1
Therapeutic Antibodies – from Past to Future

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1.1
An Exciting Start – and a Long Trek

In the late nineteenth century, the German army doctor Emil von Behring (1854–1917), later the first Nobel Laureate for Medicine, pioneered the therapeutic application of antibodies. He used blood serum for the treatment of tetanus and diphtheria (''Blutserumtherapie''). When his data were published in 1890 [1], very little was known about the factors or mechanisms involved in immune defense. Despite this, his smart conclusion was that a human body needs some defense mechanism to fight foreign toxic substances and that these substances should be present in the blood – and therefore can be prepared from serum and used for therapy against toxins or infections. His idea worked, and the success allowed him to found the first ''biotech'' company devoted to antibody-based therapy in 1904 – using his Nobel Prize money as ''venture capital.'' The company is still active in the business today as part of CSLBehring.

In 1908, Paul Ehrlich, the father of hematology [2] and the first consistent concept of immunology (''lateral chain theory,'' Figure 1.1d [3]), was awarded the second Nobel Prize related to antibody therapeutics for his groundbreaking work on serum, ''particularly to the valency determination of sera preparations.'' Ehrlich laid the foundations of antibody generation by performing systematic research on immunization schedules and their efficiency, and he was the first to describe different immunoglobulin subclasses. He also coined the phrases ''passive vaccination'' and ''active vaccination.'' His lateral chain theory (''Seitenketten,'' sometimes misleadingly translated to ''side-chain theory'') postulated chemical receptors produced by blood cells that fitted intruding toxins (antigens). Through these chemical receptors, cells combine with antigens and the receptors are eventually released as circulating antitoxins (antibodies). Without any knowledge of molecular structure or biochemical binding mechanisms, Ehrlich anticipated much of today's knowledge on immunoglobulin generation and antibody–antigen interaction, including class switching (Figure 1.1d).

Passive and active vaccines were developed in rapid succession at the beginning of the twentieth century, and were successful in saving many lives. Snake and

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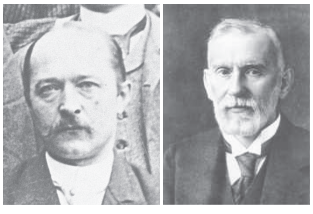
(a)

(b)


(c)

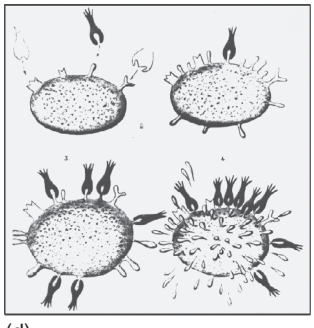
(d)

Figure 1.1 We have come a long way since the first methods for the generation of antibody based therapeutics were established (c), pioneered by (a) Emil von Behring and (b) Paul Ehrlich in the last decade of the nineteenth century. (d) Drawing from Paul Ehrlich on the lateral chain theory (lateral chains = antibodies). He anticipated principles confirmed on a molecular basis many decades later, like the binding of antigens by different specific antibodies (the ''lock and key'' principle), the differentiation and maturation of B cells, and the class switch, allowing the initially cell-bound antibodies to be released in large amounts. (Photos: Deutsches Historisches Museum, Berlin.)

spider bites could be treated specifically, and beneficial effects were even observed with human serum immunoglobulin G (IgG) preparations without prior specific immunization (e.g., protection against hepatitis A).

The enormous success of these blood products for the prevention and treatment of infections and intoxications, however, could not be expanded to other disease areas. Particularly for cancer and immune-mediated disorders, understanding of molecular processes in their etiology, or at least the ability to identify molecules strongly correlated to their onset, stimulated the desire to produce antibodies for therapeutic intervention in these diseases. Unlike a snake bite, cancer and autoimmune diseases are typically chronic, and it was rapidly understood from animal models that antibodies have to be administered more than once. Immunologists knew very well at that time that repeated application of antigen during antiserum preparation is a good strategy to ''boost'' the immune response, and, similarly, an immune response to the therapeutic agent was observed when animal serum antibodies were used to treat chronically ill patients. It was recognized that an antibody with defined specificity would limit side effects and allow sufficient concentrations to reach the target site, but the well-established methods of serum antibody preparation could not be used to produce such a drug.

