We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 3

Display Technologies for the Selection of Monoclonal Antibodies for Clinical Use

Lilian Rumi Tsuruta, Santos Mariana Lopes dos and Ana Maria Moro

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70930

Abstract

The development and improvement of strategies related to discovery technologies of monoclonal antibodies (mAbs) (phage display, yeast display, ribosome display, bacterial display, mammalian cell surface display, mRNA display, DNA display, transgenic animal, and human B-cell derived) opened perspectives for the screening and the selection of therapeutic antibodies for, theoretically, any target from any kind of organism. The implantation of a robust platform of antibody discovery technologies allows reaching this goal. Additionally to recombinant antibody selection, antibody engineering technologies were developed and explored to obtain desired characteristics of selected leading candidates such as high affinity, low immunogenicity, improved functionality, improved protein production, improved stability, and others. mAb humanization methods emerged as alternative for generating humanized variants of promising candidates obtained from non-human organism that could elicit an immune response. This chapter contains an overview of discovery technologies, mainly display methods and antibody humanization methods for the selection of therapeutic humanized and human mAbs that appeared along the development of these technologies and thereafter. The increasing applications of phage display technology will be highlighted in the antibody engineering area (affinity maturation, guided selection to obtain human antibodies) giving promising perspectives for the development of future therapeutics.

Keywords: phage display, yeast display, mRNA display, DNA display, bacterial display, mammalian cell surface display, ribosomal display, fully human mAbs, humanization, therapeutic mAbs

INTECH
open science | open minds

© 2018 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

1. Introduction

The sale of therapeutic monoclonal antibodies (mAbs) is increasing yearly in relation to other class of biological products [1], and pharmaceutical/biotechnological companies are pursuing all opportunities to develop this product. Therapeutic mAbs are indicated to diverse clinical conditions such as treatment of cancers, immune-mediated disorders and infectious diseases, among others. Every year, new mAbs are approved while a crescent number of other mAbs advance to the next phase of clinical study [2].

Generation of mAbs started with the discovery of the hybridoma technology by Köhler and Milstein [3] in 1975. The fusion of B cells from immunized animals with myeloma cells originated hybrid cells producing unlimited quantities of antibodies with unique specificities. The potential of this technique for clinical and diagnostic use became evident with the approval of the first therapeutic mAb 10 years later, Orthoclone OKT3 targeting the CD3 receptor present in T lymphocytes to control renal transplantation rejection [4]. The clinical success conducted immediately to the development of other mAbs derived from this technology. However, due to the non-conforming sequence of murine mAbs, the generation of an immune response in humans was observed and the use of higher and multiple doses for prolonged time was not possible. Murine mAbs can induce immunogenicity, human anti-mouse antibodies (HAMA, including human anti-idiotype antibodies) affecting the safety and therapeutic efficacy [5]. The evaluation of mAb immunogenicity is crucial during clinical trials and it is recommended by regulatory agencies.

With advances in the understanding of antibody structure and in molecular biology techniques, the field of antibody engineering emerged with objective to change antibody properties by altering its primary sequence. Antibody humanization techniques use antibody engineering approaches to produce antibodies with less immunogenicity and preservation of affinity and specificity of the parental antibody of non-human origin [6]. The first humanization technique led to the combination of variable region domains of a murine antibody with human constant region domains resulting in chimeric antibodies with 70% of human content [7]. Chimeric antibodies maintained the specificity of parental murine antibody and demonstrated decreased immunogenicity, however, they generated human anti-chimeric antibodies (HACA) in approximately 40% of patients [5]. Efforts to obtain mAbs with a minimum of immunogenicity resulted in the development of a technique where sequences responsible for the antibody specificity to the antigen called complementarity-determining regions (CDRs) were transplanted to human framework sequences. This technique was designated CDR grafting and generated humanized antibodies [8–10]. However, it was observed that most of the antibodies obtained by CDR grafting did not preserve the affinity of the parental murine antibody. This fact is due to the influence of the human framework on the structure of the transplanted mouse CDRs [11, 12]. Through 3-dimensional modeling, key residues were identified in the murine framework sequences that interacted with CDRs and the antigen representing the integrity of the antigen-binding site. In a maneuver of back mutating the identified critical framework residues to the mouse framework sequence, antibodies with affinities close to the parental murine antibody were obtained. Using this approach Zenapax® (daclizumab) was approved by FDA in 1997 for therapeutic use for the prevention of renal transplantation rejection [13]. Soon the antibody humanization technique became viable for the clinical application

of mAbs of non-human origin. In relation to immunogenicity, approximately 9% of humanized antibodies induced human anti-humanized antibodies (HAHA) in different clinical trials [5]. A less frequent humanization technique aiming to retain the original antibody affinity by altering only the mouse surface-exposed residues of the framework (veneering) was used with success for an anti-NaPi2b oncologic antibody where the humanized version (Rebmab200) demonstrated a slightly improved affinity in relation to the murine version (MX35) [14, 15].

Concomitant to the utilization of antibody humanization techniques started the development of *in vitro* display technologies and the exploration of the molecular diversity of the antibody genes present in a determined library. The first technology available was phage display by presenting exogenous peptides on filamentous bacteriophage (phage) surfaces [16]. The peptide sequences were fused to the amino-terminus of the p3 protein of phages and the fusion protein was expressed on the phage surface, further purified by affinity through the binding to a specific antibody. Selected phages were amplified in bacteria. Then, a biopanning process, a classical cyclic procedure to select phage clones presenting peptides by affinity binding was described [17]. The occurrence of the phage display technology to select mAbs depended on the development of two techniques. Firstly, the expression of functional antibody fragments (scFv and Fab fragment) in the periplasm of *Escherichia coli* was reported [18, 19]. The second technique to mention is the PCR for amplification of antibody genes (heavy and light chains) from hybridomas, a pool of prokaryotic and eukaryotic cells transfected with antibody genes, human peripheral blood cells or tissues rich in B cells [20–22]. The amplification of human immunoglobulin genes directly from immune or naïve human materials opened the possibility to select human antibodies from these sources, either for diagnostic or clinical purposes. In phage display technology, amplified antibody genes are cloned into appropriate phage display vectors to construct the library. Antibody fragments present in the library are expressed on the phage surface and then are submitted to biopanning to select phages by the binding to a specific peptide epitope or antigen. After some cycles of biopanning, the phages encoding the antibody fragments are analyzed individually to select specific clones. Soluble antibody fragments are expressed allowing the characterization of the antibodies and isolation of lead clones [23, 24]. Using phage display technology, six human mAbs were approved for therapeutic use and other candidates are in advanced phases of clinical studies [25]. Other *in vitro* display technologies were developed such as yeast display, ribosome display, bacterial display, mRNA display, mammalian cell surface display and DNA display and, although mAbs with therapeutic potential were obtained by using these other *in vitro* display technologies, no one reached the clinical approval so far.

In the present chapter, we describe discovery technologies to select human therapeutic mAbs.

2. Display technologies to obtain recombinant monoclonal antibodies

In vitro display technologies such as phage display, yeast display, ribosome display, bacterial display, mammalian cell surface display, mRNA display and DNA display represent *in vitro* selection platforms of specific molecules presented in a determined library. These technologies are used mainly to isolate peptides and antibody fragments in scFv, single domain

antibody (sdAb) or Fab fragment formats. These technologies mimic the *in vivo* process for antibody generation and have four key steps as it occurs *in vivo* inside the immune system: (1) generation (or cloning) of genotypic diversity; (2) linkage between the genotype and phenotype; (3) application of selective pressure and (4) amplification. This process was initially developed for the collection of recombinant antibody genes from B lymphocytes from immunized mice and naïve or infected humans. The immunoglobulin gene repertoire is cloned into a vector to provide the connection between the genotype and the phenotype of each antibody, and clones are selected through the binding to the specific antigen [26]. The isolated clones are expressed in sufficient amounts to characterize them and to select the best candidate.

The main advantage of the *in vitro* display technology is the possibility to obtain antibodies to any kind of targets and epitopes because the construction of a naïve or synthetic antibody repertoire is not dependent on an *in vivo* immune response. Even antibodies against self-antigens, toxic, unstable and non-immunogenic antigens could be isolated by selection from a combinatorial antibody library [25].

Phage display technology is one of the main platforms for generation of human therapeutic antibodies together with transgenic immunized mice, antibody humanization techniques and single B cell expression cloning [26, 27].

2.1. Phage display technology

Phage display technology was the first *in vitro* display technology developed by presenting an exogenous peptide on the filamentous phage surface [16].

The first antibody combinatorial library was constructed on a λ lytic bacteriophage vector that releases Fab fragments from the periplasm after cell lysis. The screening of Fab fragments was successfully done by transferring the material to nitrocellulose filters followed by binding to radiolabeled antigen [28]. The same methodology was applied for cloning a human antibody library and selection of specific antibodies [29]. The library was constructed from antibody genes of peripheral blood lymphocytes of individuals that had recently received a tetanus toxoid immunization.

Biopanning procedures to select antibody fragments/peptides presented on the phage surface are more efficient for clone screening, since it allows to isolate clones with defined specificity and affinity [17]. Phage display libraries were initially established by the construction of peptide libraries on filamentous phages [30–32], not lytic to bacteria, and recombinant phages are released at the time they are assembled in the bacterial membrane. The expression of scFvs on the filamentous phage surface was described, allowing antibody selection from the library through binding to the antigen [33, 34]. Fab fragments can be presented on the phage surface and, in this case, one antibody chain is fused to the phage p3 protein and directed in the periplasm and the other antibody chain is secreted directly in the periplasm where the Fab fragment is assembled [35–37]. The presentation of single domain antibodies (dAbs) on phage surfaces was also reported [38]. The p3 protein is expressed on the external phage surface, presented in five copies, being involved in the bacterial infection through the binding of bacterial F pilus with the N-terminus of p3 protein [39–41]. The p3 protein vector system is mostly used for the selection of scFv, Fab fragments and single domain antibodies. The advantages are explained in the next section.

http://dx.doi.org/10.5772/intechopen.70930

2.1.1. Vector systems to display exogenous molecules

Phage was initially used as a cloning vector and exogenous genes were inserted in its genome. This system leads to multiple presentations of exogenous peptides fused with phage p3 or p8 protein, making it difficult to select specific antibodies due to the avidity effect [16]. Antibody fragment genes were fused to the N-terminus of p3 phage protein and it was observed that phages continued to be infective [33, 35]. Phagemid vectors emerged as alternative of direct cloning into the phage genome (**Figure 1**).

