PERSPECTIVES

TIMELINE

Strategies and challenges for the next generation of therapeutic antibodies

Alain Beck, Thierry Wurch, Christian Bailly and Nathalie Corvaia

Abstract | Antibodies and related products are the fastest growing class of therapeutic agents. By analysing the regulatory approvals of IgG-based biotherapeutic agents in the past 10 years, we can gain insights into the successful strategies used by pharmaceutical companies so far to bring innovative drugs to the market. Many challenges will have to be faced in the next decade to bring more efficient and affordable antibody-based drugs to the clinic. Here, we discuss strategies to select the best therapeutic antigen targets, to optimize the structure of IgG antibodies and to design related or new structures with additional functions.

The research and development of monoclonal antibodies is a rapidly progressing field[1,2]. In the past 25 years, more than 30 immunoglobulins (IgGs) and their derivatives have been approved for use in various indications[3,4] (TIMELINE). The currently marketed antibody-based drugs have been approved not only to treat diseases affecting large numbers of patients (such as cancer and inflammatory diseases) but also for more specialized indications owing to special regulatory procedures for rare medical conditions (orphan diseases), such as paroxysmal nocturnal haemoglobinuria (for which, eculizumab (Soliris; Alexion pharmaceuticals) therapy was approved in 2007). Interestingly, 9 out of the 26 antibodies currently in Phase III clinical trials (35%) have 'orphan drug' designation[4].

Since the first generation of mouse, chimeric and humanized IgG1 antibodies reached the market in the late 1990s, the variety of antibody structures has been considerably extended. Humanized and human antibodies of other IgG isotypes (IgG2 and IgG4)[5] have been developed, as well as a large number of IgG-related products[6]. By analysing the successful regulatory approvals of IgG-based biotherapeutic agents in the past 10 years (TIMELINE), we can gain insights into the strategies developed by biopharmaceutical companies. Here, we discuss strategies to select therapeutic antigen targets based on previous clinical or experimental validation or on functional approaches; strategies to optimize the antibody structure and to design related or new structures with additional functions; as well as challenges to bring more affordable treatments to the most appropriate patient populations screened for validated biomarkers.

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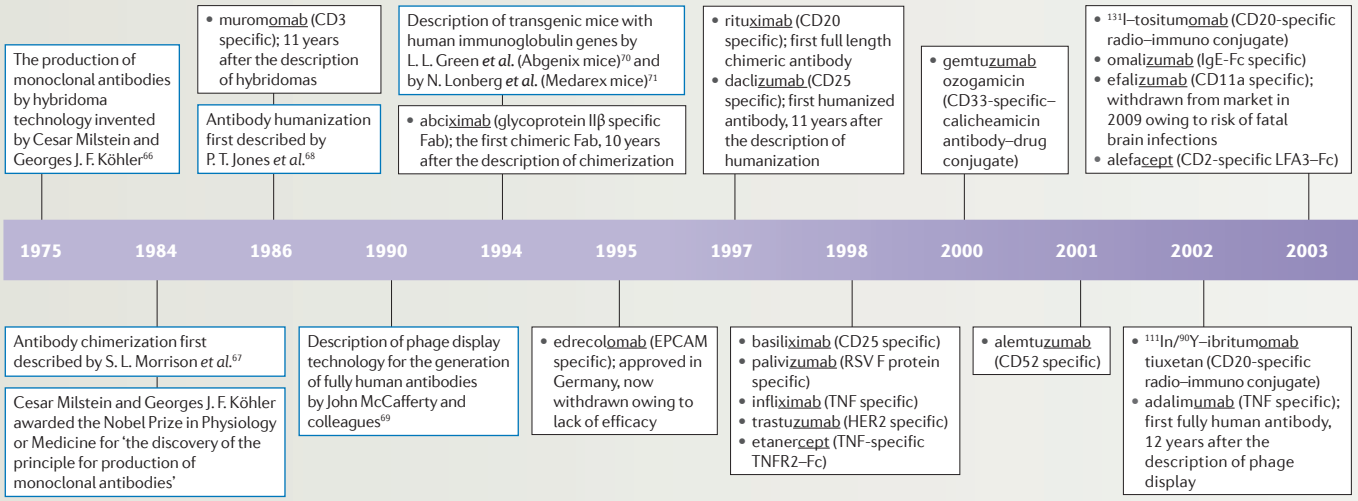
Strategies to select the best targets

Antigen target selection can be classified in broad terms into two main approaches. The first approach involves the development of antibodies directed against so-called 'validated targets', either because prior antibodies have clearly shown proof of activity in humans (first-generation approved antibodies on the market for clinically validated targets) or because a vast literature exists on the importance of these targets for the disease mechanism in both *in vitro* and *in vivo* pharmacological models (experimental validation; although this does not necessarily equate to clinical validation). Basically, the strategy consists of developing new generations of antibodies specific for the same antigens but targeting other epitopes and/or triggering different mechanisms of action (second- or third-generation antibodies, as discussed below) or even specific for the same epitopes but with only one improved property ('me better' antibodies). This validated approach has a high probability of success, but there are many groups working on this class of target proteins and freedom to operate is decreased. By contrast, one can identify new or less well studied target proteins that confer particular functions to cells that might be involved in pathogenic disorders. This second 'functional approach' — in which antibodies are selected based on a functional screen, and the targets to which they bind are then identified using proteomic or cell-based approaches (reverse pharmacology), for example — is associated with greater potential for innovation and intellectual property rights but increased risk of development failure.