Much has since been learned about the antibody structure (Figure 1.2) and its function (see Chapter 2). Hopes for advancement of the field were high when Cesar

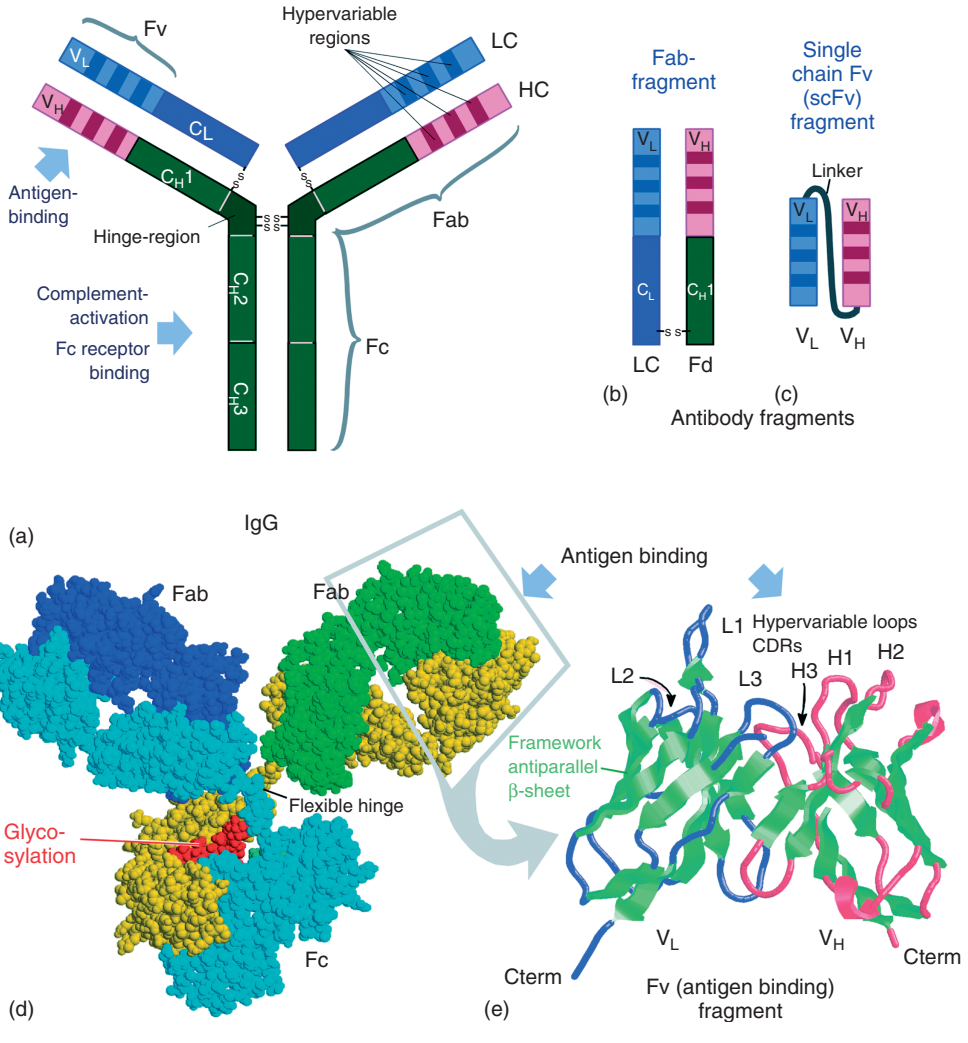

Figure 1.2 Introduction to antibody structure. (a, d) IgG is a heterotetrameric protein assembled from two identical heavy and light chains (HC, LC), assembled by disulfide bonds. (b) Fab fragments contain the antigen-binding region, and can be generated by proteolysis or recombinant production. (c) In single-chain Fv fragments (scFv), the two antigen-contacting domains (variable regions of the heavy and light chains, V_H, V_L) are connected by an oligopeptide linker to form a single polypeptide. They can be produced in *E. coli* and are typically employed for the selection of human antibodies by phage display and other display systems. (d) Space fill model based on X-ray crystallographic data of an antibody. The typical Y shape is only one of the many conformations the Fab fragments of an antibody can assume relative to each other. T-shaped structures can be assumed, and the hinge region to the Fc part can also bend significantly relative to the Fab fragments. (e) Alpha carbon backbone of an Fv fragment, the antigen-binding fragment of an antibody located at the two tips of the Y- or T-shaped complex, emphasizing the typical antiparallel beta sheet framework structure which holds together the hypervariable loops (L1–3, H1–3) composing the antigen-binding surface (CDRs).

Milstein and Georg Köhler demonstrated that monoclonal antibodies could be produced in mouse cell culture (see Chapter 2). The excitement of the late 1970s cooled rapidly, however, when almost all murine-derived monoclonal therapeutics failed during clinical evaluation. Only one of these products received US Food and Drug Administration approval in the 1980s – muromonab (Orthoclone) (see Chapter 9). This anti-CD3 antibody represented a special case because the typical transplant patient receiving it was already immune suppressed, a situation not commonly present in cancers or immune-mediated diseases. Even more important, it was realized that simple binding of an antibody to its target (inducing neutralization) may be insufficient for treatment of diseases. Effector functions such as complement activation or cellular responses triggered by Fc receptor binding are obviously needed, but are not fully provided by mouse monoclonal antibodies.