Phagemids are plasmids containing the intergenic region (IR) of filamentous phage. The IR allows replication of the phagemid mediated by helper phage and packing of phagemid single-strand DNA. Phagemid vectors present some advantages in relation to phage genome cloning: double-strand DNA is obtained for library construction, the copy number of the protein presented on the phage surface may be controlled, easy conversion to soluble protein production by insertion of an amber stop codon between recombinant protein and p3 protein [35], and higher stability due to the resistance of exogenous molecules toward deletions [43]. The main protein fusions presented by phagemids are minor p3 or major p8 coat proteins and phagemids contain g3 or g8 genes near to the cloning site of exogenous proteins. Phagemids efficiently express recombinant proteins; however, they do not amplify phages. When bacteria containing phagemids are infected by a helper phage, it is possible to amplify phages since the helper phage synthesizes all phage proteins. Helper phages are filamentous phages with inactive packing signal, replication-deficient origin and generally carry an antibiotic resistance gene. Helper phage superinfection leads to the expression of both wild type protein and fusion protein on the phage surface. During the phage assembly, there is a

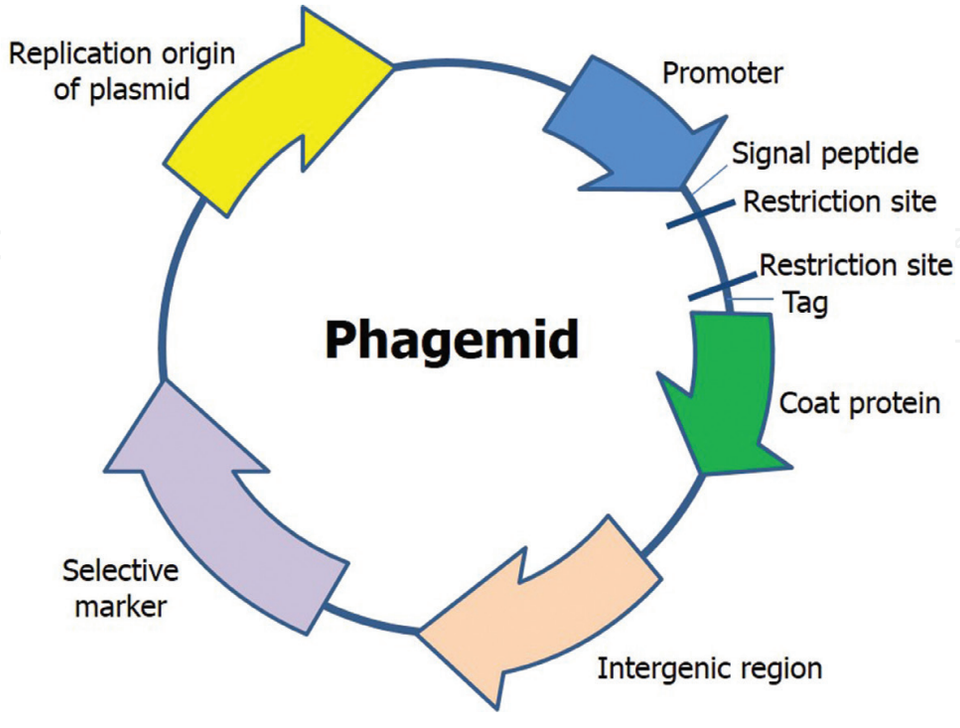


Figure 1. Scheme of phagemid vector used in phage display technology. Adapted from Qi et al. [42].

competition between the two proteins for virion incorporation. Phagemids are preferentially packed by helper phage due to the defective origin of the helper phage [44]. Phage p8 protein is the main surface protein, presenting 2700 copies per phage and has been used to present peptides [45–47] and antibody fragments [48–50]. The phage population using p8 fusion protein is multivalent, presenting approximately 900 peptides [46] and 24 Fab fragments [51] per phage particle. In the latter work, it was verified that the avidity effect of multiple copies on the phage surface did not allow selection of antibodies with high affinity. On the other hand, fusion proteins formed by antibody fragments and p3 proteins are presented in 1-3 copies because the expression of the fusion protein can be repressed by an inducible promoter.

Phage incorporates the fusion protein (antibody fragment and p3 protein) in the virion, while the wild-type p3 protein is produced by the helper phage. The presence of wild-type p3 protein is necessary for the bacteria infection. It was observed that when the N-terminal domain of p3 protein binds to bacteria F′ pili, the bacteria cell infected with the phage acquires immunity to the superinfection of other phages such as helper phage [23]. For this reason, the N-terminal domain of the p3 protein was deleted from fusion protein of the first phagemid vector presenting an Fab fragment, pComb3 [36] and other vectors presenting Fab fragments [37, 52].

2.1.2. Antibody library construction

Antibody gene combinatorial libraries with light and heavy chain genes can be constructed from various sources; human peripheral blood is the most used and spleen, lymph nodes and bone marrow are used if possible. The libraries do not represent the repertoire of antibody gene pairs because the heavy and the light chain genes are amplified separately and then paired randomly by PCR or cloning. This feature increases the diversity of the library and consequently the chance to obtain antibody clones with the desired specificity. Therefore, each antibody library represents a unique repertoire.

Basically, there are two kinds of antibody libraries to select recombinant antibodies: the library derived by a source which immune system had not been activated to recognize a specific antigen (*naïve*), and the immune library derived from donors who received immunization, have been infected or chronically diseased or suffer from cancer [26]. The immune library contains an affinity-matured antibody repertoire and therefore, higher affinity antibodies can be selected in comparison to a naïve library. In the development of human therapeutic antibodies, it is not possible to construct immune libraries to each disease due to ethical issues. Moreover, the construction of a specific library is laborious, high-costing and time-consuming to be considered for each antigen. The option in these cases has been the use of a naïve human library formed by the V, D-J-genes of the IgM repertoire from not intentionally immunized donors. The B cell repertoire can potentially contain also memory cells to previous immunization/infections of donors. In principle, a naïve library can be applied for mAb selection to any kind of antigen, since the library comprises a high variability of immunoglobulin genes and comprises antibody genes from many donors. Some antibodies isolated from a human naïve library presented good specificity to the target and low affinity being necessary to increase it for clinical applications [53]. Naïve human libraries later constructed presented larger sizes

and higher affinity clones could be selected. As examples, we can cite Dyax (now Shire) [54], CAT [55, 56], XOMA [57] and HAL [58, 59] naïve human libraries. To increase the variability and size, human semi-synthetic and synthetic libraries [60–73] were constructed based on germline framework sequences, framework sequences of known antibody sequences, consensus framework sequences and human germline VH and VL gene segments. The diversity of antibody sequences was particularly introduced by randomization of CDR sequences, mainly CDR3. A semi-synthetic library was obtained by random mutation of the CDR3 sequence of the heavy chain from a single antibody clone [60] resulting in higher affinity derived clones. An alternative to randomization of CDRs was devised, introducing any codon combination at a specific position to obtain more nature-like antibodies [74] using two strategies: trinucleotide phosphoramidites [66, 68, 70] and Slonomics approaches [71, 72]. HuCAL, HuCAL GOLD and HuCAL PLATINUM libraries are examples of synthetic libraries obtained by trinucleotide phosphoramidites [66, 68, 70] and the Ylanthia library was constructed by the Slonomics method [72].

2.1.3. Antibody selection from library

The scheme of antibody phage display technology is shown in **Figure 2**. The selection of phages presenting specific antibodies occurs by the specific binding to the antigen by a biopanning process. Only phages expressing antibodies on the surface are amplified and after the biopanning process, the library is enriched with clones presenting moderate or high affinity to the antigen. In principle, only a single round of selection would be sufficient, however, unspecific binding of antibodies presented by phages limits the library enrichment and in practice, two to five rounds of a biopanning process are performed for antibody isolation. Enriched phages obtained at the first round can be amplified in bacteria culture and then submitted to subsequent rounds of biopanning process. Some methodologies were used to isolate phage clones presenting specific antibodies. Phages can be selected by antibody binding to the antigen coated in microplates [36, 53], to antigen-coupled to resins [33], in solution using biotinylated antigens [75], or to antigen present on a cell surface [76]. After antibody phages are bound to the antigen, they are washed and then eluted by soluble antigen [77], acid solution [36] or alkali solution [53].

After antibody clone selection, the candidates are expressed and characterized individually to evaluate their affinity and specificity. Phagemid vectors can display or secrete antibody fragments through incorporation of an amber stop codon between the antibody fragment gene and the p3 protein. Antibody fragments are displayed on the phage surface when the suppressor *E. coli* strain is transfected and antibodies can be expressed when the non-suppressor *E. coli* strain is used because the amber codon is read as stop codon in this case [35]. Another option would be the conversion from a phage display vector to an expression vector of a soluble antibody fragment [36] or recloning antibody genes to an expression vector [77].

In general, in the case of naïve or synthetic human antibody libraries, the antibody affinity is proportional to the library size and, in the case of naïve human antibody libraries, the dissociation constant (K_D) was in the 10^{-6} to 10^{-7} M range for small libraries [53] and in the order of 10^{-9} M for larger libraries [55, 57].