Clinically validated targets. 'Blockbuster' antibodies such as rituximab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec), infliximab (Remicade; Centocor/Merck), trastuzumab (Herceptin; Genentech/Roche) and cetuximab (Erbitux; ImClone Systems), directed against now highly clinically validated targets such as CD20, tumour necrosis factor (TNF), human epidermal growth factor receptor 2 (HER2; also known as ERBB2) and epidermal growth factor receptor (EGFR), respectively, are tremendous success stories[1]. Second-generation antibodies directed against these same antigens have alterations such as improved variable domains to decrease immunogenicity and/or to

Timeline | **The regulatory approval of IgG-based products in the past 24 years***



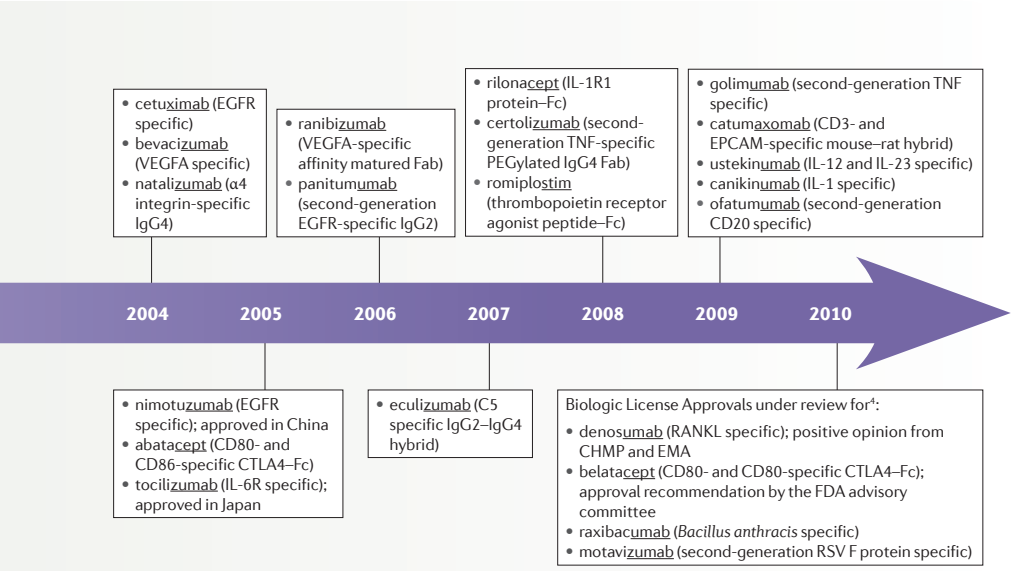
*Approved by the United States Food and Drug Administration (FDA), the European Medicines Agency (EMA), China's State Food and Drug Administration and/or the Japanese Ministry of Health. The suffix of the international non-proprietary names for monoclonal antibodies denotes the antibody format: -omab, mouse IgG2 (4 approved products); -ximab, mouse–human chimeric IgG1 (5 approved products); -zumab, humanized IgG1 (14 approved products); -umab, human antibodies from phage display or transgenic mice (7 approved products); -cept, Fc-fusion protein (4 approved products); -stim, Fc-fusion peptide (1 approved product); -axomab, trifunctional (bispecific) mouse–rat hybrid (1 approved product). C5, complement component C5; CHPM, Committee for Medicinal Products for Human Use; CTLA4, cytotoxic T lymphocyte antigen 4; EGFR, epidermal growth factor receptor; EPCAM, epithelial cell adhesion molecule; HER2, human epidermal growth factor receptor 2; ^{131}I, iodine-131; IL, interleukin; IL-1RAP, IL-1R accessory protein; ^{111}In, indium-111; LFA3, lymphocyte function-associated antigen 3; PEG, polyethylene glycol; R, receptor; RANKL, receptor activator of nuclear factor-κB ligand; RSV, respiratory syncytial virus; TNF, tumour necrosis factor; TNFR2, TNF receptor 2; VEGFA, vascular endothelial growth factor A; ^{90}Y, yttrium-90.

target distinct epitopes with higher or lower affinity for their antigens[7], and/or have different antibody formats (such as conjugating the Fab domain to polyethylene glycol (PEGylation) and Fc-fusion proteins). These antibodies have been investigated in the clinic and recently approved for use in several diseases — for example, ofatumumab (Arzerra; Genmab/GlaxoSmithKline) following rituximab, and adalimumab (Humira/Trudexa; Abbott), certolizumab pegol (Cimzia: UCB) and golimumab (Simponi; Centocor) following infliximab (TIMELINE). In addition, third-generation antibodies, targeting different epitopes, triggering other mechanisms of action and that are often engineered for improved Fc-associated immune functions or half-life[7], have also reached Phase I to III clinical trials[8,9]. For example, the third-generation CD20-specific antibody obinutuzumab (GA101; Biogen Idec/Roche/Glycart) is less immunogenic than rituximab, has a different mechanism of action and is glyco-engineered to trigger increased cytotoxicity[8,9]. Another example is the respiratory syncytial virus-specific monoclonal antibody palivizumab (Synagis; MedImmune/Abbott), which has been followed by the second-generation antibody motavizumab (MEDI-524; MedImmune) — which has affinity matured complementarity-determining regions (CDRs) and is under review by the United States Food and Drug Administration (FDA) — and then by the third generation antibody MEDI-557 (MedImmune) (a version of motavizumab with engineered Fc domains for a longer serum half-life), which is in Phase I trials[7].