As a consequence, substantial efforts were undertaken to exchange the antibody's effector domains (constant regions) for human ones, thereby also removing the most immunogenic parts from the mouse IgG. By using the then available methods of molecular cloning and recombinant expression, most antibodies intended for use as therapeutics were constructed from a combination of human and mouse sequences. Various methods, with chimerization and CDR-exchange-based (complementarity-determining region) humanization being the most widely used, were employed (see Chapter 5). When tinkering with the amino acid sequences, most candidate antibodies were also affinity matured, typically to nanomolar and subnanomolar affinities (see Chapter 6). The first technologies for humanization and affinity maturation became available during the mid-1980s and have been improved continuously since then. Because drug development, testing, and regulatory review typically takes ~8–12 years, a growing number of therapeutic antibodies were approved starting from the mid-1990s.

Early in the 1990s, two novel enabling technologies were developed that revolutionized the generation of therapeutic antibodies because, for the first time, they provided a robust and reliable method to prepare specific antibodies of human origin. Phage display (see Chapter 3) and transgenic mice (see Chapter 4) allowed the production of antibodies that are genetically 100% identical to human immunoglobulins. These approaches, as well as new ideas such as yeast display or ribosomal display, also allowed for *in vivo* evolution to improve various antibody properties. The methods are all based on the selection of antibodies from a large antibody gene repertoire in a heterologous expression system (Figure 1.3).

The experience with non-antibody recombinant human protein drugs (e.g., insulin) raised hopes that the immune system would not recognize human antibodies as foreign protein. It was soon realized, however, that immunogenicity was not reduced to zero in most cases for a number of reasons. For example, Fc-glycosylation patterns of recombinant IgG manufactured in nonhuman cell lines can be very different from that found on endogenous human IgG. In addition, variable human antibody regions derived from display technologies, with ''lottery''-derived CDRs generated outside of the context of the human immune system, may be quite immunogenic. These problems were in most cases minor compared