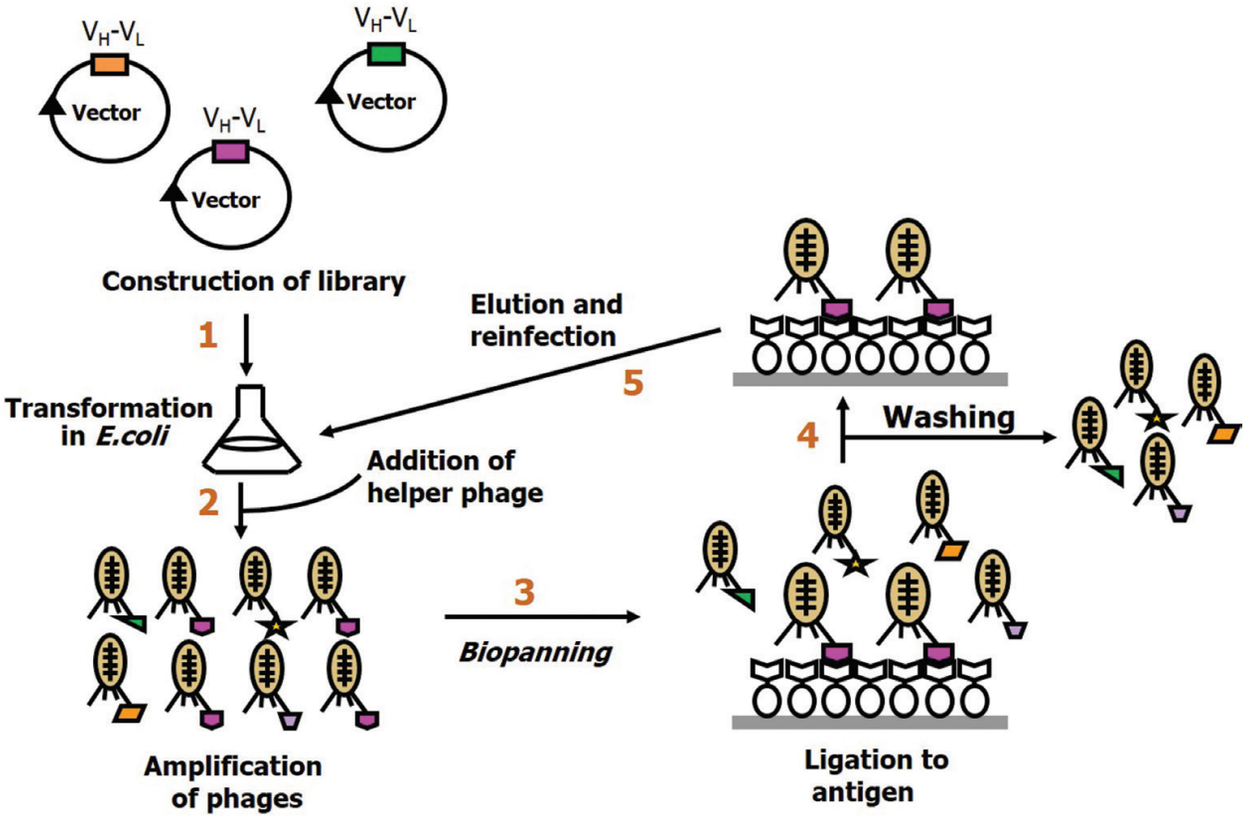


Figure 2. General scheme of phage display technology for selection of scFv fragments. (1) After scFv library construction, an aliquot is transformed in *E. coli* and grown up to OD_{600} of 0.6. (2) Helper phage is introduced to the culture and phages displaying antibody fragments are obtained. (3) Binding of scFvs displayed by phages to the antigen coated in wells of a microtiter plate. (4) Non-binding phages are washed away. (5) Phages are eluted and then reinfected into *E. coli* for a new round of antibody selection.

2.1.4. Affinity maturation of antibodies

The affinity of antibodies selected by naïve or synthetic phage display antibody libraries is generally adequate for research purposes; however they present low affinity for therapeutic applications. In these cases, selected clones should be submitted to the affinity maturation process [43]. Phage display technology allows the construction of a second antibody library originating from a selected clone, facilitating the selection of human antibodies for therapeutic use. Mutagenesis strategies have been applied to improve antibody affinity [23]. A point mutation library was constructed using error-prone PCR [78] simulating random point mutations generated by natural somatic mutation. This approach was used for anti-progesterone scFv fragments obtained from a naïve antibody library and the antibody affinity increased 30-fold. Other work constructed mutant scFv libraries against digoxigenin with low, moderate and high PCR error rate and showed that the highest antibody affinity improvement was related to higher mutation rates in the positions outside of the CDRs [79]. In addition, introduction of hot spot mutations in antibody germline sequences led to higher affinities of antibodies [80]. This approach presents advantage in relation to previous mutagenesis strategy due to the construction of smaller size libraries allowing parallel screening of multiple mutant libraries.

Mammalian cell surface display of antibody libraries coupled with *in vitro* somatic hypermutation (SHM) by the action of the activation-induced cytidine deaminase (AID) enzyme emerged as an interesting approach for affinity maturation of human antibodies [81–83]. In mammalian cell surface display, full-length and glycosylated IgGs are displayed by a transmembrane domain on the cell surface and antibody clones are isolated by screening techniques such as magnetic bead selection followed by *in vitro* SHM induction of clones which had been selected further by flow cytometry [82]. *In vitro* SHM has the ability to reproduce the *in vivo* human antibody maturation in non-B cells and introduces both point mutations and insertion/deletion to the antibody sequence in the region related to antigen contact [83]. One special characteristic of AID is that this enzyme acts preferentially toward germline hotspot motifs (WRCH) present in the antibody DNA sequence, specifically in the variable domains, and promotes point mutations at high rates in amino acid residues which change the antibody binding features [83].

2.1.5. Guided selection technique for antibody humanization

The technique of guided selection has the advantage of allowing selection of a human-nature antibody from a non-human original antibody sequence without the analysis of the antibody structure using *in vitro* display technology. Two publications demonstrated the viability of this technique using an "in series" process by different approaches [84, 85]. One publication used a rodent heavy chain (MAb32), Fd fragment, as a template and ligated it into a vector containing a human light chain repertoire [84]. A phage repertoire was selected by the antigen and the isolated human light chains were paired to a repertoire of human heavy chains. The selection of phages by the antigen was performed again and a human antibody clone was obtained recognizing the N-terminal region of the mouse antibody's target and demonstrating a similar affinity constant as the original clone. [84]. Figini et al. constructed a hybrid library combining the light chain of a murine anti-hapten antibody and the human heavy chain repertoire, from which human heavy chain sequences were isolated based on the light-heavy chain pairing ability to combine and bind to the antigen. The selected sequences were paired to the human light chain repertoire to obtain the human antibody [85].

Humira (adalimumab), an anti-TNF-α mAb, was isolated using this technique by an "in parallel" process, and it represents the first fully human antibody and also the first mAb derived by phage display technology approved by FDA in 2002 [86]. This anti-TNF-α mAb was obtained using the rodent antibody sequence (variable domains of heavy and light chains) as a template. Initially, two hybrid libraries were constructed; one formed by pairing the murine heavy chain sequence and a human light chain variable domain repertoire, and the second library was composed of the murine light chain sequence paired to a human heavy chain variable domain repertoire. Both hybrid libraries were selected against TNF-α and the antibody clones were isolated. The human antibody sequences obtained by the screening of each library were combined and the resulting library was reselected against the antigen. A human anti-TNF-α antibody was obtained and, after submitting to CDR mutagenesis, the high affinity D2E7 mAb was isolated with main indications for rheumatoid arthritis and Crohn's disease [86]. One disadvantage of the guided selection technique is that the construction of hybrid libraries can result in changes of the paratope altering the antibody fine specificity as observed previously [50, 87, 88]. This phenomenon was observed by using an "in series"

process of guided selection technique for human interferon γ receptor 1 (IFNGR-1) [89]. To obtain a human antibody with the same paratope of the original rodent antibody, a variation of this technique was performed by the preservation of the CDR3 sequence of the heavy chain from the non-human template sequence [90, 91].

2.1.6. Advantages of phage display technology

Antibody phage display technology is more utilized in the discovery of therapeutic mAbs than any other display technologies due to many advantages: (1) it was the first display technology developed so the methodology is well-established; (2) it uses the low cost *E. coli* expression system; (3) it is possible to construct large naïve libraries for selection of human antibodies against most antigens and desired epitopes; (4) selection process is versatile with the ability to determine the epitope of the antibody; (5) it can be used for affinity maturation of antibodies; and (6) antibody humanization by guided selection technique to obtain human antibodies is possible [25, 92]. As disadvantages, it can be mentioned: (1) selection of an antibody with low affinity from naïve and synthetic libraries that implies that *in vitro* affinity maturation for therapeutic application is recommendable; and (2) low antibody expression in *E. coli* for some isolated antibody sequences.

2.2. Yeast display

The yeast display technology uses yeast cells to present exogenous peptides or antibody fragments on the cell surface. In this technology, exogenous molecules are fused to the a-agglutinin adhesion receptor of *Saccharomyces cerevisiae* which is localized on the yeast surface and the cell repertoire can be screened by flow cytometry [93]. A-agglutinin receptor acts as adhesion molecule that stabilizes cell-cell interactions and promotes fusion between an "a" and α haploid cell to obtain a diploid cell. This receptor is composed of the Aga1 and Aga2 protein. Aga1 protein is secreted and binds covalently to b-glucan present in the extracellular matrix of the yeast cell wall. Aga2 protein binds to Aga1 protein through two disulfide bonds and the antibody fragment is fused to the C-terminal end of Aga2 protein (**Figure 3**) [94]. Aga1 protein and the fusion protein are expressed by induction of the GAL1 promoter with galactose [95]. ScFv is the most frequent antibody format displayed on yeast surface. Other formats include Fab fragment, whole IgG and single domain antibodies [38, 96].