Experimentally validated targets. Most cytokines and associated receptors seem to be valuable targets for the treatment of immunological disorders, as shown by the large number of antibodies that have already been approved (such as those specific for TNF, interleukin-1 (IL-1), IL-2 receptor (IL-2R), IL-6R, IL-12, IL-23 and receptor activator of nuclear factor-κB ligand (RANKL)), as well as the numerous candidates in clinical trials (such as antibodies specific for IL-4, IL-6, IL-13 and IL-17)[10–12]. In this area, there is a lesser need to identify new targets, as the mechanisms driving at least some inflammatory disorders are reasonably well known. By contrast, diversification and validation of new targets in oncology is a challenging issue[13] as the causes of malignancies are often multifactorial, redundant and frequently poorly understood. In addition, patients are becoming resistant to current cancer treatments, leading to the expression of new molecules (potential targets) that drive the tumour growth[14]. Another difficulty in oncology is to determine the best combination of drugs and drug targets, which is not always predictable from pre-clinical studies, as shown by the adverse events (such as skin toxicity, diarrhoea and infection) reported for patients with colorectal cancer who were administered with both EGFR- and vascular endothelial growth factor A (VEGFA)-specific antibodies[15]. Target selection will also require an understanding of cooperative signalling involving, for example, growth factor receptor heterodimers[16] or integrin crosstalk with growth factors[17].

> "functional approaches… allow the discovery of unknown cell surface antigens, but these new targets need extensive and careful clinical validation"

A source of potential new experimentally validated targets in oncology is the abundant literature documenting the important role of tyrosine kinase receptors in malignancies. For example, insulin-like growth factor 1 receptor (IGF1R) was proposed to be an interesting oncoprotein more than 20 years ago[18], but patients have had to wait until now to benefit from experimental treatments involving IGF1R-specific antibodies; by the end of 2009, nearly 100 clinical trials were ongoing with at least 9 different IgGs specific for IGF1R[19]. A similar lag phase existed historically for the development of cetuximab,



a chimeric antibody that inhibits EGFR activation. Today, fortunately, the translation from research to clinic tends to occur more rapidly owing to the increasing knowledge of structure–function relationships for the newest monoclonal antibodies, such as those targeting VEGF receptors or the hepatocyte growth factor receptor MET. As a common feature, a tumour must be fully dependent on the antibody target for the therapeutic antibody to affect growth[20], and the target must be overexpressed on tumour cells to avoid toxic effects.

Functionally validated targets. A more challenging approach is to select monoclonal antibodies with a defined biological effect on tumour cells (such as the inhibition of proliferation or the induction of apoptosis) and to identify the recognized antigens by proteomics and alternative techniques[21–23]. These functional approaches (or reverse pharmacology) allow the discovery of unknown cell surface antigens, but these new targets need extensive and careful clinical validation, which is a high development risk and involves longer research timelines before entering into the clinic.

Using this type of approach, the pathways that control partial or complete resistance to current therapies should be better investigated to uncover putative targets that might translate into new and efficient therapies[24]. For example, it has been reported that treatment with EGFR inhibitors leads to MET overexpression[25]. Similarly, resistance to HER2-specific antibodies has been reported to be related to IGF1R overexpression[19].

In our opinion, companies should carry out more research in these high-risk areas if they are expecting new therapeutic breakthroughs. In addition to identifying new antigen targets, another option to extend the therapeutic use of antibodies is to modulate their structure and format, which is discussed in the next section.

Strategies to optimize structures

A detailed knowledge of antibody structure and activity now allows researchers to engineer primary antibodies on a more rational basis. This can yield more homogeneous and stable molecules with additional properties such as increased cytotoxicity or dual targeting, as well as IgG-related structures with additional functions and specificities.

Improving pharmaceutical properties. Most approved antibodies are chimeric, humanized or human IgGs with similar constant domains. Numerous studies looking at the structure–function relationships of these antibodies have been published in the past five years with the aim of identifying antibody microvariants[26–28] and investigating the influence of these variants on antigen binding[23], stability, pharmacokinetics[29] and pharmacodynamics (FIG. 1). This knowledge is now being used to increase homogeneity and mitigate the chemistry, manufacture and control (CMC) liabilities of pre-clinical antibody candidates by genetic engineering[30–32]. The removal by mutation of instability or aggregation hot spots in the antibody CDRs, and the use of hinge-stabilized or aglycosylated IgG4, are just a few examples of antibodies with improved pharmacological properties (such as decreased heterogeneity) that are currently in development.

