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REVIEW

Frontier of therapeutic antibody discovery: The challenges and how to face them

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Abstract

Therapeutic monoclonal antibodies have become an important class of modern medicines. The established technologies for therapeutic antibody discovery such as humanization of mouse antibodies, phage display of human antibody libraries and transgenic animals harboring human IgG genes have been practiced successfully so far, and many incremental improvements are being made constantly. These methodologies are responsible for currently marketed therapeutic antibodies and for the biopharma industry pipeline which are concentrated on only a few dozen targets. A key challenge for wider application of biotherapeutic approaches is the paucity of truly validated targets for biotherapeutic intervention. The efforts to expand the target space include taking the pathway approach to study the disease correlation. Since many new targets are multi-spanning and multimeric membrane proteins there is a need to develop more effective methods to generate antibodies against these difficult targets. The pharmaceutical properties of therapeutic antibodies are an active area for study concentrating on biophysical characteristics such as thermal stability and aggregation propensity. The immunogenicity of biotherapeutics in humans is a very complex issue and there are no truly predictive animal models to rely on. The in silico and T-cell response approaches identify the potential for immunogenicity; however, one needs contingency plans for emergence of anti-product antibody response for clinical trials.

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Key words: Antibody; Biotherapeutics; Biophysical property; Bispecific; Humanization; Immunogenicity; Transgenic rodent; Phage display; Yeast

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INTRODUCTION

Biotherapeutics have become an important component of modern medicine, pursued not only by emerging biotech firms but also by well-established research-driven pharmaceutical companies. In fact, nowadays biotherapeutic projects comprise up to one-third of the R and D pipelines in many major pharmaceutical companies. Further, these protein therapeutic projects are found in some important disease areas such as immunology/inflammation, oncology, metabolic diseases, neuro-degenerative diseases, tissue repair/regeneration, thrombosis/hemostasis and many forms of genetic deficiencies. The composition matter of the industry-wide biotherapeutic pipeline includes engineered therapeutic proteins, classic monoclonal antibodies

(mAb), antibody drug conjugates (ADC) and novel engineered binding proteins based on immunoglobulin domains and other scaffolds in a variety of configurations[1]. In the past two decades the industry accumulated vast amount of experience in selecting biotherapeutic entities of different kinds for better pharmaceutical properties and safety profile, and also established effective processes to move candidates through preclinical studies to clinical trials[2]. It is remarkable for such a young discipline to become a reliable source of novel medicines. Nevertheless, in order to achieve sustained success, it is time to prudently reflect the challenges currently facing the biotherapeutics field[3].

TARGET SPACE AND APPROACHES FOR TARGET DISCOVERY

The last decades of the 20th century was the golden years of the pharmaceutical industry when many transforming therapeutics were discovered. The targets of these innovative medicines were so called "low-hanging fruits" because their biological functions and disease correlations were relatively simple and had been studies for years or even decades. It was widely anticipated that the successful human genome sequencing at the beginning of the new millennium would greatly expand the target space for pharmaceutical industry[4]. However, more than a decade later the whole industry still struggles with the paucity of new targets that are truly validated and druggable. This situation was first recognized for small molecule drug discovery, and here we contend that it is increasingly so for biotherapeutics as well. The general reason is that the gene structure and sequence alone does not reveal pleiotropic biological functions or the pathological consequences of its abnormal expression and regulation. For current forms of biotherapeutics, the immediate target interaction takes place in extracellular space or on cell membrane, which further limits their potential targets to this fraction of the whole proteome.

There are many approaches aimed at establishing the correlation of genes/gene products with diseases, or to validate them as targets for therapeutic intervention. We think there are two trends that represent broad industry effort to break the target discovery/validation bottleneck, one is the pathway approach and the other extensive collaboration with academics.

The pathway approach for target discovery

If genome sequencing projects accomplished putting genes as entries into a dictionary, all the "omics" studies annotate these entries: assigning the functions to the genes/gene products. The best way for annotating all genes/gene products is to organize them into molecular pathways whose spatial/temporal response to stimuli and dynamic interactions with one another lead to biological outputs[4]. Abnormalities of one node (gene/gene product) in a pathway may lead to the malfunction of this pathway or the pathways that intersect with it, which then causes diseases. In this view the drug discovery efforts may focus on correcting or compensating the abnormality of the pathway to achieve normal output. Therefore, the targets for drug intervention can be the defective node itself or other related nodes in the pathway[5].

Target discovery activities within the pathway approach include pathway construction and disease linkage. Modern technologies for studying systems biology, such as transcriptomics, proteomics, metabolomics and interactomics, are deployed to sketch out or to confirm pathways. Using carefully designed phenotypical assays that recapitulate pathway defects, one can screen for compounds that affect desired outcome. Since a large number of pathways are actuated initially by the interaction of the cell surface receptors and their cognate ligands, antibodies that specifically block such receptor-ligand interactions are naturally good tools for pathway studies aimed at target discovery and validation. If these antibodies lead to desired outcome then they can be optimized into drug candidates.

In the pathway approach, clinical studies are a critical step for target validation, where not only the particular diseases linkage is established to the targeted pathways, but also extrapolations are attempted for relevance in other diseases. As such, the results are meaningful for the indications being tested and for revealing other nodes in the pathway as potential alternatives. Case in point, mTOR was initially being targeted for immune suppression by rapamycin[6]. The very growth inhibition by targeting mTOR led to new and better drug entities for treating cancers[7,8]. Then other nodes in the same pathway such as IGF-R and PI3K are being targeted for cancer treatment[9,10]. It seems that companies adopting the pathway approach for target discovery do have more robust drug pipeline.