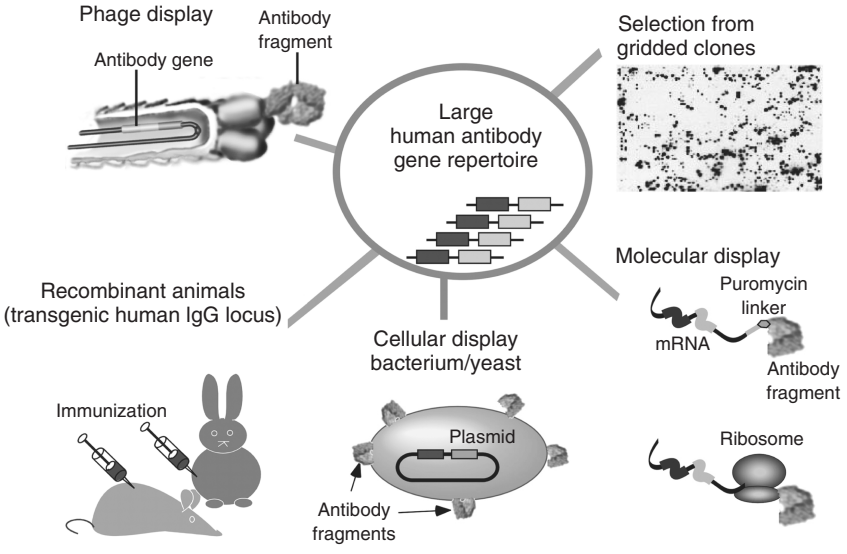

Figure 1.3 Systems employed for the generation of human antibodies. They all include a heterologous expression of a repertoire of human immunoglobulin genes. Selection is achieved either *in vivo* by immunization (in case of the recombinant animals) or *in vitro* by binding to the antigen, allowing clonal selection of the gene host.

with those encountered previously with animal serum immunoglobulins or fully murine-derived antibodies.

The first IgG antibody with a completely ''human'' sequence origin, adalimumab (Humira®), reached the market in 2002 (see Chapter 45). It was genetically assembled entirely *in vitro*, with an antigen-binding region selected from an *Escherichia coli*-hosted gene repertoire by phage display (see Chapter 3). As of early 2013, an additional nine recombinant human IgG therapeutics (panitumumab, golimumab, canakinumab, ustekinumab, ofatumumab, denosumab, belimumab, ipilimumab, and raxibacumab) have been approved, and many more antibodies derived from human gene repertoires, selected by display technologies or generated in transgenic animals, have entered clinical testing. The *in vitro* technologies, such as phage display, offer an additional advantage when antibody generation in animals is difficult, for example, owing to the high homology (resulting in low immunogenicity) of the human antigen used for immunization to a mouse protein, or in case of highly toxic or deadly pathogenic antigens. While some animal or even human serum-derived antibody products are still available, the majority of the approved antibodies and those in current clinical studies are recombinant and contain human or humanized sequences. Overall, approval success rates for chimeric, humanized, and human antibody therapeutics are higher than those for small-molecule drugs [4, 5], mainly due to the more predictable pharmacokinetics and lower risk of toxicity and other side effects when using

molecules almost identical to the IgG in our veins. There are of course exceptions, usually due to effects caused by the antigen binding itself (e.g., as seen with TGN1412).

1.2 The Gold Rush

The emphasis by the biopharmaceutical industry on development of antibodies as new therapeutic agents started when it was realized that all enabling technologies are in place to develop and produce monospecific, nearly human antibodies that may be mildly immunogenic, but provide high-affinity target binding and human effector functions, long serum half-life, and other pharmacologic advantages. Many promising new concepts for the treatment of a huge variety of diseases were envisaged. In fact, there are only few theoretical restrictions that apply to most antibody treatments. The first is the necessity to find a molecular target (antigen) accessible from the bloodstream (i.e., typically a target at the cell surface that is located solely or in a higher concentration on the cell compartment to be effected). Second, the antibody may need to activate some immune reaction at the binding site (e.g., to kill a tumor cell). Exceptions are antibodies that act by neutralization of an infectious agent or a soluble ligand (e.g., tumor necrosis factor (TNF)), which can be achieved by simple steric inhibition of the binding of the agent to its natural receptor. Affinity is not a practical limitation anymore because antibodies usually can be engineered to provide affinities better than those needed for a maximal therapeutic effect (see Chapter 6). Specificity is always an issue because no antibody is a priori unsusceptible to a cross reaction, but many strategies have been developed to tackle this problem. Most simply, large numbers of different human antibody clones can now easily be evaluated in parallel using high-throughput assays.