The library is constructed by cloning the antibody genes into the yeast display vector and has smaller size than phage display libraries. After yeast transformation and growth, antibody expression is induced and then flow cytometry screening is performed with specific ligands to enrich binders of the library. After about three rounds of antibody selection, the enriched library is transformed in *E. coli* for analysis of plasmid DNA by sequencing [96].

Yeast display technology is used to improve the ligand affinity of scFv fragments and thermal stability of proteins [97–99] and also to isolate novel antibodies with suitable affinity and specificity [94]. One advantage of this technology is the characterization of binding properties such as affinity of one clone displaying an antibody fragment on the yeast surface without sub-cloning, expression and purification steps [94]. The display level is diverse and the binding

http://dx.doi.org/10.5772/intechopen.70930

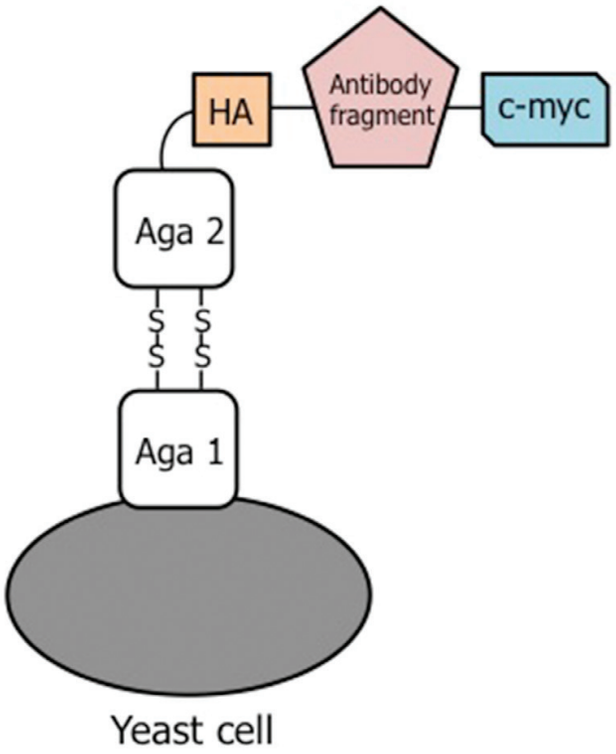


Figure 3. Scheme of the a-agglutinin receptor of yeast cell surface presenting a recombinant antibody fragment as fusion protein with Aga2 protein. Hemagglutinin (HA) tag is fused to the C-terminal end of the Aga2 protein followed by antibody fragment and c-myc tag that is fused to the C-terminal end of the recombinant antibody fragment. The presentation of the fusion protein can be monitored by anti-tags antibodies and/or binding to antigen labeled with fluorophore. Adapted from Boder and Wittrup [93].

avidity is reduced due to the cell sorting potential of flow cytometry. Yeast cells can be sorted according to the antibody binding range to the antigen and also by antibody expression range on the yeast surface through labeling the cell with fluorophore-conjugated antigen and with reagents to identify epitope of tags [94]. Boder's group used kinetic screening for a yeast display library containing random mutations and they isolated scFv clones with high affinity (48 fM) [100]. A naïve human scFv library composed by more than 10^9 clones was constructed by yeast display technology; antibody clones were firstly selected by magnetic bead screening followed by flow cytometry screening, isolating antibodies with an affinity of nM range [101]. Magnetic bead screening was applied to remove yeast cells not presenting antibody fragments like the biopanning process used in the phage display technology. As a consequence, the yeast display library size became suitable for the antibody selection by flow cytometry [96].

The construction of a yeast display vector displaying a Fab fragment was reported [102] and a single vector presenting two expression cassettes with the GAL1 promoter was used. The heavy chain fragment was expressed as a fusion protein to the N-terminal end of Aga2 protein and the light chain gene was expressed as a soluble fragment, resulting in a Fab fragment assembled on the yeast surface. The affinity matured library allowed the selection of high affinity antibodies.

A novel approach combining phage and yeast display technologies was tested to select Fab fragments against antigen 85 of *Mycobacterium tuberculosis* [103]. Initially, a large naïve scFv

library was selected by antigen binding using phage display technology. The enriched library with compatible size to flow cytometry screening was cloned into yeast display vector and highly specific antibodies were obtained. This strategy could be applied to any antigen and will serve as an alternative approach to isolate high affinity antibodies.

The major advantage of yeast display technology is the library screening using flow cytometry that allows defining specific parameters to select antibodies displayed on yeast surface. Yeast displaying antibodies can be sorted by binding to fluorescently-conjugated antigen and it is possible to separate clones quantitatively using binding affinity or dissociation kinetics parameters. Yeast cells can display about 10^5 antibodies and the expression range can be evaluated also by binding of epitope tags using fluorophore-labeled reagents [104].

Another advantage is that this platform uses yeast and therefore presents post-translational modifications like existing in the mammalian system but not present in bacteria. Post-translational modifications include glycosylation of antibodies, appropriate protein folding in the endoplasmic reticulum in the presence of chaperones and improved solubility of the product [96, 104].

In comparison to phage display technology, a yeast display platform has a limitation of the library size, due to lower efficiency of yeast transformation [96, 104]. Efforts to circumvent this problem led to the improvement of yeast transformation efficiency, allowing the construction of libraries with 10^{10} clones [105]. Another disadvantage of this platform is the multivalent display of antibodies on yeast surface and the selection based on avidity parameters [96, 104].

Among cell surface display-based technologies, we mentioned yeast and mammalian display technologies in this chapter. A prokaryotic system such as bacterial cell surface display (either Gram-negative or Gram-positive bacteria) has also been developed with the selection of high affinity binders [106–108]. The library screening is performed through the binding of the protein displayed on the cell surface to a specific fluorescently labeled ligand and analyzed mainly by flow cytometry as occurs in other cell surface display technologies. An *E. coli* display system was first extensively studied for antibody fragment isolation, then variations of this methodology were developed by genetic engineering [108–110], and full-length IgG was also displayed on bacteria [111]. There are few works considering Gram-positive bacterial cell surface display. A Gram-positive *Staphylococcus* display system was investigated [108, 112] and high affinity small proteins known as affibody molecules were selected using this system [113, 114], opening an attractive perspective for future development.

2.3. mRNA and ribosome display technologies

mRNA display technology emerged together with ribosome display technology as a revolutionary *in vitro* display platform to obviate cell transformation steps. Both technologies are centered in a fully cell-free strategy to select specific binders with great potential for diagnostic and clinical use.

The characteristics of mRNA display and ribosome display technology allows construction of libraries much larger than other *in vitro* display technologies because it avoids the library size limitation of phage and yeast display technologies which are dependent on the efficiency

of cell transformation steps. Another advantage of this technology is the extensive use of PCR that contributes to the introduction of more diversity to the library by mutations. The mRNA and ribosome display platform can be applied for selection of novel molecules, including antibody fragments, and for affinity maturation of antibodies [115].

During ribosome display, an antibody repertoire is constructed *in vitro* comprising mRNA, ribosome and translated antibody fragment [116]. The vector used contains no stop codon so that the translated antibody fragment is not released from ribosome and mRNA. After biopanning and ribosome disruption, mRNA is isolated and RT-PCR follows. Then, the cDNAs of the antibody fragments are amplified by PCR to obtain DNA for the next round of selection.

One special feature of mRNA display technology is the use of an adaptor molecule between the mRNA and translated proteins that binds them covalently forming a very stable complex to thermal and physicochemical stress that could be present during the selection step [117, 118]. In mRNA display technology, DNA molecules are initially *in vitro* transcribed to mRNA, then mRNA is translated and covalently ligated to the translated protein through puromycin, an adaptor molecule. The number of mRNA-protein complexes formed determines the functional library size. After the translation, the ribosome forms a peptide bond between the puromycin and the C-terminal residue of the polypeptide and the complex obtained is purified from the ribosome. It is followed by cDNA synthesis of the mRNA-protein complex by reverse transcription so that the library composed of cDNA/mRNA-protein complexes is build. This library is submitted to affinity selection by incubation with the target using affinity chromatography or immunoprecipitation techniques. The enriched library is recovered and mRNA hydrolysis at high pH is performed to release the cDNAs. These cDNAs are amplified by PCR to obtain DNA molecules for another round of selection and specific binders are isolated [115]. mRNA display technology presents monovalent complexes during selection and thus specific binders can be selected without interference of avidity parameters [119]. Furthermore, the diversity of libraries generated by mRNA or ribosome display can be increased by involving error-prone PCR, DNA shuffling and using an error-prone RNA-dependent RNA polymerase during *in vitro* transcription [115].

Using ribosome display, scFv fragments and Fab fragments with high affinities have been selected [120–124]. The construction of an affinity matured library for antibody mimics using mRNA display with the selection of high affinity molecules was described by Xu's group [125]. In relation to antibody fragments, mRNA display technology was used for the selection of an anti-fluorescein antibody in scFv format [126] and also for Fab fragments [127]. The selection of Fab fragments was possible by combination of mRNA display technology and emulsion PCR.

2.4. DNA display technology

DNA display technology is another complete *in vitro* display platform using a cell free system that was initially developed for the selection of peptides linked to their coding DNA present in a library [128–130]. Doi and Yanagawa established *in vitro* transcription and translation reactions in an emulsion compartment—STABLE (Streptavidin-biotin linkage in emulsions) [128]. A random decapeptide library comprising fusion proteins formed by the peptides and streptavidin-His (STA-His, His tag fused to the C-terminus of Streptavidin) was synthesized and ligated to the biotinylated DNA by a stable bond. The library was submitted to affinity selection

using nickel resin. Isolated DNAs were cloned into a vector, amplified and sequenced. A limited affinity selection efficiency was observed so that the method needed improvement [128]. The selection of diverse anti-FLAG Tag peptides from a peptide-DNA library was performed using monoclonal antibody anti-FLAG M2 [129]. The same group also demonstrated that folded proteins can be displayed on the DNA display platform by the introduction of linkers between streptavidin and fused proteins [130]. It could be shown that the GST gene could be enriched by this method by the selection of the DNA-protein conjugates with glutathione-coupled beads. The application of DNA display technology to select Fab fragments using the STABLE method was described later [131]. The selection of a Fab fragment gene was performed using a new approach: randomized hydrophobic core in the constant region with heat treatment application for selective pressure.

