptor 2 (VEGFR2) | naïve, Fab | Dyax | IgG1 | ImClone/ Lilly | FDA approved 2014 | Gastric cancer, colorectal cancer and non-small cell lung cancer |
| Ranibizumab | Lucentis | Vascular endothelial growth factor A (VEGFA) | affinity maturation of bevacizumab by phage display | Genentech | Fab | Genentech | FDA approved 2006 | Macular degeneration |
| Raxibacumab | Abthrax | Protective antigen (PA) | naïve, scFv | CAT | IgG1$\lambda$ | GSK | FDA approved 2012 | Anthrax |
| PC-mAb (M99-B05) | — | Phosphorylcholine | naïve, Fab | Dyax | IgG1 | Athera | Phase 1 | Cardiovascular disease |
| Seribantumab (MM121) (SAR256212) | — | Epidermal growth factor receptor 3 (ErbB3) | naïve, Fab | Dyax | IgG2 | Merrimack | Phase 2 | Breast cancer, Ovarian cancer, NSCLC |
| Tanibirumab (TTAC0001) | — | Vascular endothelial growth factor receptor 2 (VEGFR-2) | naïve, scFv | PharmAbcine | IgG1 | PharmAbcine | Phase 1 | Advanced cancer, metastatic cancer |
| Tarextumab (OMP59R5) | — | Notch2/3 | synthetic, Fab | MorphoSys | IgG2$\kappa$ | OncoMed | Phase 2 | SCLC, Pancreatic cancer, ssolid tumors |
| Teleukin (F16-IL2) | — | Extra-domain A1 of tenascin-C | semi-synthetic, scFv | ETH-2 | scFv-IL2 fusion | Philogen | Phase 2b | Merkel cell carcinoma, Angiomyolipoma |
| Tesidolumab (LFG316) | — | Complement C5 | synthetic, Fab | MorphoSys | IgG1$\lambda$ | Novartis | Phase 2 | Geographic atrophy, Paroxysmal nocturnal hemoglobinuria, Macular degenaration, Panuveitis |
| Tralokinumab (CAT354/ BAK1.1) | — | Interleukin 13 | naïve, scFv, BAK1 scFv affinity maturation by phage display | CAT | IgG4 | Astra Zeneca/ Abbott | Phase 3 (currently terminated) | Asthma, Atopic dermatitis, Ulcerative Colitis |
| Utomilumab (PF05082566) | — | 4-1BB (CD137, TNFRSF9) | synthetic, Fab | MorphoSys | IgG2 | Pfizer | Phase 1 | Solid tumors |
| Vantictumab (OMP18R5) | — | frizzled family receptor 7 (Fzd7) | synthetic, Fab | MorphoSys | IgG2$\lambda$ | OncoMed/ Bayer | Phase 1 | NSLC, Breast cancer, Pancreatic cancer, solid tumors |
| VAY-736 (NOV-5) | — | B-cell-activating factor receptor (BAFF-R) | synthetic, Fab | MorphoSys | IgG1$\kappa$ | Novartis | Phase 2 | MS, Primary Sjögren's syndrome, Pemphigus vulgaris, Chronic lymphocytic leukemia |

Abbreviations: ADC, antibody-drug conjugate; EMA, European Medicines Agency; Fab, fragment antigen-binding; FDA, Food and Drug Administration; IgD, immunoglobulin D; IgE, immunoglobulin E; IgG, immunoglobulin G; IL2, interleukin 2; $\kappa$, kappa light chain; $\lambda$, lambda light chain; MS, Multiple sclerosis; ND, not determined; NHL, NonHodgkin lymphoma; NSCLC, Non-small cell lung cancer; scFv, single chain fragment variable; SCLC, Small cell lung cancer; SLE, Systemic lupus erythematosus.

was possible,[89] and today 5 different biological TNF antagonists are on the market, i.e., adalimumab (Humira®), infliximab (Remicade®), golimumab (Simponi®), certolizumab pegol (Cimzia®) and etanercept (Enbrel®).