Extensive collaboration with academics

While pharmaceutical/biotech industry spends significant resources on research which is tilted toward drug discovery and development, government/non-governmental organization also provides considerable support to academic medical/life science research with more emphasis on the understanding of basic mechanisms of biology. In fact, these supports have fueled many important discoveries in the life sciences[11] and yielded some remarkable drug targets[12]. The interest from pharmaceutical industry to form research collaborations increased in the past decades, with notable arrangements between Sandoz (now Novartis) and Dana Farber Cancer Institute, and between Merck and Harvard Medical School as well as others struck by Pfizer, GSK, AstraZeneca and Johnson & Johnson. Many of these collaborations focus on target validation/early drug discovery. To further harness the scientific discoveries in the research community, the pharmaceutical industry started setting up units to co-localize with academic research clusters. For example, Pfizer established Centers for Technology Innovation in Boston, New York and San Francisco. These centers pro-

Table 1 Comparison of major technology platforms for therapeutic antibody discovery

Platforms	Main applications	Major advantages	Key disadvantages	FDA approved therapeutic antibodies
Hybridoma	Generate hits and research reagents	Mature technology and cost effective	Potential immunogenicity	Orthoclone, Zevalin, Bexxar
Humanization	Generate therapeutic candidates	Well established and low cost	Not fully human antibodies	ReoPro, Rituxan, Simulect, Remicade, Erbitux, Adcetris, Zenapax, Synagis, Herceptin, Mylotarg, Mabcampath, Xolair, Actemra, Avastin, Tysabri, Lucentis, Soliris, Cimzia, Perjeta
Phage display	Generate hits and therapeutic candidates	Large library size (> 10E10) and robust screening; fully human antibodies	Not all antibodies express well in Escherichia coli and require engineering	Humira and Benlysta
Yeast display	Improve affinity and stability	Eukaryotic host; targeted sorting by FACS	Relatively small library size	None
Transgenic Rodents	Generate therapeutic candidates	High affinity fully human antibodies	Technology accessibility	Vectibis, Ilaris, Simponi, Stelara, Arzerra, Prolia, Yervoy

FDA: Food and Drug Administration; FACS: Fluorescence activated cell sorting.

vide technology know-how to support target validation by academic researchers. It is anticipated that such close collaborations will lead to more validated targets and will help replenish the early drug discovery pipeline.

PLATFORMS FOR ANTIBODY HIT DISCOVERY

Hybridoma

Among all the approved antibody therapeutics, the majority are humanized rodent antibodies. Nowadays, however, fully human antibodies including entities from phage displayed human antibody libraries and from transgenic rodents with human antibody genes are a more common approach and have become a bigger fraction of the industry wide discovery/development pipeline[13]. This phenomenon reflects technology evolution for therapeutic antibody discovery coinciding with the looming expiration of some key technology patents. Table 1 lists a comparison of major antibody discovery technology platforms.

Rodents with wild-type immunoglobulin (Ig) genes remain an important source for therapeutic antibody discovery. With established protocols[14] and many improvements, some industrial laboratories very often obtain desirable hit clones of low pico-molar affinity, thus setting a good start for therapeutic antibody engineering. The technical improvements are centered on enhancing immune response, increasing hybridoma fusion efficiency, incorporating activity assays early in screen and automation for higher assay throughput. Incidentally, these technical improvements are also applicable to the antibody generation using transgenic rodents (see below).

Besides incremental improvements in hybridoma technologies, next generation sequencing (NGS) has been explored for antibody discovery through animal immunization[15]. In its current form, the V-genes of relevant B-cell repertoire are "digitally immortalized" by NGS before certain presumed individual clones are recombinantly produced and phenotyped. It is also worth mentioning that efforts are made to directly identify biologically/biochemically relevant antibodies from single mouse or human B cells[16-19]. In all these novel efforts to speed up and enhance the traditional methods, it is necessary to build compatible experimental workstream for recombinant expression in order to identify desired antibodies especially those rare clones.

A significant challenge to immunization based antibody discovery is related to the nature of new targets themselves, many of which are multispanning membrane proteins. Therefore, conventional biochemistry in preparing soluble protein as immunogens does not work well for this target class. Great efforts have been made to carry out immunizations using DNA vectors, viral-like particles and even viruses in order to deliver protein antigens in their native form to the host animals. Cells of different lineage over-expression target membrane proteins were also used for immunization[20].

Humanization

Humanization is the step that converts rodent antibody leads into human biotherapeutic candidates, and after more than 20 years of practice the technology became very mature in this area. The objective of humanization is to reduce the immunogenicity risk which is thought to be borne in the amino acid sequences. Therefore, a straightforward way is to decrease the murine portion on the sequence level, for which various humanization methods have been developed. Technically, those methods can be classified into two groups: rational methods and empirical methods[21]. Rational methods, including complementarity determining region (CDR) grafting[22,23], specificity determining residue (SDR) grafting[24,25], resurfacing[26,27], superhumanization[28,29], and human string content optimization[30], rely on the iterative design cycles for generating a small number of variants to retain de-

sired properties and then validating. For these methods to be successful, in-depth knowledge of the sequence and structure information becomes a key factor. One notable advantage of rational methods is that it can directly generate antibody variants in full IgG antibody format and this can streamline the downstream process since it eliminates the potential degeneration of antibody characteristics arising from reformatting. On the other hand, empirical methods, including framework repair[31,32], framework shuffling[33], guided selection[34], humaneering[35] and CDR repair[36], rely on the library generation and subsequent screening or selection to identify the desired variants, thus unlike rational design for the single variant, the design of the library and the choice of selection strategy are critical. The advantage of empirical methods is that it can retain or even improve the affinity and thus could be combined with affinity maturation. And the disadvantage is that the antibody variants from libraries are usually in single-chain variable fragment (scFv) or antigen-binding fragment (Fab) format and then need to be converted into final IgG format. It is worth mentioning that the principles and experiences in humanizing mouse antibodies have been applied to engineering antibodies from other species such as rat and rabbit.

Phage display

Phage display, initially developed by George Smith[37] for peptide epitope mapping, is now recognized as the most powerful technology for the construction of human antibody libraries and for the directed evolution of proteins[38]. The technology has been successfully deployed to construct large diversity scFv, Fab and single-domain antibody (sdAb) libraries for the identification of traditionally hard to obtain human antibodies for large panel of antigens[39], thus circumventing the hurdle of obtaining large number of human monoclonal antibodies because of both the ethical challenge of obtaining immunized human B-lymphocytes and the technical challenge in human B cell immortalization approaches[40]. Now, phage displayed antibody libraries are one of the two major sources of fully human antibodies, the other one being the transgenic mice carrying human immunoglobulin gene locus[13,41]. Currently there are two FDA approved therapeutic mAbs (Humira and Benlysta) and at least 8 other fully human mAbs for cancer therapy alone in different stages of clinical trials[42] that are derived from phage displayed antibody libraries.