Improving antibody functions. The variable fragment (Fv) of an antibody is responsible for interactions with antigens and dictates essential properties such as binding affinity and target specificity. The origin of the Fv in therapeutic antibodies can be diverse (such as hybridomas, human antibody libraries, rodents with a human antibody repertoire, or primatized or humanized antibodies from various species). Affinity maturation allows the binding affinity of the Fv to be improved and/or target selectivity to be modulated. The constant fragment (Fc) of an antibody is responsible for interactions with immune cells[33], and the associated properties of the Fc can also be modulated by engineering at several levels: altering the glycosylation status to regulate anti- and pro-inflammatory properties[34], modulating antibody-dependent cellular cytotoxicity (ADCC) by site-directed mutagenesis to alter binding to Fc receptors, increasing the serum half-life by Fc engineering to increase binding to the neonatal Fc receptor (FcRn) (which prevents IgG degradation) and increasing complement activation by isotype chimerism[35] (FIG. 2).

Second- and third-generation antibody–drug conjugates. Additional functions can be endowed on antibodies by conjugation to other drugs. So far, the clinical success of immunoconjugates is limited; only one drug, namely gemtuzumab ozogamicin (Mylotarg; Pfizer) has been approved in the United States (but not in Europe) for the treatment of patients with acute myeloid leukaemia. Nevertheless, promising new immunoconjugates — including optimized linkers that are hydrolysable in the cytoplasm, resistant or susceptible to proteases, or resistant to multi-drug resistance efflux pumps — associated with highly cytotoxic drugs are now being studied in advanced clinical trials (such as trastuzumab–DM1 (Genentech) and inotuzumab–ozogamicin

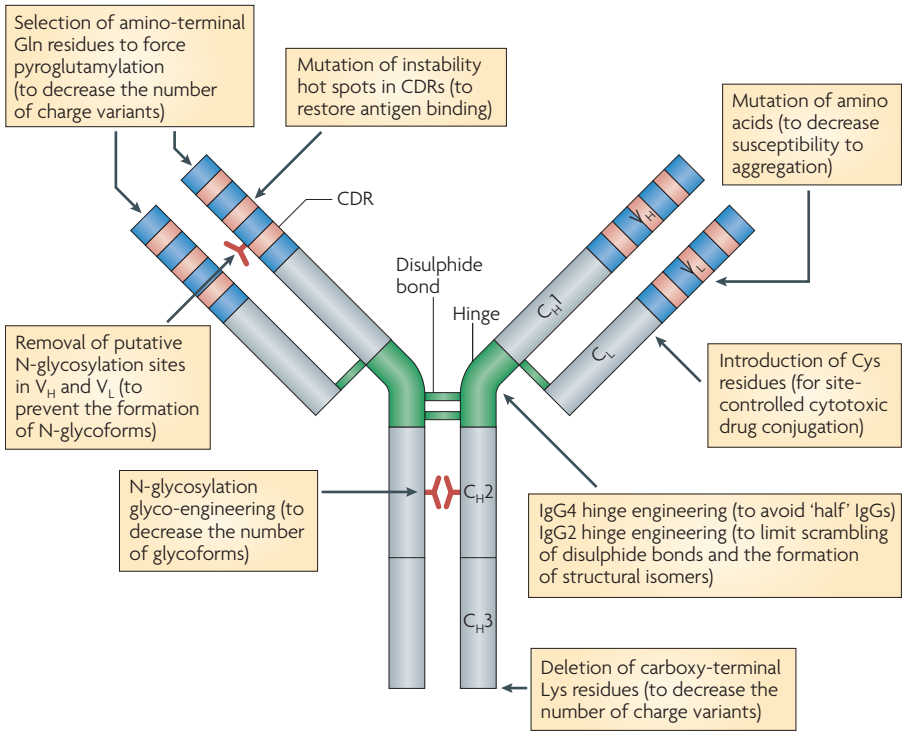


Figure 1 | **Antibody design to improve homogeneity and potential for development.** High-resolution mass spectrometry methods in combination with ultra-performance separation techniques are now routinely used at all stages of antibody discovery and development to assess antibody structure. As a consequence, these new analytical tools have resulted in the identification of minor antibody components, such as charge variants, glycoforms, disulphide bridge isoforms and other low level molecular species. As shown in the figure, lessons learned from the effects of these micro-variants on the stability and the pharmacokinetic and/or pharmacodynamic properties can be used for the design of the next generation of optimized antibodies with higher homogeneity, stability and potency. It is also important to consider the production system used to ensure low levels of xenobiotic glycans and humanized antibody glycosylation patterns. CDR, complementarity-determining region; C_H, heavy chain constant domain; C_L, light chain constant domain; V_H, heavy chain variable domain; V_L, light chain variable domain.

in Phase III trials targeting HER2$^+$ and CD22$^+$ cells in patients with breast and various B cell lymphomas, respectively[36,37]). IgGs have also been engineered to contain unique drug conjugation positions to obtain uniform and more homogeneous drug conjugates (such as thiomab–drug conjugates, which have a uniform stoichiometry of approximately two coupled drugs per antibody molecule[38]), which should open new therapeutic avenues to deliver highly cytotoxic drugs with increased tolerability[38–40].