Adalimumab was discovered through a collaboration between BASF Bioresearch Corporation/Knoll and CT in 1993. BASF/Knoll had extensive experience with TNF from a Biogen collaboration, which failed to develop TNF into a commercialized drug product. At that time, BASF also developed the anti-TNF murine monoclonal antibody MAK195 as an F(ab')2 for acute reactions like sepsis (afelimomab, Segard).[90] MAK195 mediated potent TNF inhibition, but, as a murine antibody, it was not considered for treatment of chronic autoimmune diseases. The company decided to develop a new human antibody that would have low immunogenicity and long plasma half-life. MAK195 was used as a template for guided selection of human antibody V-domains with CAT's antibody phage display technology, with the goal to achieve binding to a similar epitope on TNF with similar high affinity. Two scFv libraries were constructed, one library with MAK195 VH combined with a human variable light chain repertoire and one library with MAK195 VL and a heavy chain repertoire. Both libraries were used for panning on TNF. From the selected hybrid scFvs, human VH and VL were combined in a third library and

reselected on TNF. Subsequently, CDR mutagenesis was performed, resulting in D2E7.[70] The final clinical candidate D2E7 was taken through most of the drug development process by Knoll before BASF sold its pharma division, including Knoll, to Abbott Laboratories, which performed further development, manufacturing and marketing. In clinical trials, adalimumab demonstrated long-term efficacy and safety, and its convenient subcutaneous administration. Ready-to-use liquid formulation and every-other-week dosing with the option to increase to weekly also in combination with methotrexate (MTX) were also beneficial for patients.[91-94]

Adalimumab was first approved by the FDA on December 31, 2002 for treatment of moderate-to-severe forms of rheumatoid arthritis as monotherapy or in combination with MTX or other disease-modifying anti-rheumatic drugs, and then marketed by Abbott Laboratories (AbbVie after the company split in 2013) under the brand name Humira®, which stands for "human monoclonal antibody in rheumatoid arthritis." When Humira® was launched, it was the third TNF inhibitor on the market, after the chimeric monoclonal antibody infliximab and the TNF-R Fc fusion protein etanercept. Despite this late start in the market, adalimumab developed into the best-selling antibody drug, achieving more than 10 billion USD global sales in 2013.[2] At a dose of 40 mg per patient subcutaneously every 2 weeks, adalimumab has a lower discontinuation risk and simpler administration (autoinjector pen)[95] compared to infliximab (3–10 mg/kg intravenously every 4–8 weeks). Etanercept (50 mg per patient subcutaneously per week), however, had a better retention rate than adalimumab or infliximab as first-line biotherapy in rheumatoid arthritis, and adalimumab as second-line biotherapy.[96]

Today, adalimumab is approved in the United states (US), European Union (EU), and Japan (JP) for moderate-to-severe chronic rheumatoid arthritis in adults and polyarticular juvenile idiopathic arthritis in children, psoriasis and psoriatic arthritis, ankylosing spondylitis, pediatric and adult Crohn's disease,[97] ulcerative colitis (US and EU only), hidradenitis suppurativa, axial spondyloarthritis (EU only), intestinal Behçet disease (JP only), and it is in clinical testing for treatment of sarcoidosis and uveitis.[98] Treatment emergent adverse effects (TEAE) of adalimumab like reactivation of latent infections, such as tuberculosis, or a higher prevalence of opportunistic infections are mainly due to the TNF suppression and also occur with other TNF inhibitors.[99] The risk of lymphoma is 3-fold higher compared with the general population according to the Humira® Injection Prescribing Information and Medication Guide (Abbott Laboratories; IL, USA: 2009), but there is no significant difference in tumor risk compared to rheumatoid arthritis patients naïve to anti-TNF therapy.[100] Due to rare reports of serious liver injury, demyelinating central nervous system disorders, and cardiac failure, as well as anaphylaxis and serious allergic reactions, physicians are instructed to carefully monitor patients. Adalimumab is indicated for adult Crohn's disease patients who are already intolerant to infliximab, whereas it is not indicated for TNF-blocker resistant ulcerative colitis patients. In 2014, Zydus Cadila Healthcare Ltd., launched in India the first adalimumab biosimilar (Exemptia®) for about a fifth of Humira® price, in the Indian market, where the Humira® patent was not claimed. Humira® is protected by many patents, the main US patent US6090382 will expire in 2016.