Very often hits from panning of naive phage displayed antibody libraries need to go through affinity maturation to become therapeutic candidates. Different approaches, such as saturation mutagenesis, phage display and ribosome display[43], have been developed and successfully applied to improve the affinity of the initial hits from even sub-micro molar level to the range that meets the therapeutic requirements as long as the initial antibody hits are confirmed to bind to the right epitope. Further, more human antibody structures and sequences have become public accessible, which will enable more rational design for the affinity maturation of initial hits. Recently, a more powerful and robust affinity maturation technology namely yeast display is being developed (discussed below).

There is a potential drawback that not only affinity but also biophysical property is less desirable from naive phage displayed antibody libraries (Fabs or scFvs), because the displayed antibodies are represented as Fabs or scFvs, and expressed in *Escherichia coli* a prokaryotic system. This phenomenon can be further exacerbated with synthetic antibody libraries. To overcome this potential drawback, one can carry out initial rounds of bio-panning of phage displayed antibody libraries to generate hits, followed by displaying the hit pools on yeast. Using this approach, the large number of initial hits will be sorted by fluorescence activated cell sorting (FACS) technology according to their binding to the target, which depends on their affinity as well as their expression, post-translational processing and eventual display on the surface of yeast, a eukaryote.

In recent years, NGS has found many applications in therapeutic antibody discovery. Deep sequencing helps characterize an antibody library made from the Ig V gene pool from hundreds of people[44], or from cord blood cells[45], thus providing a better guidance for the design of new versions of phage displayed human antibody libraries to capture and represent *in vitro* the natural human Ig V gene repertoire at the maximum level[46,47]. With all this newly learned knowledge one can more easily maintain the characters of a CDR when designing a synthetic or semi-synthetic antibody library for better performance. The knowledge also helps the smarter affinity maturation design with minimum mutations in CDRs. It is generally believed that the immunogenicity of therapeutic antibodies could be reduced if they have higher identity to natural human B cell antibodies[41,48].

The ultimate power of phage displayed antibody libraries will be harnessed when both robust cell based phage biopanning and label-free cell-based assays for initial phage antibody hits are being established and optimized, which will in due course, increase the chance of identifying therapeutic antibodies for more challenging and less explored targets such as G-protein coupled receptors (7TMs) and other multi-spanning membrane proteins. In our opinion, phage display along with other two mainstream technologies, namely, mouse hybridoma technology and transgenic mice carrying human immunoglobulin locus will yield more novel therapeutic antibodies in the years to come.

Yeast display

In most cases, initial antibody hits generated through various methods need to be further improved in many aspects, such as affinity, stability and isoelectric point (pI), to meet requirements as biotherapeutics. Among the methods used for affinity maturation, yeast surface display is a convenient yet very effective approach. In this approach, an antibody or antibody fragment of interest is

displayed on yeast surface by fusing to yeast a-agglutinin subunit 2 (Aga2p). Aga2p is linked through double disulfide bonds to a-agglutinin subunit 1 (Aga1p), which is overexpressed on yeast surface in a display strain. Tags are usually fused to C-terminus of the displayed antibody, which are used for checking display levels. Desired regions of an antibody are mutagenized to create mutagenic libraries. Yeast displayed antibody mutagenic libraries are then double stained with antigen and anti-tag antibody to check antigen binding and display level. FACS is used iteratively to select strong binding population among displayed variants derived from the antibody hits of previous round[49]. Depending on the cell staining-sorting protocol, one can selectively improve a specific element of affinity such as off-rate[50]. Using such a protocol, a 10 000-fold increase of affinity can be achieved for an antibody against its target, of which 1000 fold can be gained from the off-rate improvement. In addition, non-antibody binding domains can be displayed for affinity maturation, Jin *et al*[51] reported a 200 000-fold increase of receptor α_L integrin inserted domain using yeast display.

One challenge for therapeutic antibody development is the presence of undesirable post-translational modification (PTM) sites in complimentary determining regions (CDR) which can cause detrimental modification during production, processing and storage. Using yeast display, one can include saturation mutagenesis strategies to replace the undesired amino acids in the PTM sites during mutagenic library construction for affinity maturation. In this way, the iterative selection procedure of yeast display will yield clones with required affinity and without undesired PTM sites.

As a eukaryotic host yeast has the quality control machinery for proper protein folding, which all antibodies or antibody fragments need to go through before being displayed on the cell surface. Therefore, the antibodies or antibody fragments displayed on yeast surface are most likely well folded. This feature is well suited for improvement of other pharmaceutical properties of therapeutic antibodies. For example, one can control library sorting to maintain diversity of output, and as a result, to increase likelihood of finding clones with increased Tm and desired pI.

In addition to improving antibody affinity and biophysical properties, yeast display has also been used for *de novo* selection of binders from either immune[52] or nonimmune libraries[53]. There are two obvious advantages in using yeast display for primary hit selection. Firstly, clones isolated by yeast display have to pass eukaryotic post-translational quality control machinery and are likely to have better biophysical properties. Secondly, one can use FACS to estimate affinity and dissociation rate of yeast-displayed binders without the need for recombinant expression. This saves time and cost and can increase throughput of the binders to be tested. One drawback of using yeast display for primary hit selection is its relatively smaller library size than phage display. Typical library sizes for yeast display range from 10^8 to 10^9[54], while phage library can be as large as 10^{11}[55]. However, recent efforts on improving yeast transformation efficiency have achieved 10^{10} library size for displaying human antibodies, which could increase its competitiveness against other display systems in terms of library size[56].

Transgenic rodents

The initial attempt using mice to generate fully human antibodies directly was made by immunizing SCID mice harboring reconstituted immune system from grafted human hematopoietic stem cells (HSC)[57]. Nowadays there are mouse strains that accept human hematopoietic stem grafts more readily and develop more human like immune system[58-60]. Among the challenges in using these mice is the tedious work of HSC grafting, since the successfully reconstituted immune system will not pass to the progeny.

Mice carrying partial human immunoglobulin gene loci that functionally replaces their own immunoglobulin genes were first developed in the 1990's, with KM mouse and XenoMouse as the most representative ones[61,62]. The key common features of the first generation of human Ig transgenic mice are the inclusion of partial human Ig V gene repertoire and human CH genes. It is generally thought these features contributed to the suboptimal antibody response to immunogens. As a consequence, when these animals are used for therapeutic antibody discovery, much larger cohorts have to be immunized in order to yield adequate pools of hits to screen. Antibodies generated from these mice have started to contribute to the current biotherapeutics pipeline.