Some commentators are pessimistic about the high cost of production of antibodies compared with small-molecule drugs. They have calculated that health systems could not afford all of these new, expensive drugs even if they were made available. Novel alternative production systems (e.g., microbial, eukaryotic, or plant-derived), however, may allow much cheaper production of antibodies for many applications, and may even allow the ''expensive'' antibodies to enter new, low-margin therapeutic markets. However, the ostensible advantage of cheaper production of the raw product often is counterbalanced by downstream cost and regulatory uncertainties. Continuous successes in increasing production yields of the classical CHO cells have also reduced this advantage [6]. So, despite the fact that numerous promising systems have been available for many years, their use for the production of therapeutic antibodies has not made significant progress in recent years.

1.3
Success and Setbacks

The exciting opportunities offered by the new technologies led to an explosion in the number of novel antibodies entering clinical studies. After the slow start in the 1980s and no substantial change between 1985 and 2000 [4], the rate at which novel antibodies entered their first clinical studies increased dramatically (~3×) during the 2000s. Today, hundreds of antibodies are being evaluated in clinical studies of a broad range of diseases, from cancers and immune-mediated disorders to infectious, neurological, and bone-related diseases (Table 1.1). Cancer therapeutics have traditionally dominated the field, but applications in immune-mediated disorders are on the rise, with more antibodies in Phase 3 for these disorders than for any other therapeutic area [7, 8]. By mid-2013, nearly 40 monoclonal antibodies had gained approval in Europe or the United States (Table 1.2).

The nature of the antigens is also diverse, with the approved antibodies targeting both cell surface markers (e.g., CD11a, CD20, CD25, CD33, CD52, EGFR, HER2) and soluble molecules (e.g., TNF, vascular endothelial growth factor (VEGF)) using a variety of molecular formats: canonical (i.e., full-length, unconjugated) IgG, antigen-binding fragments (Fab), antibody-drug conjugates, radioimmunoconjugates, and bispecific antibodies (Table 1.2). The utility of the formats is perfectly illustrated by the antibody variants made to block the interaction of TNF with its receptor, which ultimately leads to reduction in inflammation. Starting with mouse hybridoma-derived antibodies that failed in clinical studies, chimeric (e.g., infliximab) and human (e.g., adalimumab, golimumab) antibodies were prepared, tested, and approved for marketing. The humanized certolizumab pegol, a Fab fused to poly(ethylene glycol) to improve its pharmacokinetics, represents another marketed anti-TNF antibody construct with clinical utility, despite the fact that it is no longer a molecule present in nature. In addition, a human-soluble TNF

Table 1.1 Therapeutic antibodies in clinical studies in 2012.[a]

Therapeutic area	Clinical phase			
	1	1/2 or 2	2/3 or 3	Total
Cancer	92	75	11	178
Immune-mediated disorders	34	46	12	92
All other categories	45	40	8	93
Total	171	161	31	363

[a]Therapeutic antibodies included in the commercial clinical pipeline that had entered a first Phase 1 study prior to the end of 2012; candidates in clinical studies sponsored solely by government, non-profit or academic organizations were excluded. Clinical phase is the highest phase to which the candidates had advanced by July 2013.
Data assembled from public domain sources, including www.clinicaltrials.gov and company webpages.

Table 1.2 Therapeutic monoclonal antibodies approved or in review in the European Union or United States.