Advantages of this technology compared to other cell free *in vitro* display technologies such as ribosome and mRNA display is that RNase-free conditions are not needed for the selection step because a reverse transcription step is unnecessary. Furthermore, the removal of a stop codon between nucleic acid and peptide is not necessary. DNA display technology is simpler than other fully *in vitro* display technologies, with lesser steps [128–130]. One disadvantage of this technology is its novelty, devoid of a robust platform and knowledge in comparison to other *in vitro* display technologies.

3. Approved therapeutic monoclonal antibodies

By the end of 2016, 20 fully human mAbs were approved for therapeutic use by the US Food and Drug Administration (FDA) and often approved by the European Medicines Agency (EMA) too. The approved fully human mAbs are derived basically from two technologies: phage display or transgenic mice expressing human antibody genes [132, 133]. Among approved mAbs, five were generated by phage display with selection of antigen-specific binders from two different libraries, Cambridge Antibody Technology - CAT (MedImmune, subsidiary of AstraZeneca) based on scFv fragments or Dyax Corp's human Fab fragment libraries [25, 134]. The other 15 mAbs were developed in transgenic mice, with integrated human immunoglobulin loci.

Considering the therapeutic mAbs approved which used phage display technology to acquire the human antibody composition, there are examples of different targets for immune intervention: two (adalimumab and belimumab) are in use for autoimmune diseases, one (raxibacumab) is for control of *Bacillus anthracis* infection and two others (ramucirumab and necitumumab) address growth factor receptors for cancer treatment.

The first fully human mAb approved for therapy in 2002 (FDA) and 2003 (EMA)—adalimumab (Humira®, AbbVie Inc., formerly Abbott Laboratories)—was generated by phage display technology [25, 134]. Adalimumab is an anti-TNFα mAb developed in 1993 through a collaboration between BASF Bioresearch Corporation and CAT using an anti-TNF murine antibody (MAK195) from BASF as a template for guided selection of human antibody V-domains with CAT's antibody phage display technology [25]. Adalimumab was first

approved for treatment of rheumatoid arthritis (RA) under the brand name Humira (human monoclonal antibody in rheumatoid arthritis) [25, 135]. Besides RA, adalimumab is currently used in the treatment of plaque psoriasis, ulcerative colitis, Crohn's disease, non-infectious uveitis, hidradenitis suppurativa, psoriatic arthritis, juvenile idiopathic arthritis and ankylosing spondylitis [136].

Nine years after the approval of the first human mAb developed by phage display technology (adalimumab), in 2011 belimumab (Benlysta, GSK) was approved for the treatment of systemic lupus erythematosus [25, 134, 137]. Belimumab was selected using CAT library as a result of the collaboration between CAT and Human Genome Sciences (now GlaxoSmithKline), and targets the soluble form of human B-lymphocyte stimulator (BLyS) [25, 134]. Under a license to use the CAT library, Human Genome Sciences (now GSK) discovered Raxibacumab (Abthrax) also using phage display. Raxibacumab binds the *Bacillus anthracis* protective antigen (PA) and was approved by FDA in 2012 for the prophylaxis and treatment of anthrax [25, 134, 138, 139].

In addition to the three previously described antibodies, which were discovered using scFv-fragment CAT library by antibody phage display, two mAbs—ramucirumab and necitumumab—were derived from the same technology but from a different library, a human Fab-fragment library constructed by de Haard and colleagues at Dyax [25, 134]. Ramucirumab (Cyramza, Eli Lilly) is an anti-VEGFR-2 (vascular endothelial growth factor receptor 2) mAb approved for the treatment of cancer (gastric or gastroesophageal junction, non-small cell lung and colorectal) in 2014 [140]. Necitumumab (Portrazza, Eli Lilly) targets epidermal growth factor receptor (EGFR) and blocks binding to epidermal growth factor (EGF) [25, 134]. This mAb gained first marketing approval for FDA and EMA in 2015 and 2016, respectively, for the treatment of non-small cell lung cancer [25, 141, 142].

Ranibizumab (Lucentis, Genentech), an anti-VEGF-A (vascular endothelial growth factor A) Fab fragment, was generated by the humanization of the murine mAb A4.6.1 [134]. Based on a random mutation library, clones were selected by phage display [143]. Afterward, an alanine scanning study and information of the crystal structure of the Fab-VEGF complex were used for affinity maturation [144, 145]. This mAb was approved in the US in 2006 for the treatment of age-related macular degeneration [25, 134], and posteriorly for the treatment of macular edema after retinal vein occlusion, diabetic macular edema and diabetic retinopathy [25].

4. Future perspectives of the development of recombinant antibodies

This chapter is focused on fully human therapeutic mAbs, mainly those derived from phage display technologies. Other technologies emerged in recent years for the obtainment of human mAbs with high promises of success, derived directly from human B lymphocytes by two main approaches, immortalization of memory B cells by polyclonal stimulation followed by EBV transformation and/or the capture and sorting of memory B cells or plasmablasts followed by amplification of the mAb variable chains expressed by the single B cell, which can be transfected to mammalian cells [27]. The natural pairing of rearranged heavy chain and light chains regions can

be found only in B cells. Display technologies add complexity to the repertoire by pairing light and heavy chains by chance. A very promising new class of antibodies consists of single domain antibodies from camels and sharks comprising only one variable domain of the heavy chain [146, 147]. These molecules are stable, non-aggregating molecules both *in vitro* and *in vivo*. Carrying the ability to bind antigens intracellularly as intrabodies inside the nucleus or cytosol makes them an important platform for antigen trafficking and knockdown, meaning a promise for the future [38].

It draws attention when analyzing the therapeutic mAbs in the market that the majority of them target cancer and autoimmune diseases, while some are directed to other conditions and very few are directed to infectious diseases treatment. It is also more astonishing since the first use of antibodies in immunotherapy fought against infectious diseases by the end of the nineteenth century. One obvious reason is that bacterial infections can be treated with antibiotics and many can be prevented by vaccination. Viral infections, on the other hand, are more complicated to treat by vaccination or treatment with antibodies as some viruses exhibit high mutation rates.

Broadly neutralizing mAbs to influenza viruses can be used to probe *in vitro* vaccine candidates and provide useful information for understanding data generated by preliminary *in vivo* studies, contributing to a universal influenza virus vaccine strategy [148, 149]. The identification of new conserved epitopes resulted from analysis with two broadly neutralizing mAbs, specific for the HA2 subunit of influenza virus belonging to different clades. Both mAbs were generated by phage display from B cells of an influenza vaccinated individual [150] and from a "non-immune" human antibody library [151]. Contrary to the disadvantages listed for phage display libraries, these are high affinity mAbs, in the range of nanomolar and picomolar, respectively.

Generation of mAbs by phage display technology was a breakthrough since this technology opened possibilities to isolate human antibodies for any kind of epitope/antigen without immunization. The success of this technology can be observed by the approval of some drugs, mAbs and other kinds of proteins, and many more are under clinical studies at different stages. There are some candidates for Phase 3 clinical trial, bringing promises of new drugs in the near future [25, 134]. As a perspective for infectious diseases, this technology should be more widely applied for rapid screening of antibodies for diagnostic or therapeutic purposes based on immune or naïve human libraries when epidemic infectious disease breaks without an available drug for its treatment. Other perspective concerns the combinatorial library construction. Recently, the human antibody repertoire derived from blood of human naïve or immunized donors has been intensively analyzed by next-generation sequencing [152, 153]. Advances in the knowledge of the human antibody repertoire would help in the designing of antibody libraries and for antibody maturation of existing human libraries, making possible the selection of mAbs with higher affinity for clinical use.

Author details

Lilian Rumi Tsuruta*, Mariana Lopes dos Santos and Ana Maria Moro

*Address all correspondence to: lilian.tsuruta@butantan.gov.br

Lab Biopharmaceuticals in Animal Cells, Instituto Butantan, São Paulo, SP, Brazil

References

[1] Aggarwal RS. What's fueling the biotech engine-2012 to 2013. Nature Biotechnology. 2014;**32**(1):32-39

[2] Reichert JM. Antibodies to watch in 2017. MAbs. 2017;**9**(2):167-181

[3] Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;**256**(5517):495-497

[4] Norman DJ, Shield CF3rd, Barry J, Henell K, Funnell MB, Lemon J. A U.S. clinical study of Orthoclone OKT3 in renal transplantation. Transplantation Proceedings. 1987;**19**(2 Suppl 1):21-27

[5] Hwang WY, Foote J. Immunogenicity of engineered antibodies. Methods. 2005;**36**(1):3-10

[6] Almagro JC, Fransson J. Humanization of antibodies. Frontiers in Bioscience: A Journal and Virtual Library. 2008;**13**:1619-1633

[7] Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains. Proceedings of the National Academy of Sciences of the United States of America. 1984;**81**(21):6851-6855

[8] Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature. 1986; **321**(6069):522-525

[9] Verhoeyen M, Milstein C, Winter G. Reshaping human antibodies: Grafting an antilysozyme activity. Science. 1988;**239**(4847):1534-1536

[10] Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature. 1988;**332**(6162):323-327

[11] Queen C, Schneider WP, Selick HE, Payne PW, Landolfi NF, Duncan JF, et al. A humanized antibody that binds to the interleukin 2 receptor. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**(24):10029-10033

[12] Caldas C, Coelho V, Kalil J, Moro AM, Maranhão AQ, Brígido MM. Humanization of the anti-CD18 antibody 6.7: An unexpected effect of a framework residue in binding to antigen. Molecular Immunology. 2003;**39**(15):941-952

[13] Tsurushita N, Hinton PR, Kumar S. Design of humanized antibodies: From anti-Tac to Zenapax. Methods. 2005;**36**(1):69-83

[14] Lopes dos Santos M, Yeda FP, Tsuruta LR, Horta BB, Pimenta Jr AA, Degaki TL, et al. Rebmab200, a humanized monoclonal antibody targeting the sodium phosphate transporter NaPi2b displays strong immune mediated cytotoxicity against cancer: A novel reagent for targeted antibody therapy of cancer. PLoS One. 2013;**8**(7):e70332.