### Belimumab (Benlysta®)

The technologies for DNA sequencing that emerged in the early 1990s led to the discovery of a new member of the tumor necrosis factor (TNF) ligand family, B-lymphocyte stimulator (BLyS, now TNFSF13B) by Human Genome Sciences (HGS). BLyS was suggested to be involved in monocyte-driven B-cell activation.[101,102] In 2001, several groups reported a connection between elevated levels of circulating BlyS and systemic lupus erythematosus (SLE).[103,104] Patients with SLE generate autoreactive antibodies that deregulate inflammatory responses and form immune complexes, the deposition of which causes target organ failure. Probable inherent abnormalities in the immune system and an environmental trigger, e. g., ultraviolet radiation, infection with the Epstein-Barr virus, may lead to ineffective clearance of apoptotic nuclear fragments, which are processed by antigen-presenting cells (APC) and presented to autoreactive T cells. These T cells activate autoreactive B cells to produce anti-self antibodies. These act as APCs and release proinflammatory cytokines such as interferon-$\alpha$, interleukin (IL)-6, IL-17, TNF, a proliferation-inducing ligand (APRIL) and BLyS.[105-107]

BLyS was chosen as a target for the development of an antagonistic antibody that is able to block B-cell activation, but also affects Th1 and/or Th17 cells.[108-110] In a collaboration with CT, HGS selected about 1,200 antibodies from a scFv phage display library[39] that were able to inhibit BLyS activity.[111] Affinity maturation lead to the development of LymphoStatB, subsequently named belimumab, which was able to deplete B cells in spleen and lymph nodes of cynomolgus monkeys.[112] A Phase 1 clinical study of belimumab in individuals with mild-to-moderate SLE disease was initiated in 2001. Efficacy was not shown in this study due to small number of patients and brief treatment studies. Nevertheless, it was considered successful, and 2 Phase 2 studies in SLE and rheumatoid arthritis patients were initiated.[113,114] The Phase 2 study also did not show a significant effect of belimumab in SLE patients, which could have led to the discontinuation of the development of this antibody, but a deeper analysis revealed a deficiency in the study design. A modified analysis revealed that belimumab-treated patients had a significantly better response than patients treated with the standard of care plus placebo.[115] The FDA accepted the new interpretation of the results, and a pivotal trial was initiated in 2007.[116] Belimumab was ultimately approved for SLE in 2011. The drug was marketed as Benlysta® by GlaxoSmithKline (GSK), which purchased HGS one year later. At the same time the transgenic mouse-derived human antibody tabalumab, which also targets BlyS and was developed by Eli Lilly, was terminated in clinical Phase 3 studies due to lack of efficacy.[117]

### DX-2930

In classical hereditary angioedema (HAE), patients have a deficiency of the acute phase protein C1 esterase inhibitor, which regulates plasma kallikrein (pKal), factor XIIa and other

proteases of the plasma contact system. pKal liberates bradykinin involved in vasodilation and inflammation. This results in local edema affecting the skin, gastrointestinal tract, genitourinary tract and larynx[118-120]. A potential treatment is the inhibition of pKal, which is also involved in the complement system and coagulation cascade. Using phage display, Dyax selected a 66 amino acid Kunitz domain that blocks pKal activity. The Kunitz domain DX-88 (ecallantide) was approved by the FDA in 2009 and marketed by Dyax under the brand name Kalbitor®.[121-123] As a follow-on to the peptide program, human antibodies were selected from the Dyax FAB310 phage library using biotinylated pKal immobilized on streptavidin beads. The Fab M0162-A04 inhibits active pKal, but not prekallikrein or any other serine protease and was further affinity matured resulting in the Fab M0199-A08. The human IgG1 variant DX-2930 showed good pharmacokinetic and pharmacodynamic properties in cynomolgus monkey, as well reduced edema in a rat model.[124] Subsequently, a successful clinical phase 1 study was performed.[119] In October 2015, a Phase 3 study was started (ClinicalTrials.gov Identifier: NCT02586805). In January 2016, Shire acquired Dyax mainly because of DX-2930 and DX-88.