International non-proprietary name	Brand name	Target; Format	Indication first approved	First EU (US) approval year
Muromonab-CD3	Orthoclone Okt3	CD3; Murine IgG2a	Reversal of kidney transplant rejection	1986[a] (1986[b])
Nebacumab	Centoxin	Endotoxin; Human IgM	Gram-negative sepsis	1991[a,b] (NA)
Abciximab	Reopro	GPIIb/IIIa; Chimeric IgG1 Fab	Prevention of blood clots in angioplasty	1995[a] (1994)
Edrecolomab	Panorex	EpCAM; Murine IgG2a	Colon cancer	1995[a,b] (NA)
Rituximab	MabThera, Rituxan	CD20; Chimeric IgG1	Non-Hodgkin's lymphoma	1998 (1997)
Basiliximab	Simulect	IL2R; Chimeric IgG1	Prevention of kidney transplant rejection	1998 (1998)
Daclizumab	Zenapax	IL2R; Humanized IgG1	Prevention of kidney transplant rejection	1999 (1997)[b]
Palivizumab	Synagis	RSV; Humanized IgG1	Prevention of respiratory syncytial virus infection	1999 (1998)
Infliximab	Remicade	TNF; Chimeric IgG1	Crohn disease	1999 (1998)
Trastuzumab	Herceptin	HER2; Humanized IgG1	Breast cancer	2000 (1998)
Gemtuzumab ozogamicin	Mylotarg	CD33; Humanized IgG4; ADC	Acute myeloid leukemia	NA (2000[b])
Alemtuzumab	MabCampath, Campath-1H	CD52; Humanized IgG1	Chronic myeloid leukemia	2001 (2001)[c]
Adalimumab	Humira	TNF; Human IgG1	Rheumatoid arthritis	2003 (2002)
Tositumomab-I131	Bexxar	CD20; Murine IgG2a; Radiolabeled	Non-Hodgkin lymphoma	NA (2003)
Efalizumab	Raptiva	CD11a; Humanized IgG1	Psoriasis	2004 (2003)[b]
Cetuximab	Erbitux	EGFR; Chimeric IgG1	Colorectal cancer	2004 (2004)
Ibritumomab tiuxetan	Zevalin	CD20; Murine IgG1	Non-Hodgkin's lymphoma	2004 (2002)
Omalizumab	Xolair	IgE; Humanized IgG1	Asthma	2005 (2003)
Bevacizumab	Avastin	VEGF; Humanized IgG1	Colorectal cancer	2005 (2004)
Natalizumab	Tysabri	α4 integrin; Humanized IgG4	Multiple sclerosis	2006 (2004)
Ranibizumab	Lucentis	VEGF; Humanized IgG1 Fab	Macular degeneration	2007 (2006)
Panitumumab	Vectibix	EGFR; Human IgG2	Colorectal cancer	2007 (2006)
Eculizumab	Soliris	C5; Humanized IgG2/4	Paroxysmal nocturnal hemoglobinuria	2007 (2007)
Certolizumab pegol	Cimzia	TNF; Humanized Fab, pegylated	Crohn disease	2009 (2008)

Table 1.2 (*Continued*)

International non-proprietary name	Brand name	Target; Format	Indication first approved	First EU (US) approval year
Golimumab	Simponi	TNF; Human IgG1	Rheumatoid and psoriatic arthritis, ankylosing spondylitis	2009 (2009)
Canakinumab	Ilaris	IL1b; Human IgG1	Muckle-Wells syndrome	2009 (2009)
Catumaxomab	Removab	EPCAM/CD3; Rat/mouse bispecific mAb	Malignant ascites	2009 (NA)
Ustekinumab	Stelara	IL12/23; Human IgG1	Psoriasis	2009 (2009)
Tocilizumab	RoActemra, Actemra	IL6R; Humanized IgG1	Rheumatoid arthritis	2009 (2010)
Ofatumumab	Arzerra	CD20; Human IgG1	Chronic lymphocytic leukemia	2010 (2009)
Denosumab	Prolia	RANK-L; Human IgG2	Bone loss	2010 (2010)
Belimumab	Benlysta	BLyS; Human IgG1	Systemic lupus erythematosus	2011 (2011)
Ipilimumab	Yervoy	CTLA-4; Human IgG1	Metastatic melanoma	2011 (2011)
Brentuximab vedotin	Adcetris	CD30; Chimeric IgG1; ADC	Hodgkin lymphoma, systemic anaplastic large cell lymphoma	2012 (2011)
Pertuzumab	Perjeta	HER2; Humanized IgG1	Breast cancer	2013 (2012)
Raxibacumab	(Pending)	*B. anthrasis* PA; Human IgG1	Anthrax infection	NA (2012)
Trastuzumab emtansine	Kadcyla	HER2; Humanized IgG1; ADC	Breast cancer	2013
Vedolizumab	(Pending)	α4β7 integrin; Humanized IgG1	Ulcerative colitis, Crohn disease	In review (In review)
Ramucirumab	(Pending)	VEGFR2; Human IgG1	Gastric cancer	(NA) In review
Obinutuzumab	(Pending)	CD20; Humanized IgG1; Glycoengineered	Chronic lymphocytic leukemia	In review (In review