Bispecific antibodies. For most diseases, several mediators contribute to overall pathogenesis by either unique or overlapping mechanisms. The simultaneous blockade of several targets might therefore yield better therapeutic efficacy than inhibition of a single target. After many years of unsuccessful trials, the first bispecific antibody, catumaxomab (Removab; Fresenius Biotech/TRION Pharma), which binds to both epithelial cell adhesion molecule (EPCAM) on tumour cells and CD3 on effector immune cells, was approved by the European Medicines Agency (EMA) in 2009 for the treatment of malignant ascites[41]. Another promising example of a bispecific antibody is blinatumomab (MT103; Micromet/MedImmune), specific for tumour-associated CD19 and effector cell-expressed CD3, which is being investigated in Phase II clinical trials for the therapy of minimal residual disease of B cell-precursor acute lymphoblastic leukaemia indication. Bispecific antibodies directed against two different tumour-associated or immunological antigen targets are another strategy that has been investigated, but with only limited success owing partly to the highly heterogeneous mixtures that result from the multiple possibilities of immunoglobulin chain association and also to scale-up and purification issues[42].

These difficulties have been recently overcome by the dual variable domain IgG (DVD-IgG) technology. This new type of immunoglobulin was obtained by combining the variable domains of two already characterized monoclonal antibodies (two V_L domains on the light chain and two V_H domains on the heavy chain), as exemplified by an IL-12- and IL-18-specific antibody or by an IL-1α- and IL-1β-specific antibody[33,43]. This technology enables the different specificities of two monoclonal antibodies to be engineered into a single functional, dual-specific, tetravalent IgG-like molecule, and these antibodies can be made with good production yields in a scalable Chinese hamster ovary (CHO) cell line. Another elegant approach consisted of engineering an additional paratope in the variable domain of an existing antibody, which resulted in simultaneous binding to HER2 and VEGFA[44]. In these two examples, the resulting proteins can be produced as a homogeneous single, functional species and with productivities similar to conventional IgGs, which is not the case for the previous bispecific antibody formats.

Polyclonal or oligoclonal antibodies. Another interesting concept is to design recombinant polyclonal or oligoclonal antibodies directed against the same or different targets: for example, the Rhesus D blood group antigen-specific polyclonal antibody rozrolimupab (Sym001; Symphogen A/S), which is a mixture of 25 unique recombinant monoclonal antibodies[45], is currently in Phase II clinical trials for the treatment of chronic and acute idiopathic thrombocytopenic purpura. Synergistic preclinical *in vivo* antitumour efficacy was also recently reported for Sym004 (Symphogen A/S), a controlled mixture of two EGFR-specific antibodies that produces a superior response to cetuximab and panitumumab (Vectibix; Amgen) alone[46], which has led to a Phase I clinical trial in patients with EGFR$^+$ breast cancer. As these recombinant mixtures are produced by a single cell type and are co-purified, this should result in a less expensive drug product than the use of two or more separately produced monoclonal antibodies[47].

Engineering new protein scaffolds. As an alternative to antibodies, several small protein-based drugs and alternative antibody formats are currently being investigated. These may be cheaper to produce and have advantages such as

deeper tumour penetration associated with smaller size. Such protein scaffolds, with highly specific binding properties derived from natural human proteins, have now entered clinical trials[48,49] (TABLE 1). Among them, ecallantide (Kalbitor/DX-88; Dyax) — a Kunitz domain-based scaffold that targets human plasma kallikrein — was approved in December 2009 by the FDA for the treatment of attacks of hereditary angioedema[50,51]. More than 10 protein scaffolds are currently in clinical trials, of which 6 are in Phase II trials. Of interest, as one of the main disadvantages of these new drugs is their potential immunogenicity and safety profile[52,53], it is encouraging that none of them elicited severe adverse reactions or anti-drug antibody responses during Phase I clinical trials. Nevertheless, these structures might have their own limitations in terms of pharmacokinetic and pharmacodynamic properties and potential for development, as each scaffold will need its own unique CMC package[54].