Efforts have been made to create new strains of transgenic mice. One area of focus is to include more complete human Ig repertoire coded by larger portions of human immunoglobulin loci. The other area of focus is to keep more murine *cis* elements required for efficient B-cell receptor (BCR) assembly and signaling[63]. New strains of mouse and rat that incorporate the above improvements have been or being created by firms such as Ablexis (www.ablexis.com), Harbour Antibodies (www.harbourantibodies.com), Kymab (www.kymab.com), and OMT (www.openmonoclonaltechnology.com). Further, many of these companies attempt new technology access models that potentially provide more benefits to industry-wide therapeutic antibody discovery.

BISPECIFIC ANTIBODIES

Conventional therapeutic antibodies have a large molecular weight, and are bivalent and mono-specific for target binding. For accessing many potential new targets and harnessing body's disease fighting power, it is highly desirable to have biotherapeutic entities that are smaller and capable of binding to multiple different targets at right compartment, biochemically stable yet having required serum half-life. The "bispecific" biotherapeutics not only has sound scientific rationale but also has achieved clinical efficacy[64,65].

The most successful class of bispecific antibodies to date involved T-cell recruitment by anti-CD3 moiety and another moiety for tumor cell targeting. Catumaxomab was the first approved bispecific antibody targeting EpCam and CD3. In patients with malignant ascite due to epithelial carcinomas, it can improve puncture free survival to 46 d versus 11 d in the control group, and overall survival to 71 d *vs* 44 d in the control[66]. There are now five programs in early stage clinical trials that shared this similar concept--Blinatumomab (CD19 × CD3), MT110 (EpCam × CD3) and MT111 (CEA × CD3) by Micromet, FBTA05 (CD20 × CD3) by Trion and IMCgp100 (gp100 × CD3) by Immunocore. Remarkably, Blinatumomab showed 80% response rate at a dose level of 0.06 mg per square meter[67] in a phase II trial. Blintumomab also led to clearance of tumor cells in bone marrow and liver. The clinical benefits therefore provide strong validation for the effective tumor cell eradication by T-cell recruitment using bispecific antibodies. Initial formats such as triomab and quandroma had issues in manufacturing due to heterogeneity[68]. BiTE (for bispecific T-cell engager) based on the fusion of two scFvs was used to build Blinatumomab. However, the shorter half-life of BiTE requires continuous infusion. Improvement in the format may further help for efficacy and manufacturability.

There are other classes of bispecific antibodies that are in clinical trials[68]. They are either simultaneous targeting of multiple receptors-examples are anti-EGFR × anti-HER3, anti-Her2 × anti-Her3, anti-Ang2 × anti-VEGF, or targeting two ligands-such as anti-IL4 × anti-IL13. MEHD7945A, a two-in-one antibody against EGFR and HER3, was developed based on that complete inhibition of downstream MAPK and AKT pathways can only be achieved when both EGFR and HER3 are inhibited simultaneously. This bispecific antibody is expected to be efficacious for broader patient populations. Interestingly, MEHD7945A provided impressive tumor killing in multiple tumor models, and was more active than either EGFR antibody or HER3 antibody in several tumor models[69]. Yet it would be critical to see if the benefits can be realized in the more complicated patient populations. The validation of this class of bispecific antibodies could be another important leap forward. It would bring up a whole spectrum of possible combinations. Different combinations would require different formats. It is equally encouraging to see a huge effort and availability of a large number of formats that can potentially support the various needs.

STABILITY AND AGGREGATION

Stability and aggregation level of biologic drugs are the two critical factors affecting their pharmaceutical properties such as protein production, formulation, shelf life, dosing route, *in vivo* half-life and immunogenicity. Therefore, great efforts are made on stability enhancement and aggregation reduction for biotherapeutics.

Both sequence- and structure-based approaches have been successfully applied to improve the biotherapeutic stability. Sequence based analyses such as the germlining analysis[70,71], sequence consensus analysis[72], and sequence covariance analysis[73] have all revealed potential amino acid changes to improve protein stability. These methods are based on the contemplation that more frequent amino acids or amino acid pairs in germline or a large number of homologous sequences are more likely than other amino acid combinations to increase protein stability. The covariance method, in particular, has improved the melting temperature (Tm) of a scFv by an impressive 11.2 ℃ by a single point mutation in the VH framework[74].

The structure based engineering attempts to stabilize fragile regions either by inserting extra stabilizing interactions, such as salt bridges[74] or disulfide bonds[75], or by eliminating incompatible interactions such as charges being buried in hydrophobic core[76]. On the experimental side, the clever deployment of display technology[77] has also produced stabilizing amino acid changes.

With all the point mutation data collected from sequence design, structure based design, random screening and display, it is more likely than ever to establish a set of empirical rules to predict the effects of mutations on protein stability. Algorithms like I-Mutant[78] are the pioneers, though the accuracy of these algorithms is still less than desired[79].

The progress in thermal stability engineering has largely benefited from the high throughput screening techniques such as differential scanning fluorimetry[80]. Unfortunately, a similar high throughput approach has not been established to screen for mutations affecting aggregation. The low throughput HPLC technology is still the status quo for quantifying protein aggregation. Further, aggregation is sensitive to growth condition and purification procedure, which makes it more complicated for studying intrinsic factors influencing protein aggregation.

Mutations designed on structure or sequence considerations have been reported to significantly reduce the aggregation level of target proteins. For instance, a single F29D mutation could make a single domain VH antibody totally soluble even when heated to 95 ℃[81]. The encouraging examples in literature demonstrate that our current understanding of the aggregation mechanisms can guide us operationally in designing a small mutagenesis library that covers several point mutations to suppress protein aggregation.

Two major mechanisms work independently or collaboratively to induce aggregation. The first is the non-native interaction among proteins in their fully or partially unfolded states. The second is the hydrophobic attraction among charge-neutral monomers in their native states. If the first mechanism is the major cause, the aggregation can be remedied by mutations that enhance protein stability. In this scenario, the higher the protein stability is, the lower the protein aggregation propensity is[82]. If the second mechanism dominates, the aggregation can be reduced by mutations that either drive the isoelectric

point away from the buffer pH or reduce solvent exposed hydrophobic surface area. The introduction of highly soluble glycan is also an alternative[83]. In this scenario aggregation and stability are not necessarily correlated in any fashion.