### Anti-TRAIL receptor antibodies

Mapatumumab (HGS-ETR1) is a human monoclonal antibody that binds and activates the tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1; also known as, TNFRSF10A, APO2, CD261, and death receptor 4). TRAIL-R1 (and 2) are death receptors that activate the second key signaling mechanism for caspase activation and apoptosis induction. The natural ligand TRAIL (Apo2L) binds and activates both TRAIL-R1 and TRAIL-R2. Overexpression of TRAIL-R1/2 in some tumor types, as well as their distinct, p53-independent mode of apoptosis activation via extracellular ligand binding makes these receptors attractive targets for agonistic ligands and antibodies designed as cancer therapies.[125]

The agonistic TRAIL-R1 antibody mapatumumab (HGS-ETR1) was discovered by phage display by CAT using the Vaughan library[39] in collaboration with HGS (now GSK) in 1999.[126] It induces cell death in multiple tumor cell lines *in vitro* and *in vivo*, which can be enhanced by combination with chemotherapeutic agents like camptothecin, cisplatin, carboplatin, or 5-fluorouracil.[126] Mapatumumab demonstrated safety and tolerability in cancer patients with advanced solid tumors or non-Hodgkin's lymphoma (NHL), both as a single agent and in combination with chemotherapy.[127-129] Although no clinical activity of single-agent mapatumumab was observed in patients with refractory colorectal cancer in a Phase 2 trial,[130] its favorable safety profile and pre-clinical evidence of potential synergy with chemotherapy was the rationale for further clinical testing. Mapatumumab in combination with paclitaxel and carboplatin in unselected patients with advanced non-small-cell lung cancer (NSCLC) did not reveal any clinical benefit compared to chemotherapy alone.[131,132] However, a Phase 1b/2 trial conducted in patients with relapsed or refractory non-Hodgkin's lymphoma as monotherapy with 6 doses of either 3 or 10 mg/kg mapatumumab every 21 days achieved clinical responses in 3 patients with follicular lymphoma (FL), 2 of them with complete responses and one with partial response.[133] A randomized Phase 2 trial of mapatumumab in combination with the proteasome inhibitor bortezomib for treatment of advanced multiple myeloma did not demonstrate efficacy compared to the control group.[134]

Lexatumumab (HGSETR2) targets pro-apoptotic TRAIL-receptor 2 (TRAIL-R2, TNFRSF10B, CD253, DR5), and is another phage display derived human antibody from the collaboration of CT and HGS.[126] Like mapatumumab, lexatumumab mediated synergistic effects in combination with chemotherapeutics.[135] In combination with radiation, lexatumumab showed some clinical activity in pediatric solid tumors.[136]

Drozitumab (Apomab, PRO95780, Roche/Genentech) was also generated by phage display from a human scFv library against TRAIL-R2.[137] A Phase 1 study demonstrated safety in humans at doses up to 20 mg/kg and evidence of activity in several different tumor types at 4 and 10 mg/kg.[138] In contrast to these and other agonistic antibodies, soluble recombinant human TRAIL (dulanermin) did not result in objective responses in patients with indolent B-cell Non-Hodgkin lymphoma (NHL)[139] or NSCLC.[140]

### Granulocyte-macrophage colony-stimulating factor antibodies

MOR103 is a human monoclonal antibody specific for granulocyte-macrophage colony-stimulating factor (GM-CSF) developed by MorphoSys in collaboration with GSK (GSK3196165). The proinflammatory cytokine GM-CSF is produced by a variety of cells including activated T and B cells, monocytes/macrophages, endothelial cells, and fibroblasts and it stimulates myeloid differentiation of haematopoietic stem cells, proliferation and activation of macrophages, monocytes, neutrophils, eosinophils, dendritic cells, and microglia.[141] GM-CSF plays a critical role in autoimmune diseases as shown in a murine collagen-induced arthritis model,[142,143] transgenic mice constitutively expressing GM-CSF develop autoimmune gastritis[144] and elevated levels of GM-CSF correlate with the active phase of multiple sclerosis (MS).[145,146]