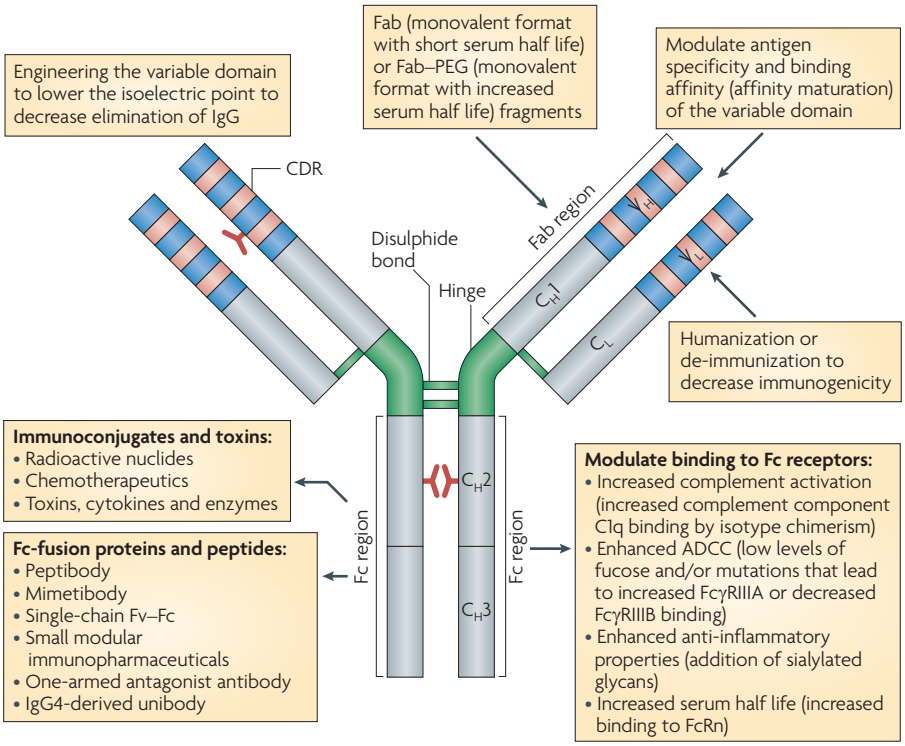


Figure 2 | **Antibody design to improve the pharmacological functions.** A better knowledge of the structure–function relationships of antibody molecules allows fine-tuning of their associated pharmacological properties. The variable domain, which is associated with antigen binding (Fab moiety), can be tailored to modulate binding affinity and specificity using well-described phage display techniques. Fab fragments can be used as a monovalent non-activating format with a long half-life (conjugated to polyethylene glycol (PEGylated)) or with a short half-life (naked). Depending on its origin, humanization or de-immunization (that is, the substitution of key amino acids predicted to abrogate binding to human MHC class II molecules in order to reduce a T cell immune response) techniques can greatly decrease the potential immunogenicity of an antibody. With regard to the antibody Fc portion, better knowledge of the Fc receptors present on immune cells allows the tailored engagement of associated effector functions (such as antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity or phagocytosis) by modulation of the binding affinities to these Fc receptors through mutations and/or glyco-engineering. The antibody Fc domain is also the major binding region to develop immunoconjugates, by association with a radioactive label, cytotoxic drug or protein. CDR, complementarity-determining region; C_H, heavy chain constant domain; C_L, light chain constant domain; FcγR, Fc receptor for IgG; FcRn, neonatal Fc receptor; V_H, heavy chain variable domain; V_L, light chain variable domain.

Strategies to provide affordable treatments

Antibodies are a successful class of therapeutic agents, but many treaments remain costly, which may limit their use, particularly when synergistic combinations of IgGs are required[55]. Thus, decreasing costs is an important part of drug development.

Decreasing production and processing costs. Although increased productivity is an important factor in decreasing costs, the greatest effect comes from combining this with improved and less costly downstream processing. Improving the production yields of mammalian cell lines that produce already approved antibodies[56] and improving the selection of alternative purification and formulation methods (such as the use of chemical mimotopes rather than protein A for purifying IgG or large scale precipitation, which are much less expensive) are key steps that are being actively investigated by the biotechnology industry[57], with significant progress in downstream processing already having been achieved. In addition, the design of less heterogeneous antibody structures will help to facilitate scale-up and process comparability and limit the need for extensive validation of new protocols. Alternative cell lines with simpler culture media, higher productivity, shorter production times and no viral inactivation steps are also important features to consider in terms of cost reduction. These might include, for example, the use of engineered yeast with humanized glycosylation enzymes or plant cells (for the production of fully functional glycosylated antibodies) or the use of *Escherichia coli* (for the production of Fab fragments or non-glycosylated IgGs when effector functions are not required)[58]. Such non-mammalian production systems will not require costly viral inactivation validation steps, as are required for mammalian cell lines that might be contaminated with viruses that could infect humans.

Biosimilar or 'me better' antibodies? In contrast to the low-cost generic versions of small molecules that are off patent, it is so far not possible to produce exact copies of large proteins and glycoproteins, such as antibodies, owing to their structural complexity[59]. Nevertheless, since 2005, the EMA has initiated regulatory approval pathways for biosimilar products, currently resulting in marketing authorization for 12 products encompassing three product classes (human growth hormone, erythropoietin and granulocyte colony-stimulating factor)[60]. Furthermore, biosimilar antibodies (identical amino-acid sequence but only a similar glycosylation profile compared with a reference product), such as a biosimilar antibody of rituximab, are approved in countries such as India, China and South Korea. Their possible emergence on European markets was recently discussed at a workshop organized by the EMA in London[61]. Several laboratories also plan to

Table 1 | **Alternative protein and antibody scaffolds: early clinical proof-of-concept**

Name	Scaffold or format	Developer or licensee	Parent protein structure	Clinical trial phase	Disease	Target
Ecallantide (Kalbitor/DX88)	Kunitz domain	Dyax	Human lipoprotein-associated coagulation inhibitor (LACI)	FDA approved (December 2009)	Hereditary angioedema	Kallikrein inhibitor
TRU-015	SMIP	Trubion/Pfizer	Various origin and length	Phase IIb	NHL	CD20
Dom-0200/ART621	Domain antibody	Domantis (now GlaxoSmithKline)/Cephalon	V_H or V_L antibody domain; 100–130 amino acids	Phase II	Rheumatoid arthritis and psoriasis	TNF
MT103	BiTE	Micromet	scFv–scFv; 200–260 amino acids	Phase II	ALL	CD19 and CD3
				Phase I	NHL	
Angiocept (BMS-844203/CT-322)	Adnectin	Adnexus (owned by Bristol-Myers Squibb)	10th FN3 domain of fibronectin; 94 amino acids	Phase II	Colorectal cancer, NSCLC and glioblastoma	VEGFR2
ALX-0081	Nanobody	Ablynx	VHH; ~100 amino acids	Phase II	ACS and TTP	vWF
ESBA105	Stable scFv	ESBATech/Alcon	scFv with hyperstable properties	Phase II	Uveitis	TNF
AMG-220 (C326)	Avimer	Avidia (owned by Amgen)	Domain A of LDL receptor; a repeating motif of ~35 amino acids	Phase I	Crohn's disease	IL-6
MT110	BiTE	Micromet	scFv–scFv; ~500 amino acids	Phase I	Lung and gastric cancers	EPCAM and CD3
ABY-002	Affibody	Affibody	Z domain of protein A from *Staphylococcus aureus*; 58 amino acids	Phase I	Breast cancer imaging	HER2
MP0112	DARPin	Molecular Partners	Ankyrin repeat proteins; 67 amino acids plus a repeating motif of 33 amino acids	Phase I	Ophthalmological diseases	VEGF
PRS-050 (Angiocal)	Anticalin	Pieris	Lipocalin; 160–180 amino acids	Phase I starts early 2010	Solid tumours	VEGF