In antibody therapeutics sometimes CDRs epitomize the issues for stability and aggregation. Changing residues within and around CDRs have improved the stability and reduced the aggregation on several Fv domains[81] without changing the affinity, indicating in these cases not every residue affecting biophysical properties in CDRs participates in target binding. This strategy may become necessary when there are no other alternatives.

IMMUNOGENICITY

Approximately 40% of the approved chimeric antibodies and 9% of humanized antibodies induce marked anti-drug antibody responses in vivo[84]. On the other hand, fully human antibody therapeutics can still be immunogenic. For example, 12% of patients treated with the fully-human antibody adalimunab (Humira) developed anti-drug antibodies[85,86]. Neutralizing anti-drug antibodies interfere with drug-target interaction, thus decreasing the efficacy directly, while non-neutralizing anti-drug antibodies may lead to abnormal pharmacokinetics in affected individuals. In rare cases anti-drug antibodies might lead toxicity due to the formation of immune complexes. The concern and consequence of immune responses to the non-human sequences has led to the development of fully human antibodies using human antibody sequences or using transgenic mice bearing the human antibody genome. Yet even in fully human antibodies the antigen-specific CDRs still can break the tolerance and become immunogenic. This is exemplified by the fact that human sera from healthy donors contain detectable levels of anti-idiotype antibody to a wide variety of autoantibodies, indicating that the immune tolerance system is actually leaky[87]. The presence of CD4+ T helper cell epitopes in the V region have been described as correlating with immune responses to antibodies.

The main approach to minimizing the immunogenicity of antibody therapeutics has been replacing as much of the non-human part of the sequences as possible with the human antibody sequences. Identification and modification of CD4+ T cell epitopes may lead to a reduced immunogenicity. Computational models and *in vitro* T cell stimulation tests have been developed to predict CD4+ T cell epitopes, each of these methods has limitations and tends to under- or over-predict CD4+ T cell epitopes[88]. Additionally, antibody aggregation and post-translational modifications such as glycosylation, glycation, deamidation and oxidation of amino acid side chains can confer immunogenicity as well and should be avoided[89].

Efforts have also been made to predict immunogenicity of biotherapeutic candidates using various animal models. Although these animal models are helpful in understanding the mechanisms underlying immunogenicity of therapeutic proteins, they have limited predictive value because of species differences[90]. Therefore, at the present time the immunogenicity is still an important domain of translational studies in clinical development of biotherapeutics.

CONCLUSION

This review summarized ongoing strategies actively pursued by both biopharma and academics to overcome some of current challenges in biotherapeutics discovery. These efforts will inevitably lead to new opportunities for the biopharma industry and will benefit patients. With the increased understanding of antibody structure and function, more rationale design approaches for further engineering the initial hits identified by contemporary screening/selection technologies are enabling the generation of antibodies with improved pharmacological and physicochemical properties. Further, the ongoing efforts in discovery of new biologics targets aided by the advances in systems biology, extensive investigation of bi-specific antibodies and ADC for enhancement of therapeutic effects, wide application of NGS for harnessing the power of natural human antibody repertoires, together with increased investment and enthusiasm from both industry and academia will ultimately bring new safer and more effective biologic therapies to patients in the years ahead.

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REFERENCES

1 **Caravella J**, Lugovskoy A. Design of next-generation protein therapeutics. *Curr Opin Chem Biol* 2010; **14**: 520-528
2 **Projan SJ**, Gill D, Lu Z, Herrmann SH. Small molecules for small minds? The case for biologic pharmaceuticals. *Expert Opin Biol Ther* 2004; **4**: 1345-1350
3 **Berggren R**, Møller M, Moss R, Poda P, Smietana K. Outlook for the next 5 years in drug innovation. *Nat Rev Drug Discov* 2012; **11**: 435-436
4 **Rask-Andersen M**, Almén MS, Schiöth HB. Trends in the exploitation of novel drug targets. *Nat Rev Drug Discov* 2011; **10**: 579-590
5 **Fishman MC**, Porter JA. Pharmaceuticals: a new grammar for drug discovery. *Nature* 2005; **437**: 491-493
6 **MacDonald AS**. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 2001; **71**: 271-280
7 **Hudes G**, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007; **356**: 2271-2281
8 **Baselga J**, Campone M, Piccart M, Burris HA, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, Beck JT, Ito Y, Yardley D, Deleu I, Perez A, Bachelot T, Vit-