[15] Lindegren S, Andrade LN, Back T, Machado CM, Horta BB, Buchpiguel C, et al. Binding affinity, specificity and comparative biodistribution of the parental murine monoclonal

antibody MX35 (anti-NaPi2b) and its humanized version Rebmab200. PLoS One. 2015;**10**(5):e0126298

[16] Smith GP. Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. Science. 1985;**228**(4705):1315-1317

[17] Parmley SF, Smith GP. Antibody-selectable filamentous fd phage vectors: Affinity purification of target genes. Gene. 1988;**73**(2):305-318

[18] Better M, Chang CP, Robinson RR, Horwitz AH. *Escherichia coli* secretion of an active chimeric antibody fragment. Science. 1988;**240**(4855):1041-1043

[19] Skerra A, Pluckthun A. Assembly of a functional immunoglobulin Fv fragment in *Escherichia coli*. Science. 1988;**240**(4855):1038-1041

[20] Larrick JW, Danielsson L, Brenner CA, Abrahamson M, Fry KE, Borrebaeck CA. Rapid cloning of rearranged immunoglobulin genes from human hybridoma cells using mixed primers and the polymerase chain reaction. Biochemical and Biophysical Research Communications. 1989;**160**(3):1250-1256

[21] Orlandi R, Gussow DH, Jones PT, Winter G. Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**(10):3833-3837

[22] Sastry L, Alting-Mees M, Huse WD, Short JM, Sorge JA, Hay BN, et al. Cloning of the immunological repertoire in *Escherichia coli* for generation of monoclonal catalytic antibodies: Construction of a heavy chain variable region-specific cDNA library. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**(15):5728-5732

[23] Burton DR, Barbas CF3rd. Human antibodies from combinatorial libraries. Advances in Immunology. 1994;**57**:191-280

[24] Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR. Making antibodies by phage display technology. Annual Review of Immunology. 1994;**12**:433-455

[25] Frenzel A, Schirrmann T, Hust M. Phage display-derived human antibodies in clinical development and therapy. MAbs. 2016;**8**(7):1177-1194

[26] Hoogenboom HR. Selecting and screening recombinant antibody libraries. Nature Biotechnology. 2005;**23**(9):1105-1116

[27] Wilson PC, Andrews SF. Tools to therapeutically harness the human antibody response. Nature Reviews Immunology. 2012;**12**(10):709-719

[28] Huse WD, Sastry L, Iverson SA, Kang AS, Alting-Mees M, Burton DR, et al. Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda. Science. 1989;**246**(4935):1275-1281

[29] Persson MA, Caothien RH, Burton DR. Generation of diverse high-affinity human monoclonal antibodies by repertoire cloning. Proceedings of the National Academy of Sciences of the United States of America. 1991;**88**(6):2432-2436

[30] Cwirla SE, Peters EA, Barrett RW, Dower WJ. Peptides on phage: A vast library of peptides for identifying ligands. Proceedings of the National Academy of Sciences of the United States of America. 1990;**87**(16):6378-6382

[31] Scott JK, Smith GP. Searching for peptide ligands with an epitope library. Science. 1990;**249**(4967):386-390

[32] Devlin JJ, Panganiban LC, Devlin PE. Random peptide libraries: A source of specific protein binding molecules. Science. 1990;**249**(4967):404-406

[33] McCafferty J, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: Filamentous phage displaying antibody variable domains. Nature. 1990;**348**(6301):552-554

[34] Breitling F, Dübel S, Seehaus T, Klewinghaus I, Little M. A surface expression vector for antibody screening. Gene. 1991;**104**(2):147-153

[35] Hoogenboom HR, Griffiths AD, Johnson KS, Chiswell DJ, Hudson P, Winter G. Multi-subunit proteins on the surface of filamentous phage: Methodologies for displaying antibody (Fab) heavy and light chains. Nucleic Acids Research. 1991;**19**(15):4133-4137

[36] Barbas CF3rd, Kang AS, Lerner RA, Benkovic SJ. Assembly of combinatorial antibody libraries on phage surfaces: The gene III site. Proceedings of the National Academy of Sciences of the United States of America. 1991;**88**(18):7978-7982

[37] Garrard LJ, Yang M, O'Connell MP, Kelley RF, Henner DJ. Fab assembly and enrichment in a monovalent phage display system. Bio/Technology. 1991;**9**(12):1373-1377

[38] Boldicke T. Single domain antibodies for the knockdown of cytosolic and nuclear proteins. Protein Science. 2017

[39] Lubkowski J, Hennecke F, Pluckthun A, Wlodawer A. The structural basis of phage display elucidated by the crystal structure of the N-terminal domains of g3p. Nature Structural Biology. 1998;**5**(2):140-147

[40] Lubkowski J, Hennecke F, Pluckthun A, Wlodawer A. Filamentous phage infection: Crystal structure of g3p in complex with its coreceptor, the C-terminal domain of TolA. Structure. 1999;**7**(6):711-722

[41] Holliger P, Riechmann L. A conserved infection pathway for filamentous bacteriophages is suggested by the structure of the membrane penetration domain of the minor coat protein g3p from phage fd. Structure. 1997;**5**(2):265-275

[42] Qi H, Lu H, Qui HJ, Petrenko V, Liu A. Phagemid vectors for phage display: Properties, characteristics and construction. Journal of Molecular Biology. 2012;**417**:15

[43] Bradbury AR, Marks JD. Antibodies from phage antibody libraries. Journal of Immunological Methods. 2004;**290**(1-2):29-49

[44] Vieira J, Messing J. Production of single-stranded plasmid DNA. Methods in Enzymology. 1987;**153**:3-11

[45] Il'ichev AA, Minenkova OO, Tat'kov SI, Karpyshev NN, Eroshkin AM, Ofitserov VI, et al. The use of filamentous phage M13 in protein engineering. Molekuliarnaia Biologiia. 1990;**24**(2):530-535

[46] Greenwood J, Willis AE, Perham RN. Multiple display of foreign peptides on a filamentous bacteriophage. Peptides from *Plasmodium falciparum* circumsporozoite protein as antigens. Journal of Molecular Biology. 1991;**220**(4):821-827

[47] Felici F, Castagnoli L, Musacchio A, Jappelli R, Cesareni G. Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent exposition vector. Journal of Molecular Biology. 1991;**222**(2):301-310

[48] Chang CN, Landolfi NF, Queen C. Expression of antibody Fab domains on bacteriophage surfaces. Potential use for antibody selection. Journal of Immunology. 1991;**147**(10):3610-3614

[49] Huse WD, Stinchcombe TJ, Glaser SM, Starr L, MacLean M, Hellstrom KE, et al. Application of a filamentous phage pVIII fusion protein system suitable for efficient production, screening, and mutagenesis of F(ab) antibody fragments. Journal of Immunology. 1992;**149**(12):3914-3920

[50] Kang AS, Jones TM, Burton DR. Antibody redesign by chain shuffling from random combinatorial immunoglobulin libraries. Proceedings of the National Academy of Sciences of the United States of America. 1991;**88**(24):11120-11123

[51] Kang AS, Barbas CF, Janda KD, Benkovic SJ, Lerner RA. Linkage of recognition and replication functions by assembling combinatorial antibody Fab libraries along phage surfaces. Proceedings of the National Academy of Sciences of the United States of America. 1991;**88**(10):4363-4366

[52] Soderlind E, Simonsson AC, Borrebaeck CA. Phage display technology in antibody engineering: Design of phagemid vectors and in vitro maturation systems. Immunological Reviews. 1992;**130**:109-124

[53] Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G. By-passing immunization. Human antibodies from V-gene libraries displayed on phage. Journal of Molecular Biology. 1991;**222**(3):581-597

[54] de Haard HJ, van Neer N, Reurs A, Hufton SE, Roovers RC, Henderikx P, et al. A large non-immunized human Fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies. The Journal of biological chemistry. 1999;**274**(26):18218-18230

[55] Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC, et al. Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library. Nature Biotechnology. 1996;**14**(3):309-314

[56] Lloyd C, Lowe D, Edwards B, Welsh F, Dilks T, Hardman C, et al. Modelling the human immune response: Performance of a 1011 human antibody repertoire against a broad

panel of therapeutically relevant antigens. Protein Engineering, Design & Selection. 2009;**22**(3):159-168

[57] Schwimmer LJ, Huang B, Giang H, Cotter RL, Chemla-Vogel DS, Dy FV, et al. Discovery of diverse and functional antibodies from large human repertoire antibody libraries. Journal of Immunological Methods. 2013;**391**(1-2):60-71

[58] Hust M, Meyer T, Voedisch B, Rülker T, Thie H, El-Ghezal A, et al. A human scFv antibody generation pipeline for proteome research. Journal of Biotechnology. 2011;**152**(4):159-170

[59] Kugler J, Wilke S, Meier D, Tomszak F, Frenzel A, Schirrmann T, et al. Generation and analysis of the improved human HAL9/10 antibody phage display libraries. BMC Biotechnology. 2015;**15**:10

[60] Barbas CF3rd, Bain JD, Hoekstra DM, Lerner RA. Semisynthetic combinatorial antibody libraries: A chemical solution to the diversity problem. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**(10):4457-4461