ACS, acute coronary syndrome; ALL, acute lymphoblastic lymphoma; BiTE, bispecific T cell engager; DARPin, designed ankyrin repeat protein; EPCAM, epithelial cell adhesion molecule; FDA, United States Food and Drug Administration; HER2, human epidermal growth factor receptor 2; IL, interleukin; LDL, low-density lipoprotein; NHL, non Hodgkin's lymphoma; NSCLC, non-small-cell lung carcinoma; R, receptor; scFv: single-chain variable domain antibody fragment; SMIP, small modular immunopharmaceutical; TNF, tumour necrosis factor; TTP, thrombotic thrombocytopenic purpura; VEGF, vascular endothelial growth factor; V_H, heavy chain variable domain; VHH, heavy chain variable domain (in camelids); V_L, light chain variable domain; vWF, von Willebrand factor.

bring 'me better' antibodies to the clinic, such as those with controlled and optimized glycosylation by producing them in glyco-engineered yeast strains[58] (for example, a copy of the rituximab amino acid sequence but with afucosylated glycoforms resulting in a 100-fold increase in ADCC) and/or with increased plasma half-life[62] (for example, a copy of rituximab but with a mutation of three amino acids in the Fc domain resulting in extended pharmacokinetics). In both cases, the cost of treatment should decrease because of lower cost of the product or a less frequent administration regimen. Nevertheless, the development of biosimilar and 'me better' antibodies needs new regulations that must be discussed and validated by regulatory authorities[63]. The first wave of biosimilar antibodies are copies of current important therapeutic antibodies such as a biosimilar rituximab (Reditux; Dr Reddy's Laboratories), which is approved in India, and a biosimilar abciximab (Clotinab; Abu Abxis), which is appoved in South Korea; further biosimilar candidates include copies of infliximab, etanercept (Enbrel; Amgen/Pfizer), cetuximab and trastuzumab.

Biomarker identification and selection of patients. The screening of patients with breast cancer for HER2 expression status before trastuzumab (HER2-specific) treatment is the paradigm of subset selection for targeted treatment; in this case a subset of women with a $HER2^+$ type of breast cancer (around 20%) are selected for HER2-targeted treatment[16]. For EGFR-targeted therapy of colorectal cancer, it was originally thought that, because EGFR is overexpressed in tumour cells from more than 95% of patients, there was no need for patient stratification; however, it has recently been found that only patients carrying tumours with a wild-type *KRAS* phenotype (60% of patients) will benefit from EGFR-specific cetuximab or panitumumab treatment[64]. Similar observations apply to lung-targeted anti-cancer drugs[63] and to anti-angiogenic therapies[65]. The identification of biomarkers and patient selection is becoming a paradigm for the development of targeted therapies that requires further investigation.

Conclusions and perspectives

The IgG-based biotherapeutic agents that have been approved in the past decade show that pharmaceutical laboratories have worked on the diversification and fine tailoring of antibody structures to bring new antibodies to the market. Following the success of the first generation of

Glossary

Antibody-dependent cellular cytotoxicity
(ADCC). A mechanism of cell-mediated immunity whereby effector cells of the immune system (mainly natural killer cells) actively lyse a target cell that has been bound by specific antibodies. It is one of the mechanisms by which antibodies, as part of the humoral immune response, can limit and contain infection.

Biosimilar antibody
A generic version of an 'innovator' antibody with the same amino-acid sequence but produced from a different clone and manufacturing process, resulting in differences in glycosylation and other microvariations. Biosimilar antibodies are known as follow-on biologics in the United States.

Bispecific antibody
(Also known as a bifunctional antibody). A monoclonal antibody that binds to two different epitopes. These can be on the same antigen or two different antigens, thereby triggering two different functions. Bispecific antibodies do not usually occur naturally.

Chemistry, manufacture and control
(CMC). A part of pharmaceutical development that deals with the nature of the antibody drug substance and drug product, as well as the manner in which both are obtained, and by which the manufacturing process is quality controlled. Unfavourable physico-chemical characteristics of an antibody molecule that might result in difficulties to translate a research lead candidate into a scalable drug with appropriate pharmacokinetic and pharmacodynamic features are known as CMC liabilities (also referred to as 'drugability' or 'developability' issues).