tori L, Xu Z, Mukhopadhyay P, Lebwohl D, Hortobagyi GN. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 2012; **366**: 520-529
9 **Yap TA**, Olmos D, Molife LR, de Bono JS. Targeting the insulin-like growth factor signaling pathway: figitumumab and other novel anticancer strategies. *Expert Opin Investig Drugs* 2011; **20**: 1293-1304
10 **Maira SM**, Pecchi S, Huang A, Burger M, Knapp M, Sterker D, Schnell C, Guthy D, Nagel T, Wiesmann M, Brachmann S, Fritsch C, Dorsch M, Chène P, Shoemaker K, De Pover A, Menezes D, Martiny-Baron G, Fabbro D, Wilson CJ, Schlegel R, Hofmann F, García-Echeverría C, Sellers WR, Voliva CF. Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor. *Mol Cancer Ther* 2012; **11**: 317-328
11 **Fire A**, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 1998; **391**: 806-811
12 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432
13 **Nelson AL**, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. *Nat Rev Drug Discov* 2010; **9**: 767-774
14 **Köhler G**, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; **256**: 495-497
15 **Reddy ST**, Ge X, Miklos AE, Hughes RA, Kang SH, Hoi KH, Chrysostomou C, Hunicke-Smith SP, Iverson BL, Tucker PW, Ellington AD, Georgiou G. Monoclonal antibodies isolated without screening by analyzing the variable-gene repertoire of plasma cells. *Nat Biotechnol* 2010; **28**: 965-969
16 **Story CM**, Papa E, Hu CC, Ronan JL, Herlihy K, Ploegh HL, Love JC. Profiling antibody responses by multiparametric analysis of primary B cells. *Proc Natl Acad Sci USA* 2008; **105**: 17902-17907
17 **Collarini EJ**, Lee FE, Foord O, Park M, Sperinde G, Wu H, Harriman WD, Carroll SF, Ellsworth SL, Anderson LJ, Tripp RA, Walsh EE, Keyt BA, Kauvar LM. Potent high-affinity antibodies for treatment and prophylaxis of respiratory syncytial virus derived from B cells of infected patients. *J Immunol* 2009; **183**: 6338-6345
18 **Jin A**, Ozawa T, Tajiri K, Obata T, Kondo S, Kinoshita K, Kadowaki S, Takahashi K, Sugiyama T, Kishi H, Muraguchi A. A rapid and efficient single-cell manipulation method for screening antigen-specific antibody-secreting cells from human peripheral blood. *Nat Med* 2009; **15**: 1088-1092
19 **Wilson PC**, Andrews SF. Tools to therapeutically harness the human antibody response. *Nat Rev Immunol* 2012; **12**: 709-719
20 **Ebersbach H**, Proetzel G, Zhang C. Antigen presentation for the generation of binding molecules. In: Proetzel G, Ebersbach H, edtors. Antibody methods and protocols. Totowa: Humana Press, 2012: 1-10
21 **Almagro JC**, Fransson J. Humanization of antibodies. *Front Biosci* 2008; **13**: 1619-1633
22 **Riechmann L**, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. *Nature* 1988; **332**: 323-327
23 **Queen C**, Schneider WP, Selick HE, Payne PW, Landolfi NF, Duncan JF, Avdalovic NM, Levitt M, Junghans RP, Waldmann TA. A humanized antibody that binds to the interleukin 2 receptor. *Proc Natl Acad Sci USA* 1989; **86**: 10029-10033
24 **Gonzales NR**, Padlan EA, De Pascalis R, Schuck P, Schlom J, Kashmiri SV. SDR grafting of a murine antibody using multiple human germline templates to minimize its immunogenicity. *Mol Immunol* 2004; **41**: 863-872
25 **Kashmiri SV**, De Pascalis R, Gonzales NR, Schlom J. SDR grafting--a new approach to antibody humanization. *Methods* 2005; **36**: 25-34
26 **Padlan EA**. A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties. *Mol Immunol* 1991; **28**: 489-498
27 **Roguska MA**, Pedersen JT, Keddy CA, Henry AH, Searle SJ, Lambert JM, Goldmacher VS, Blättler WA, Rees AR, Guild BC. Humanization of murine monoclonal antibodies through variable domain resurfacing. *Proc Natl Acad Sci USA* 1994; **91**: 969-973
28 **Tan P**, Mitchell DA, Buss TN, Holmes MA, Anasetti C, Foote J. "Superhumanized" antibodies: reduction of immunogenic potential by complementarity-determining region grafting with human germline sequences: application to an anti-CD28. *J Immunol* 2002; **169**: 1119-1125
29 **Hwang WY**, Almagro JC, Buss TN, Tan P, Foote J. Use of human germline genes in a CDR homology-based approach to antibody humanization. *Methods* 2005; **36**: 35-42
30 **Lazar GA**, Desjarlais JR, Jacinto J, Karki S, Hammond PW. A molecular immunology approach to antibody humanization and functional optimization. *Mol Immunol* 2007; **44**: 1986-1998
31 **Baca M**, Presta LG, O'Connor SJ, Wells JA. Antibody humanization using monovalent phage display. *J Biol Chem* 1997; **272**: 10678-10684
32 **Chen Y**, Wiesmann C, Fuh G, Li B, Christinger HW, McKay P, de Vos AM, Lowman HB. Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an affinity-matured Fab in complex with antigen. *J Mol Biol* 1999; **293**: 865-881
33 **Dall'Acqua WF**, Damschroder MM, Zhang J, Woods RM, Widjaja L, Yu J, Wu H. Antibody humanization by framework shuffling. *Methods* 2005; **36**: 43-60
34 **Osbourn J**, Groves M, Vaughan T. From rodent reagents to human therapeutics using antibody guided selection. *Methods* 2005; **36**: 61-68
35 **Baer M**, Sawa T, Flynn P, Luehrsen K, Martinez D, Wiener-Kronish JP, Yarranton G, Bebbington C. An engineered human antibody fab fragment specific for Pseudomonas aeruginosa PcrV antigen has potent antibacterial activity. *Infect Immun* 2009; **77**: 1083-1090
36 **Dennis M**. CDR repair: A novel approach to antibody humanization. In: Shire SJ, Gombotz W, Bechtold-Peters K, Andya J, editors. Current trends in monoclonal antibody development and manufacturing. New York: Springer, 2010: 9-28
37 **Smith GP**. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 1985; **228**: 1315-1317
38 **McCafferty J**, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* 1990; **348**: 552-554
39 **Schofield DJ**, Pope AR, Clementel V, Buckell J, Chapple SDj, Clarke KF, Conquer JS, Crofts AM, Crowther SR, Dyson MR, Flack G, Griffin GJ, Hooks Y, Howat WJ, Kolb-Kokocinski A, Kunze S, Martin CD, Maslen GL, Mitchell JN, O'Sullivan M, Perera RL, Roake W, Shadbolt SP, Vincent KJ, Warford A, Wilson WE, Xie J, Young JL, McCafferty J. Application of phage display to high throughput antibody generation and characterization. *Genome Biol* 2007; **8**: R254
40 **Li J**, Sai T, Berger M, Chao Q, Davidson D, Deshmukh G, Drozdowski B, Ebel W, Harley S, Henry M, Jacob S, Kline B, Lazo E, Rotella F, Routhier E, Rudolph K, Sage J, Simon P, Yao J, Zhou Y, Kavuru M, Bonfield T, Thomassen MJ, Sass PM, Nicolaides NC, Grasso L. Human antibodies for immunotherapy development generated via a human B cell hybridoma technology. *Proc Natl Acad Sci USA* 2006; **103**: 3557-3562
41 **Weiner LM**. Fully human therapeutic monoclonal antibodies. *J Immunother* 2006; **29**: 1-9
42 **Dantas-Barbosa C**, de Macedo Brigido M, Maranhao AQ. Antibody phage display libraries: contributions to oncology. *Int J Mol Sci* 2012; **13**: 5420-5440