[61] Hoogenboom HR, Winter G. By-passing immunisation. Human antibodies from synthetic repertoires of germline VH gene segments rearranged in vitro. Journal of Molecular Biology. 1992;**227**(2):381-388

[62] Griffiths AD, Williams SC, Hartley O, Tomlinson IM, Waterhouse P, Crosby WL, et al. Isolation of high affinity human antibodies directly from large synthetic repertoires. The EMBO journal. 1994;**13**(14):3245-3260

[63] Nissim A, Hoogenboom HR, Tomlinson IM, Flynn G, Midgley C, Lane D, et al. Antibody fragments from a 'single pot' phage display library as immunochemical reagents. The EMBO Journal. 1994;**13**(3):692-698

[64] Pini A, Viti F, Santucci A, Carnemolla B, Zardi L, Neri P, et al. Design and use of a phage display library. Human antibodies with subnanomolar affinity against a marker of angiogenesis eluted from a two-dimensional gel. The Journal of Biological Chemistry. 1998;**273**(34):21769-21776

[65] Soderlind E, Strandberg L, Jirholt P, Kobayashi N, Alexeiva V, Aberg AM, et al. Recombining germline-derived CDR sequences for creating diverse single-framework antibody libraries. Nature Biotechnology. 2000;**18**(8):852-856

[66] Knappik A, Ge L, Honegger A, Pack P, Fischer M, Wellnhofer G, et al. Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. Journal of Molecular Biology. 2000; **296**(1):57-86

[67] Silacci M, Brack S, Schirru G, Marlind J, Ettorre A, Merlo A, et al. Design, construction, and characterization of a large synthetic human antibody phage display library. Proteomics. 2005;**5**(9):2340-2350

[68] Rothe C, Urlinger S, Lohning C, Prassler J, Stark Y, Jager U, et al. The human combinatorial antibody library HuCAL GOLD combines diversification of all six CDRs according

to the natural immune system with a novel display method for efficient selection of high-affinity antibodies. Journal of Molecular Biology. 2008;**376**(4):1182-1200

[69] Yang HY, Kang KJ, Chung JE, Shim H. Construction of a large synthetic human scFv library with six diversified CDRs and high functional diversity. Molecules and Cells. 2009;**27**(2):225-235

[70] Prassler J, Thiel S, Pracht C, Polzer A, Peters S, Bauer M, et al. HuCAL PLATINUM, a synthetic Fab library optimized for sequence diversity and superior performance in mammalian expression systems. Journal of Molecular Biology. 2011;**413**(1):261-278

[71] Zhai W, Glanville J, Fuhrmann M, Mei L, Ni I, Sundar PD, et al. Synthetic antibodies designed on natural sequence landscapes. Journal of Molecular Biology. 2011;**412**(1): 55-71

[72] Tiller T, Schuster I, Deppe D, Siegers K, Strohner R, Herrmann T, et al. A fully synthetic human Fab antibody library based on fixed VH/VL framework pairings with favorable biophysical properties. MAbs. 2013;**5**(3):445-470

[73] de Kruif J, Terstappen L, Boel E, Logtenberg T. Rapid selection of cell subpopulation-specific human monoclonal antibodies from a synthetic phage antibody library. Proceedings of the National Academy of Sciences of the United States of America 1995;**92**(9):3938-3942

[74] Shim H. Synthetic approach to the generation of antibody diversity. BMB Reports. 2015;**48**(9):489-494

[75] Hawkins RE, Russell SJ, Winter G. Selection of phage antibodies by binding affinity. Mimicking affinity maturation. Journal of Molecular Biology. 1992;**226**(3):889-896

[76] Marks JD, Ouwehand WH, Bye JM, Finnern R, Gorick BD, Voak D, et al. Human antibody fragments specific for human blood group antigens from a phage display library. Bio/Technology. 1993;**11**(10):1145-1149

[77] Clackson T, Hoogenboom HR, Griffiths AD, Winter G. Making antibody fragments using phage display libraries. Nature. 1991;**352**(6336):624-628

[78] Gram H, Marconi LA, Barbas CF3rd, Collet TA, Lerner RA, Kang AS. vitro selection and affinity maturation of antibodies from a naive combinatorial immunoglobulin library. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**(8):3576-3580

[79] Daugherty PS, Chen G, Iverson BL, Georgiou G. Quantitative analysis of the effect of the mutation frequency on the affinity maturation of single chain Fv antibodies. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(5):2029-2034

[80] Ho M, Kreitman RJ, Onda M, Pastan I. vitro antibody evolution targeting germline hot spots to increase activity of an anti-CD22 immunotoxin. The Journal of Biological Chemistry. 2005;**280**(1):607-617

[81] Bowers PM, Horlick RA, Neben TY, Toobian RM, Tomlinson GL, Dalton JL, et al. Coupling mammalian cell surface display with somatic hypermutation for the discovery and maturation of human antibodies. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(51):20455-20460

[82] Bowers PM, Horlick RA, Kehry MR, Neben TY, Tomlinson GL, Altobell L, et al. Mammalian cell display for the discovery and optimization of antibody therapeutics. Methods. 2014;**65**(1):44-56

[83] King DJ, Bowers PM, Kehry MR, Horlick RA. Mammalian cell display and somatic hypermutation in vitro for human antibody discovery. Current Drug Discovery Technologies. 2014;**11**(1):56-64

[84] Jespers LS, Roberts A, Mahler SM, Winter G, Hoogenboom HR. Guiding the selection of human antibodies from phage display repertoires to a single epitope of an antigen. Bio/Technology. 1994;**12**(9):899-903

[85] Figini M, Marks JD, Winter G, Griffiths AD. In vitro assembly of repertoires of antibody chains on the surface of phage by renaturation. Journal of Molecular Biology. 1994;**239**(1):68-78

[86] Osbourn J, Groves M, Vaughan T. From rodent reagents to human therapeutics using antibody guided selection. Methods. 2005;**36**(1):61-68

[87] Zebedee SL, Barbas CF3rd, Hom YL, Caothien RH, Graff R, DeGraw J, et al. Human combinatorial antibody libraries to hepatitis B surface antigen. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**(8):3175-3179

[88] Ohlin M, Owman H, Mach M, Borrebaeck CA. Light chain shuffling of a high affinity antibody results in a drift in epitope recognition. Molecular Immunology. 1996;**33**(1):47-56

[89] Watzka H, Pfizenmaier K, Moosmayer D. Guided selection of antibody fragments specific for human interferon gamma receptor 1 from a human VH- and VL-gene repertoire. Immunotechnology: An International Journal of Immunological Engineering. 1998;**3**(4):279-291

[90] Beiboer SH, Reurs A, Roovers RC, Arends JW, Whitelegg NR, Rees AR, et al. Guided selection of a pan carcinoma specific antibody reveals similar binding characteristics yet structural divergence between the original murine antibody and its human equivalent. Journal of Molecular Biology. 2000;**296**(3):833-849

[91] Klimka A, Matthey B, Roovers RC, Barth S, Arends JW, Engert A, et al. Human anti-CD30 recombinant antibodies by guided phage antibody selection using cell panning. British Journal of Cancer. 2000;**83**(2):252-260

[92] Zhao A, Tohidkia MR, Siegel DL, Coukos G, Omidi Y. Phage antibody display libraries: A powerful antibody discovery platform for immunotherapy. Critical Reviews in Biotechnology. 2016;**36**(2):276-289

[93] Boder ET, Wittrup KD. Yeast surface display for screening combinatorial polypeptide libraries. Nature Biotechnology. 1997;**15**(6):553-557

[94] Feldhaus MJ, Siegel RW. Yeast display of antibody fragments: A discovery and characterization platform. Journal of Immunological Methods. 2004;**290**(1-2):69-80

[95] Gera N, Hussain M, Rao BM. Protein selection using yeast surface display. Methods. 2013;**60**(1):15-26

[96] Sheehan J, Marasco WA. Phage and yeast display. Microbiology Spectrum. 2015;**3**(1):17 AID-0028-2014

[97] Shusta EV, Kieke MC, Parke E, Kranz DM, Wittrup KD. Yeast polypeptide fusion surface display levels predict thermal stability and soluble secretion efficiency. Journal of Molecular Biology. 1999;**292**(5):949-956

[98] Shusta EV, Holler PD, Kieke MC, Kranz DM, Wittrup KD. Directed evolution of a stable scaffold for T-cell receptor engineering. Nature Biotechnology. 2000;**18**(7):754-759

[99] Orr BA, Carr LM, Wittrup KD, Roy EJ, Kranz DM. Rapid method for measuring ScFv thermal stability by yeast surface display. Biotechnology Progress. 2003;**19**(2):631-638

[100] Boder ET, Midelfort KS, Wittrup KD. Directed evolution of antibody fragments with monovalent femtomolar antigen-binding affinity. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(20):10701-10705

[101] Feldhaus MJ, Siegel RW, Opresko LK, Coleman JR, Feldhaus JM, Yeung YA, et al. Flow-cytometric isolation of human antibodies from a nonimmune Saccharomyces cerevisiae surface display library. Nature Biotechnology. 2003;**21**(2):163-170

[102] van den Beucken T, Pieters H, Steukers M, van der Vaart M, Ladner RC, Hoogenboom HR, et al. Affinity maturation of Fab antibody fragments by fluorescent-activated cell sorting of yeast-displayed libraries. FEBS Letters 2003;**546**(2-3):288-294.