Complement-dependent cytotoxicity
A mechanism of antibody-mediated immunity whereby antibody binding to the complement component C1q activates the classical complement activation cascade leading to formation of the membrane attack complex, the cytolytic end product of the complement cascade.

Complementarity-determining region
(CDR). A short sequence (up to 13 amino acids) found in the variable domains of immunoglobulins. The CDRs (six of which are present in IgG molecules) are the most variable part of immunoglobulins and contribute to their diversity by making contacts with a specific antigen, allowing immunoglobulins to recognize a vast repertoire of antigens with a high affinity.

Fab fragment
The fragment of antigen binding is the region of an antibody that binds to antigens. It is composed of one constant and one variable domain of each of the heavy and light chains (V_H and V_L, respectively).

Fc-fusion protein
An engineered recombinant protein carrying at its carboxy-terminal end the Fc portion (Hinge–C_H2–C_H3 domains) of an antibody and, at its amino-terminal end, any kind of protein or peptide such as a receptor-binding domain or a ligand. For example, etanercept, a product that is approved to treat rheumatoid arthritis by acting as a tumour necrosis factor inhibitor, is an Fc-fusion protein of IgG1 Fc with tumour necrosis factor receptor 2. The suffix -cept or -stim is used to identify Fc-fusion proteins or peptides, respectively.

Humanized antibody
A humanized antibody is obtained by genetic engineering to increase its similarity to antibodies produced naturally in humans, thereby decreasing its potential immunogenicity. A common humanization method is known as CDR grafting; this involves introducing the CDRs from a non-human antibody of interest into a framework acceptor sequence of a human germline V gene that is closely related to the antibody of interest. The suffix -zumab is used to identify humanized antibodies.

'Me better' antibody
(Also known as a 'bio-better' antibody). We define this as an antibody targeting the same validated epitope as an existing antibody (having the same CDRs: 'me too') but with an optimized glycosylation profile (such as low fucose levels for enhanced ADCC) or an engineered Fc domain to increase the serum half-life.

Microvariants
Antibodies with small structural differences (such as amino-terminal pyroglutamic acid residues, carboxy-terminal clipped lysine residues, different glycoforms or disulphide bridge isomers) that are present in the drug substance, which might affect the pharmacokinetic and pharmacodynamic properties and that must be kept in comparable amounts during the production scale-up (toxicology studies, Phases I, II and III clinical trials and post-marketing batches).

Orphan diseases
Rare diseases that affect only a small number of patients. Both the United States Food and Drug Administration and the European Medicines Agency have special development and regulatory procedures to stimulate research for such illnesses.

Paratope
The antigen-binding site of an antibody composed of portions of the different CDRs of the antibody's heavy and light chain variable domains.

PEGylation
The covalent attachment of polyethylene glycol polymer chains to a Fab fragment to increase the serum half-life.

Pharmacodynamics
The study of the physiological effects of the antibody, the mechanisms of drug action and the relationship between antibody concentration and effect: what an antibody does to a body.

Pharmacokinetics
The study of antibody clearance in the serum: what the body does to an antibody.

Protein scaffold
An engineered protein typically of small size (< 100 amino acids) and containing a highly structured core associated with variable domains of high conformational tolerance, allowing insertions, deletions or other substitutions. These domains can create a putative binding interface for any targeted protein. The structure of protein scaffolds can be highly diverse (such as immunoglobulin-like molecules, loop-containing proteins, highly structured proteins and oligomeric proteins), but they are usually of human origin.

Second-generation antibody
A first-generation follow-up antibody with improved variable domains (such as humanized or human variable domains or affinity matured CDRs).

Third-generation antibody
A second-generation follow-up antibody with improved variable domains (such as humanized or human variable domains or affinity matured CDRs) and improved Fc domains (for example, glyco- or amino-acid engineered to increase effector functions or to improve half-life).

monoclonal antibody blockbusters, second-generation antibodies were recently approved. In addition, many third-generation antibodies designed to trigger different mechanisms of action simultaneously (such as targeting growth factors, inhibiting angiogenesis and restoring apoptosis) and associated with enhanced or silenced effector functions (ADCC or complement-dependent cytotoxicity) are being investigated in clinical trials.

Among the challenges to be faced in the next 10 years are the identification and validation of new targets, addressing the resistance to current drug treatments and understanding target cross talk and regulation. In the meantime, efforts have to be made to decrease the costs of industrial production by increasing the productivity of the current cell lines, by developing alternative production systems and purification processes and by optimizing the design of more homogeneous and stable IgGs. The availability of regulatory pathways to register biosimilar antibodies might be another way to decrease healthcare costs and to generalize the use of monoclonal antibodies. As an alternative to antibodies, proof-of-concept of the clinical efficacy of new protein scaffolds with different pharmacological properties and less expensive manufacturing processes might also help to bring more affordable targeted biotherapies to the market.

Alain Beck, Thierry Wurch, Christian Bailly and Nathalie Corvaia are at the Centre d'Immunologie Pierre Fabre (CIPF), 5 Avenue Napoléon III, F74160, Saint-Julien-en-Genevois, France.

Correspondence to A.B.
e-mail: alain.beck@pierre-fabre.com

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Competing interests statement
The authors declare competing financial interests: See Web version for details.

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