MorphoSys developed MOR103 with phage display selection from the HuCal Gold library[67] followed by affinity maturation with tri-nucleotide cassette mutagenesis[147] of CDR-L3 and CDR-H2, affinity-driven selection and cross-cloning of the best candidates.[148] MOR103 (MOR04357) inhibits human GM-CSF with an $IC_{50}$ in the low picomolar range and cross-reacts with rhesus and rat GM-CSF.[148] MOR103 was tested for treatment of moderate rheumatoid arthritis in a Phase 1b/2a trial using 0.3, 1.0 or 1.5 mg/kg once a week for 4 weeks and with follow-up to 16 weeks. MOR103 was well tolerated and showed preliminary evidence of clinical efficacy.[149] In Phase 1b study, MOR103 was well-tolerated in patients with relapsing-remitting MS and secondary progressive MS without any evidence of immunogenicity.[150]

Namilumab/MT203 is another GM-CSF antagonizing antibody under clinical evaluation by Takeda. MT203 was discovered by Micromet by guided selection using a rat monoclonal antibody as template in combination with technology licensed from CAT. The antibody contains a human VL domain and humanized VH with parental rat HCDR3.[151,152]

Mavrilimumab, which inhibits the GMCSF receptor, is undergoing evaluation in Phase 2 studies of patients with rheumatoid arthritis.[153] This antibody was developed by CAT, which was acquired by AstraZeneca in 2006, using phage display.[154]

## Antibodies targeting epidermal growth factor receptor, vascular endothelial growth factor or vascular endothelial growth factor receptors

### Necitumumab (Portrazza®)

The epidermal growth factor receptor (EGFR) plays an important role in many cancers, including NSCLC. In 40 to 80% of lung cancer patients, EGFR is overexpressed and a high copy number can be found in up to 60% of the patients.[155-157] Stimulation of the receptor by ligand binding leads to activation of different signaling cascades, such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3k)/Akt pathway. This results in cell proliferation, differentiation, invasion and metastasis, which promotes the malignant phenotype.[158]

Consequently, EGFR is the target of different types of anticancer therapeutics, including small-molecule inhibitors of the kinase domain (e. g., gefitinib, erlotinib) or monoclonal antibodies to the receptor domain (e. g., chimeric cetuximab).[159]

Necitumumab (clone name IMC-11F8) was developed by ImClone Systems, Eli Lilly and BristolMyers Squibb (BMS) from the "de Haard" Dyax Fab phage display library.[40] In this case, epidermal carcinoma cells (A431) were used to screen for antibodies that blocked EGFR activation. Necitumumab binds to the receptor with an affinity of 3.3 ± 0.5 nM.[160] The antibody blocks the binding of several relevant ligands and inhibits the proliferation of different cancer cell lines.[161] Clinical studies were initiated in 2004, and necitumumab was finally approved by the FDA in 2015 for combinatory therapy with gemcitabine and cisplatin of squamous NSCLC.[162] Necitumumab treatment resulted in increased toxicity and mortality in non-squamous NSCLC. Necitumumab is marketed by Eli Lilly under the brand name Portrazza®. Currently, there are ongoing clinical trials to investigate safety and efficacy in other NSCLC-related indications.[160] Necitumumab was also tested in combination with chemotherapy (modified chFOLFOX6) in first-line treatment of locally advanced or metastatic colorectal cancer. The combination therapy was active and toxicity was manageable.[164]

### Ranibizumab (Lucentis®)

Endothelial cells can be stimulated by angiogenic factors to form new blood vessels[165] to generate new vascularization. Additionally, angiogenic factors are known to play an important role in diverse cancer diseases, rheumatoid arthritis and macular degeneration.[166-168] Bevacizumab (Avastin®, Roche) is a humanized antibody derived from the murine antibody A.4.6.1. which binds to vascular endothelial growth factor (VEGF).[169] Avastin® is approved for the treatment of colon, lung, breast, kidney and ovarian cancer. Bevacizumab was affinity maturated using phage display in the Fab format.[170] The results of an alanine-scanning study and the crystal structure[171] of the Fab-VEGF complex were used to build different antibody gene libraries. For each CDR of the heavy chain a single mutation library was constructed by randomizing only those amino acid positions shown to be important for the interaction with VEGF. Additionally, another mutation library was constructed with mutations at particular sites in the framework 3 of the VH domain. Off-rate selection was used to identify antibody clones with higher affinity. Finally, the beneficial mutations were combined and screened for high affinity antibodies. Clone Y0317 (subsequently named ranibizumab) had an affinity to VEGF of about 0.1 nM and contained only 6 mutations compared to the parental antibody. Ranibizumab demonstrated a 100-fold improvement for inhibition of VEGF-dependent cell proliferation compared to the parental antibody.[170] The Fab of ranibizumab is produced in *E. coli* and was first approved by the FDA in 2010 for the treatment of macular edema following retinal vein occlusion. In 2012, it was approved for the treatment of diabetic macular edema, and in 2015 for treating diabetic retinopathy. Novartis markets ranibizumab under the brand name Lucentis®. The humanized parental IgG bevacizumab and the affinity-matured Fab ranibizumab have equivalent effects on visual acuity for the treatment of neovascular age-related macular degeneration (AMD) when administered according on the same schedule.[172] Bevacizumab is not approved for treatment of AMD, but is used off-label for this purpose.