43 **Finlay WJ**, Cunningham O, Lambert MA, Darmanin-Sheehan A, Liu X, Fennell BJ, Mahon CM, Cummins E, Wade JM, O'Sullivan CM, Tan XY, Piche N, Pittman DD, Paulsen J, Tchistiakova L, Kodangattil S, Gill D, Hufton SE. Affinity maturation of a humanized rat antibody for anti-RAGE therapy: comprehensive mutagenesis reveals a high level of mutational plasticity both inside and outside the complementarity-determining regions. *J Mol Biol* 2009; **388**: 541-558

44 **Glanville J**, Zhai W, Berka J, Telman D, Huerta G, Mehta GR, Ni I, Mei L, Sundar PD, Day GM, Cox D, Rajpal A, Pons J. Precise determination of the diversity of a combinatorial antibody library gives insight into the human immunoglobulin repertoire. *Proc Natl Acad Sci USA* 2009; **106**: 20216-20221

45 **Prabakaran P**, Chen W, Singarayan MG, Stewart CC, Streaker E, Feng Y, Dimitrov DS. Expressed antibody repertoires in human cord blood cells: 454 sequencing and IMGT/HighV-QUEST analysis of germline gene usage, junctional diversity, and somatic mutations. *Immunogenetics* 2012; **64**: 337-350

46 **Zhai W**, Glanville J, Fuhrmann M, Mei L, Ni I, Sundar PD, Van Blarcom T, Abdiche Y, Lindquist K, Strohner R, Telman D, Cappuccilli G, Finlay WJ, Van den Brulle J, Cox DR, Pons J, Rajpal A. Synthetic antibodies designed on natural sequence landscapes. *J Mol Biol* 2011; **412**: 55-71

47 **Glanville J**, Kuo TC, von Büdingen HC, Guey L, Berka J, Sundar PD, Huerta G, Mehta GR, Oksenberg JR, Hauser SL, Cox DR, Rajpal A, Pons J. Naive antibody gene-segment frequencies are heritable and unaltered by chronic lymphocyte ablation. *Proc Natl Acad Sci USA* 2011; **108**: 20066-20071

48 **Lonberg N**. Fully human antibodies from transgenic mouse and phage display platforms. *Curr Opin Immunol* 2008; **20**: 450-459

49 **Boder ET**, Wittrup KD. Yeast surface display for screening combinatorial polypeptide libraries. *Nat Biotechnol* 1997; **15**: 553-557

50 **Siegel RW**, Baugher W, Rahn T, Drengler S, Tyner J. Affinity maturation of tacrolimus antibody for improved immunoassay performance. *Clin Chem* 2008; **54**: 1008-1017

51 **Jin M**, Song G, Carman CV, Kim YS, Astrof NS, Shimaoka M, Wittrup DK, Springer TA. Directed evolution to probe protein allostery and integrin I domains of 200,000-fold higher affinity. *Proc Natl Acad Sci USA* 2006; **103**: 5758-5763

52 **Bowley DR**, Labrijn AF, Zwick MB, Burton DR. Antigen selection from an HIV-1 immune antibody library displayed on yeast yields many novel antibodies compared to selection from the same library displayed on phage. *Protein Eng Des Sel* 2007; **20**: 81-90

53 **Wang XX**, Cho YK, Shusta EV. Mining a yeast library for brain endothelial cell-binding antibodies. *Nat Methods* 2007; **4**: 143-145

54 **Gera N**, Hussain M, Rao BM. Protein selection using yeast surface display. *Methods* 2012; Epub ahead of print

55 **Dufner P**, Jermutus L, Minter RR. Harnessing phage and ribosome display for antibody optimisation. *Trends Biotechnol* 2006; **24**: 523-529

56 **Benatuil L**, Perez JM, Belk J, Hsieh CM. An improved yeast transformation method for the generation of very large human antibody libraries. *Protein Eng Des Sel* 2010; **23**: 155-159

57 **McCune JM**, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 1988; **241**: 1632-1639

58 **Legrand N**, Huntington ND, Nagasawa M, Bakker AQ, Schotte R, Strick-Marchand H, de Geus SJ, Pouw SM, Böhne M, Voordouw A, Weijer K, Di Santo JP, Spits H. Functional CD47/signal regulatory protein alpha (SIRP(alpha)) interaction is required for optimal human T- and natural killer- (NK) cell homeostasis in vivo. *Proc Natl Acad Sci USA* 2011; **108**: 13224-13229

59 **Chen Q**, Khoury M, Chen J. Expression of human cytokines dramatically improves reconstitution of specific human-blood lineage cells in humanized mice. *Proc Natl Acad Sci USA* 2009; **106**: 21783-21788

60 **Suzuki M**, Takahashi T, Katano I, Ito R, Ito M, Harigae H, Ishii N, Sugamura K. Induction of human humoral immune responses in a novel HLA-DR-expressing transgenic NOD/Shi-scid/γcnull mouse. *Int Immunol* 2012; **24**: 243-252

61 **Green LL**, Hardy MC, Maynard-Currie CE, Tsuda H, Louie DM, Mendez MJ, Abderrahim H, Noguchi M, Smith DH, Zeng Y, David NE, Sasai H, Garza D, Brenner DG, Hales JF, McGuinness RP, Capon DJ, Klapholz S, Jakobovits A. Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. *Nat Genet* 1994; **7**: 13-21

62 **Lonberg N**, Taylor LD, Harding FA, Trounstine M, Higgins KM, Schramm SR, Kuo CC, Mashayekh R, Wymore K, McCabe JG. Antigen-specific human antibodies from mice comprising four distinct genetic modifications. *Nature* 1994; **368**: 856-859

63 **Lee EC**, Owen M. The application of transgenic mice for therapeutic antibody discovery. In: Proetzel G, Ebersbach H, editors. Antibody methods and protocols. Totowa: Humana Press, 2012: 137-148

64 **Kontermann R**. Dual targeting strategies with bispecific antibodies. *MAbs* 2012; **4**: Epub ahead of print

65 **Li J**, Zhu Z. Research and development of next generation of antibody-based therapeutics. *Acta Pharmacol Sin* 2010; **31**: 1198-1207

66 **Linke R**, Klein A, Seimetz D. Catumaxomab: clinical development and future directions. *MAbs* 2010; **2**: 129-136