[103] Ferrara F, Naranjo LA, Kumar S, Gaiotto T, Mukundan H, Swanson B, et al. Using phage and yeast display to select hundreds of monoclonal antibodies: Application to antigen 85, a tuberculosis biomarker. PLoS One. 2012;**7**(11):e49535

[104] Boder ET, Raeeszadeh-Sarmazdeh M, Price JV. Engineering antibodies by yeast display. Archives of Biochemistry and Biophysics. 2012;**526**(2):99-106

[105] Benatuil L, Perez JM, Belk J, Hsieh CM. An improved yeast transformation method for the generation of very large human antibody libraries. Protein Engineering, Design & Selection. 2010;**23**(4):155-159

[106] Georgiou G, Stathopoulos C, Daugherty PS, Nayak AR, Iverson BL, Curtiss R 3rd. Display of heterologous proteins on the surface of microorganisms: From the screening of combinatorial libraries to live recombinant vaccines. Nature Biotechnology. 1997;**15**(1):29-34

[107] Samuelson P, Gunneriusson E, Nygren PA, Stahl S. Display of proteins on bacteria. Journal of Biotechnology. 2002;**96**(2):129-154

[108] Lofblom J. Bacterial display in combinatorial protein engineering. Biotechnology Journal. 2011;**6**(9):1115-1129

[109] Francisco JA, Campbell R, Iverson BL, Georgiou G. Production and fluorescence-activated cell sorting of *Escherichia coli* expressing a functional antibody fragment on the external surface. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**(22):10444-10448

[110] Daugherty PS, Chen G, Olsen MJ, Iverson BL, Georgiou G. Antibody affinity maturation using bacterial surface display. Protein Engineering. 1998;**11**(9):825-832

[111] Mazor Y, Van Blarcom T, Mabry R, Iverson BL, Georgiou G. Isolation of engineered, full-length antibodies from libraries expressed in *Escherichia coli*. Nature Biotechnology. 2007;**25**(5):563-565

[112] Samuelson P, Hansson M, Ahlborg N, Andreoni C, Gotz F, Bachi T, et al. Cell surface display of recombinant proteins on Staphylococcus carnosus. Journal of Bacteriology. 1995;**177**(6):1470-1476

[113] Lofblom J, Wernerus H, Stahl S. Fine affinity discrimination by normalized fluorescence activated cell sorting in staphylococcal surface display. FEMS Microbiology Letters. 2005;**248**(2):189-198

[114] Kronqvist N, Lofblom J, Jonsson A, Wernerus H, Stahl S. A novel affinity protein selection system based on staphylococcal cell surface display and flow cytometry. Protein Engineering, Design & Selection. 2008;**21**(4):247-255

[115] Lipovsek D, Pluckthun A. In-vitro protein evolution by ribosome display and mRNA display. Journal of Immunological Methods. 2004;**290**(1-2):51-67

[116] Pluckthun A. Ribosome display: A perspective. Methods in Molecular Biology. 2012; **805**:3-28

[117] Roberts RW, Szostak JW. RNA-peptide fusions for the in vitro selection of peptides and proteins. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**(23):12297-12302

[118] Nemoto N, Miyamoto-Sato E, Husimi Y, Yanagawa H. In vitro virus: Bonding of mRNA bearing puromycin at the 3'-terminal end to the C-terminal end of its encoded protein on the ribosome in vitro. FEBS Letters. 1997;**414**(2):405-408

[119] Josephson K, Ricardo A, Szostak JW. mRNA display: From basic principles to macrocycle drug discovery. Drug Discovery Today. 2014;**19**(4):388-399

[120] Hanes J, Schaffitzel C, Knappik A, Pluckthun A. Picomolar affinity antibodies from a fully synthetic naive library selected and evolved by ribosome display. Nature Biotechnology. 2000;**18**(12):1287-1292

[121] Zhou L, Mao WP, Fen J, Liu HY, Wei CJ, Li WX, et al. Selection of scFvs specific for the HepG2 cell line using ribosome display. Journal of Biosciences. 2009;**34**(2):221-226

[122] Zhao HY, Zhang YZ, Xiao CL. Advances in the study of affinity selection-ultrafiltration/HPLC-MS. Yao Xue Xue Bao. 2009;**44**(10):1084-1088

[123] Luo Y, Xia Y. Selection of single-chain variable fragment antibodies against fenitrothion by ribosome display. Analytical Biochemistry. 2012;**421**(1):130-137

[124] Stafford RL, Matsumoto ML, Yin G, Cai Q, Fung JJ, Stephenson H, et al. In vitro Fab display: A cell-free system for IgG discovery. Protein Engineering, Design & Selection. 2014;**27**(4):97-109

[125] Xu L, Aha P, Gu K, Kuimelis RG, Kurz M, Lam T, et al. Directed evolution of high-affinity antibody mimics using mRNA display. Chemistry & Biology. 2002;**9**(8):933-942

[126] Fukuda I, Kojoh K, Tabata N, Doi N, Takashima H, Miyamoto-Sato E, et al. In vitro evolution of single-chain antibodies using mRNA display. Nucleic Acids Research. 2006;**34**(19):e127

[127] Sumida T, Yanagawa H, Doi N. In vitro selection of fab fragments by mRNA display and gene-linking emulsion PCR. Journal of Nucleic Acids. 2012;**2012**:371379

[128] Doi N, Yanagawa H. STABLE: Protein-DNA fusion system for screening of combinatorial protein libraries in vitro. FEBS Letters. 1999;**457**(2):227-230

[129] Yonezawa M, Doi N, Kawahashi Y, Higashinakagawa T, Yanagawa H. DNA display for in vitro selection of diverse peptide libraries. Nucleic Acids Research. 2003;**31**(19):e118

[130] Yonezawa M, Doi N, Higashinakagawa T, Yanagawa H. DNA display of biologically active proteins for in vitro protein selection. Journal of Biochemistry. 2004;**135**(3):285-288

[131] Sumida T, Doi N, Yanagawa H. Bicistronic DNA display for in vitro selection of Fab fragments. Nucleic Acids Research. 2009;**37**(22):e147

[132] Lonberg N. Fully human antibodies from transgenic mouse and phage display platforms. Current Opinion in Immunology. 2008;**20**(4):450-459

[133] Bruggemann M, Osborn MJ, Ma B, Hayre J, Avis S, Lundstrom B, et al. Human antibody production in transgenic animals. Archivum Immunologiae et Therapiae Experimentalis (Warsz). 2015;**63**(2):101-108

[134] Nixon AE, Sexton DJ, Ladner RC. Drugs derived from phage display: From candidate identification to clinical practice. MAbs. 2014;**6**(1):73-85

[135] Scheinfeld N. Adalimumab (HUMIRA): A review. Journal of Drugs in Dermatology. 2003;**2**(4):375-377

[136] Humira (Adalimumab). Abbvie Inc. 2016 Available from: https://www.humira.com/ [Accessed: Jan 24, 2017]

[137] Benlysta (Belimumab), GSK 2016 Available from: http://www.benlysta.com/ [Accessed: Jan 24, 2017]

[138] Mazumdar S. Raxibacumab. MAbs. 2009;**1**(6):531-538

[139] Raxibacumab. ABthrax – Prescribing Information. GSK; 2016 Available from: https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Raxibacumab/pdf/RAXIBACUMAB-PI-PIL.PDF [Accessed: Jan 24, 2017]

[140] Cyramza (Ramucirumab), Eli Lilly 2016 Available from: http://www.cyramzahcp.com/ [Accessed: Jan 24, 2017]

[141] Reichert JM. Antibodies to watch in 2016. MAbs. 2016;**8**(2):197-204

[142] Garnock-Jones KP. Necitumumab: First global approval. Drugs. 2016;**76**(2):283-289

[143] Baca M, Presta LG, O'Connor SJ, Wells JA. Antibody humanization using monovalent phage display. The Journal of Biological Chemistry. 1997;**272**(16):10678-10684

[144] Muller YA, Chen Y, Christinger HW, Li B, Cunningham BC, Lowman HB, et al. VEGF and the Fab fragment of a humanized neutralizing antibody: Crystal structure of the complex at 2.4 A resolution and mutational analysis of the interface. Structure. 1998;**6**(9):1153-1167

[145] Chen Y, Wiesmann C, Fuh G, Li B, Christinger HW, McKay P, et al. Selection and analysis of an optimized anti-VEGF antibody: Crystal structure of an affinity-matured Fab in complex with antigen. Journal of Molecular Biology. 1999;**293**(4):865-881

[146] Muyldermans S. Nanobodies: Natural single-domain antibodies. Annual Review of Biochemistry. 2013;**82**:775-797

[147] Zielonka S, Empting M, Grzeschik J, Konning D, Barelle CJ, Kolmar H. Structural insights and biomedical potential of IgNAR scaffolds from sharks. MAbs. 2015;**7**(1):15-25

[148] Burioni R, Canducci F, Mancini N, Clementi N, Sassi M, De Marco D, et al. Monoclonal antibodies isolated from human B cells neutralize a broad range of H1 subtype influenza A viruses including swine-origin Influenza virus (S-OIV). Virology. 2010;**399**(1):144-152

[149] Krause JC, Tsibane T, Tumpey TM, Huffman CJ, Basler CF, Crowe Jr JE. A broadly neutralizing human monoclonal antibody that recognizes a conserved, novel epitope on the globular head of the influenza H1N1 virus hemagglutinin. Journal of Virology 2011;**85**(20):10905-10908

[150] Ekiert DC, Bhabha G, Elsliger MA, Friesen RH, Jongeneelen M, Throsby M, et al. Antibody recognition of a highly conserved influenza virus epitope. Science. 2009;**324** (5924):246-251

[151] Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen LM, et al. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Nature Structural & Molecular Biology. 2009;**16**(3):265-273

[152] DeKosky BJ, Lungu OI, Park D, Johnson EL, Charab W, Chrysostomou C, et al. Large-scale sequence and structural comparisons of human naive and antigen-experienced antibody repertoires. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**(19):E2636-E2645

[153] DeWitt WS, Lindau P, Snyder TM, Sherwood AM, Vignali M, Carlson CS, et al. A public database of memory and naive B-cell receptor sequences. PLoS One. 2016;**11**(8):e0160853

IntechOpen

IntechOpen