### Ramucirumab (Cyramza®)

Angiogenesis is one of the most important steps for cancer progression.[167] The VEGFs and their receptors are involved in this process. To date, 4 human VEGFs[173] and 3 receptors[174-178] have been identified. The most important receptor for angiogenesis is thought to be VEGFR2 (also known as kinase insert domain receptor (KDR)), which forms a homodimer,[179,180] but also heterodimers that include other receptors involved in signal transduction.[181,182] The importance of the VEGF angiogenesis pathway for cancer progression led to the development of humanized antibodies against VEGF, such as bevacizumab[183] and ranibizumab,[170] as well as VEGF-binding recombinant fusion proteins. These consist of peptides derived from the extracellular domains of human VEGF receptors 1 and 2 (i.e. VEGF-trap) fused to human IgG1 Fc discovered by Regeneron and named aflibercept (Eylea®, approved for AMD in 2011; co-development with Bayer) or ziv-aflibercept (Zaltrap®, approved in 2012 for colorectal cancer; co-development with Sanofi).[184] Another strategy was the inhibition of the VEGF receptors.

Ramucirumab was screened from the de Haard Fab phage display library[40] against recombinantly expressed kinase insert domain-containing receptor (KDR or VEGFR2) fused to alkaline phosphatase.[185] Here, 4 different antibody candidates were identified that were able to block the interaction between VEGF and human VEGF-R2, inhibit VEGF-induced proliferation of human endothelial cells and the migration of VEGF-R2 positive leukemia cells. Three of the 4 clones contained the same heavy chain sequence. Therefore, this VH was used for the generation of a new phage display library, in which this heavy chain was paired with the variable light chain repertoire of the naïve library.[186] The new library was screened under modified conditions to enrich antibodies with higher affinities,

and clone 1121, which showed a more than 30-fold increase in affinity (100 pM), better receptor ligand binding inhibition and better inhibition of receptor phosphorylation by ligand binding, was identified.[187] Clone 1121 (later named ramucirumab) had an ~8-fold higher affinity to the receptor compared to that of the natural ligand, VEGF.[188]

The results of a Phase 1 clinical dose-escalation trial to evaluate safety, maximum-tolerated dose, pharmacokinetics and pharmacodynamics of ramucirumab appeared very promising with regard to its anti-cancer activity in patients with different kinds of cancers.[189] Starting in 2009, Phase 3 clinical trials were carried out with patients suffering from advanced gastric or gastro-esophageal junction adenocarcinoma. The results showed that ramucirumab was the first biological treatment given as a single drug that provided survival benefits in these patients.[190]

Ramucirumab was approved by the FDA in 2014 for the treatment of advanced gastric or gastro-esophageal junction adenocarcinoma and metastatic non-small-cell carcinoma. The antibody is marketed by Eli Lilly under the brand name Cyramza®. In 2013, Eli Lilly announced that Phase 3 studies of ramucirumab for the treatment of breast and liver cancer were terminated due to lack of efficacy.