67 **Klinger M**, Brandl C, Zugmaier G, Hijazi Y, Bargou RC, Topp MS, Gökbuget N, Neumann S, Goebeler M, Viardot A, Stelljes M, Brüggemann M, Hoelzer D, Degenhard E, Nagorsen D, Baeuerle PA, Wolf A, Kufer P. Immunopharmacologic response of patients with B-lineage acute lymphoblastic leukemia to continuous infusion of T cell-engaging CD19/CD3-bispecific BiTE antibody blinatumomab. *Blood* 2012; **119**: 6226-6233

68 **May C**, Sapra P, Gerber HP. Advances in bispecific biotherapeutics for the treatment of cancer. *Biochem Pharmacol* 2012; **84**: 1105-1112

69 **Schaefer G**, Haber L, Crocker LM, Shia S, Shao L, Dowbenko D, Totpal K, Wong A, Lee CV, Stawicki S, Clark R, Fields C, Lewis Phillips GD, Prell RA, Danilenko DM, Franke Y, Stephan JP, Hwang J, Wu Y, Bostrom J, Sliwkowski MX, Fuh G, Eigenbrot C. A two-in-one antibody against HER3 and EGFR has superior inhibitory activity compared with monospecific antibodies. *Cancer Cell* 2011; **20**: 472-486

70 **Luo J**, Obmolova G, Huang A, Strake B, Teplyakov A, Malia T, Muzammil S, Zhao Y, Gilliland GL, Feng Y. Coevolution of antibody stability and Vκ CDR-L3 canonical structure. *J Mol Biol* 2010; **402**: 708-719

71 **Urech DM**, Borras L, inventor; ESBATECH AG (Schlieren CH), assignee. Sequence based engineering and optimization of single chain antibodies. United States Patent Application 20100137150. 2010 Jun 3

72 **Chowdhury PS**, Vasmatzis G, Beers R, Lee B, Pastan I. Improved stability and yield of a Fv-toxin fusion protein by computer design and protein engineering of the Fv. *J Mol Biol* 1998; **281**: 917-928

73 **Wang N**, Smith WF, Miller BR, Aivazian D, Lugovskoy AA, Reff ME, Glaser SM, Croner LJ, Demarest SJ. Conserved amino acid networks involved in antibody variable domain interactions. *Proteins* 2009; **76**: 99-114

74 **Miller BR**, Demarest SJ, Lugovskoy A, Huang F, Wu X, Snyder WB, Croner LJ, Wang N, Amatucci A, Michaelson JS, Glaser SM. Stability engineering of scFvs for the development of bispecific and multivalent antibodies. *Protein Eng Des Sel* 2010; **23**: 549-557

75 **Zhao JX**, Yang L, Gu ZN, Chen HQ, Tian FW, Chen YQ,

Zhang H, Chen W. Stabilization of the single-chain fragment variable by an interdomain disulfide bond and its effect on antibody affinity. *Int J Mol Sci* 2010; **12**: 1-11
76 **Langedijk AC**, Honegger A, Maat J, Planta RJ, van Schaik RC, Plückthun A. The nature of antibody heavy chain residue H6 strongly influences the stability of a VH domain lacking the disulfide bridge. *J Mol Biol* 1998; **283**: 95-110
77 **Jung S**, Honegger A, Plückthun A. Selection for improved protein stability by phage display. *J Mol Biol* 1999; **294**: 163-180
78 **Capriotti E**, Fariselli P, Casadio R. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. *Nucleic Acids Res* 2005; **33**: W306-W310
79 **Potapov V**, Cohen M, Schreiber G. Assessing computational methods for predicting protein stability upon mutation: good on average but not in the details. *Protein Eng Des Sel* 2009; **22**: 553-560
80 **King AC**, Woods M, Liu W, Lu Z, Gill D, Krebs MR. High-throughput measurement, correlation analysis, and machine-learning predictions for pH and thermal stabilities of Pfizer-generated antibodies. *Protein Sci* 2011; **20**: 1546-1557
81 **Perchiacca JM**, Bhattacharya M, Tessier PM. Mutational analysis of domain antibodies reveals aggregation hotspots within and near the complementarity determining regions. *Proteins* 2011; **79**: 2637-2647
82 **Wörn A**, Plückthun A. Different equilibrium stability behavior of ScFv fragments: identification, classification, and improvement by protein engineering. *Biochemistry* 1999; **38**: 8739-8750
83 **Wu SJ**, Luo J, O'Neil KT, Kang J, Lacy ER, Canziani G, Baker A, Huang M, Tang QM, Raju TS, Jacobs SA, Teplyakov A, Gilliland GL, Feng Y. Structure-based engineering of a monoclonal antibody for improved solubility. *Protein Eng Des Sel* 2010; **23**: 643-651
84 **Hwang WY**, Foote J. Immunogenicity of engineered antibodies. *Methods* 2005; **36**: 3-10
85 **Bartelds GM**, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, Dijkmans BA, Tak PP, Wolbink GJ. Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis* 2007; **66**: 921-926
86 **Bender NK**, Heilig CE, Dröll B, Wohlgemuth J, Armbruster FP, Heilig B. Immunogenicity, efficacy and adverse events of adalimumab in RA patients. *Rheumatol Int* 2007; **27**: 269-274
87 **Harding FA**, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MAbs* 2010; **2**: 256-265
88 **Messitt TJ**, Terry F, Moise L, Martin W, De Groot AS. A comparison of two methods for T cell epitope mapping: "cell free" in vitro versus immunoinformatics. *Immunome Res* 2011; **7**: 6
89 **Büttel IC**, Chamberlain P, Chowers Y, Ehmann F, Greinacher A, Jefferis R, Kramer D, Kropshofer H, Lloyd P, Lubiniecki A, Krause R, Mire-Sluis A, Platts-Mills T, Ragheb JA, Reipert BM, Schellekens H, Seitz R, Stas P, Subramanyam M, Thorpe R, Trouvin JH, Weise M, Windisch J, Schneider CK. Taking immunogenicity assessment of therapeutic proteins to the next level. *Biologicals* 2011; **39**: 100-109
90 **Brinks V**, Jiskoot W, Schellekens H. Immunogenicity of therapeutic proteins: the use of animal models. *Pharm Res* 2011; **28**: 2379-2385

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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