## Antibodies for infectious diseases

### Raxibacumab (Abthrax®)

Anthrax is an infectious disease caused by *Bacillus anthracis*, an aerobic, Gram-positive, spore-forming bacterium that is found in soils around the world. The Centers for Disease Control and Prevention (CDC) classified anthrax as category A agent, indicating that it has a high risk of potential use as bioweapon.[191] *B. anthracis* secrets 2 toxins, the lethal toxin (LT) and the edema toxin (ET).[192] Both toxins are composed of 2 subunits. The LT consists of the lethal factor (LF) and the protective antigen (PA); the ET is formed by the edema factor (EF) and PA. PA is necessary for the cellular uptake of both toxins.[193] For this reason, PA was chosen as the target for the development program that yielded raxibacumab, which neutralizes PA with an $IC_{50}$ of 0.21 nM. Binding kinetics of raxibacumab to PA revealed an equilibrium binding constant ($K_d$) of 2.78 nM. Raxibacumab was selected for PA neutralization by HGS (now part of GSK) using a naïve human scFv phage display library licensed from CAT. The final format is an IgG1$\lambda$ mAb. In 2012, the FDA approved raxibacumab to treat inhalational anthrax. The use of raxibacumab in combination with antibiotics is advised.[194,195] An alternative to targeting PA is the inhibition of the lethal factor complex formation.[196]

### Foravirumab

Rabies is caused by the rabies virus, which infects the central nervous system. Before Louis Pasteur developed a rabies vaccination, the disease was always fatal. The current post-exposure therapy is based on vaccination, as performed by Pasteur in 1885, and polyclonal anti-rabies immunoglobulins.[197-199] Crucell selected a panel of recombinant antibodies against the rabies glycoprotein from an immune scFv library.[200] The neutralization of 27 wild type rabies viruses ("representative street rabies viruses") was tested *in vitro*, and the best neutralizing antibodies were tested *in vivo* in a hamster rabies infection model. Here, the antibody CR4098 showed 100% post-exposure protection with 40 IU/kg.[201] This antibody was further analyzed in combination with another human antibody, CR57, derived by somatic cell hybridization technique,[202] in *in vitro* and *in vivo* models.[199] The safety of this cocktail, named CL184, was tested in a clinical Phase 1 study,[203] and subsequently in 2 Phase 2 studies (ClinicalTrials.gov Identifier: NCT00708084, NCT00656097). The antibodies were named foravirumab (CR4098) and rafivirumab (CR57). This program demonstrated that antibody phage display using immune libraries is an efficient technology for the discovery of neutralizing antibodies to infectious pathogens and toxins.

## Summary and outlook

The large number of phage display-derived antibodies in clinical development demonstrates the value of this *in vitro* selection technology for the discovery of therapeutic antibodies. Phage display has not only proven to be a versatile and reliable platform technology for the discovery of "fully" human antibodies for over 2 decades, but it has been successfully used for the development of other types of peptide and protein therapeutics, such as ecallantide (Kalbitor®, Dyax/Shire),[122] Xyntha purification peptide[204] and the peptibody trebananib (AMG-386, Amgen)[205] (for review see Nixon *et al.*[86]).

The changing intellectual property landscape will further affect development. For many years access to antibody phage display was controlled by a few companies, based on their technology patents. But these, namely the Breitling/Dübel (EP0440147) and McCafferty/Winter (EP0774511, EP0589877) families, expired in 2011 in Europe. In the US the patent US5969108 (filed 1990, granted 1991, published 1999), US6172197 (filed 1991, granted 1995, published 2001) and US6806079 (filed 1990, granted 2000, published 2004) are most relevant. The expiration of US patents is more complex and depends on variables such as the filling date, the grant date, patent term adjustments and extensions. Together with lower pricing driven by the competition with biosimilar products, the patent expiry should spur innovation and allow more companies to advance human antibodies made by phage display into the clinic.

Antibody phage display has advantages over newer technologies like yeast or mammalian cell display, including library size ($>10^{10}$), robust and well-established *E. coli* expression system, scalability, short panning cycles and cost-efficiency, but also *in vitro* selection conditions and the ability to pan on antigen-expressing cells. Issues with *E. coli* expression and phage display of full size IgGs can be addressed with efficient expression platforms and screening technologies.[206] The power of phage display lies in the choice of the optimal *in vitro* selection conditions, which allows pre-definition of many biophysical and biochemical antibody properties at a very early step of the discovery process. For example, it is possible to target distinct antigen epitopes, even specific conformations, address interspecies cross-reactivity, and reduce off-target binding using phage display. Moreover, the technique can be combined with immunization and immune libraries to get the best of these antibody discovery approaches.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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