Abbreviations: ADC, antibody-drug conjugate; BLyS, B lymphocyte stimulator; C5, complement 5; CD, cluster of differentiation; CTLA-4, cytotoxic T lymphocyte antigen 4; EGFR, epidermal growth factor receptor; EPCAM, epithelial cell adhesion molecule; Fab, antigen-binding fragment; GP glycoprotein; HER, human epidermal growth factor receptor; IL, interleukin; NA, not approved; PA, protective antigen; RANK-L, receptor activator of NFkappab ligand; RSV, respiratory syncytial virus; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Note: Information current as of July 2013.

[a] European country-specific approval.

[b] Withdrawn from market.

[c] Withdrawn from market for first approved indication; in regulatory review for supplemental indication.

receptor–Fc fusion protein (etanercept) became an approved iteration of the idea. Recombinant antibody technology may not have reached its limits; for example, novel synthetic binders of non-antibody structure (see Chapter 17) could potentially serve as TNF agonists.

Regardless of their composition (e.g., protein, peptide, small molecule), the development of drugs is challenging, with issues occasionally arising even for approved products, and notable setbacks have certainly occurred for some antibody therapeutics. In March 2006, life-threatening adverse effects not predicted from the animal studies were observed in a Phase 1 clinical study of the anti-CD28 antibody TGN1412. An Expert Scientific Group subsequently reviewed the case and provided a number of recommendations intended to improve the safety of first-in-humans studies. In another instance, in February 2005, the marketing of natalizumab (Tysabri®), a treatment for multiple sclerosis, was voluntarily suspended on the basis of reports of progressive multifocal leukoencephalopathy that occurred in patients treated in combination with interferon beta-1a in clinical trials [9]. Natalizumab was returned to the market in 2006, but strict monitoring of patients receiving the product is required.

These events show that despite the theoretical advantages of antibody therapeutics, there are still risks associated with their use. These mainly originate from our still very incomplete understanding of molecular and immunological processes, particularly in combination therapies. Here, great hopes are put on the intense research going on worldwide into ''-omics'' and systems biology, which is intended to lead to a mathematical interaction model for all involved factors. When such a model is in common use, which is likely only many years from now, we may be able to better predict adverse effects of novel drugs and combination therapies on a truly rational basis. Interestingly, in recent years the methods first developed for the generation of human therapeutic antibodies – in particular phage display – were further developed into key enablers for gene function research by providing for the first time a viable perspective to generate a set of monoclonal antibodies to the entire proteome [10, 11].

1.4 The Gleaming Horizon

The recent success stories of recombinant human-like IgGs do not mark the end of the development, but just the start. Biosimilar antibodies are being developed in significant numbers for products going off patent, illustrating the robust markets and viability of the underlying therapeutic approach. But there is still a lot to gain beyond the well-established path. As we understand more of the complex molecular interactions between immune cells or in cancer tissues, and with additional knowledge gained from the ''-omics'' and systems biology approaches, we can endeavor to expand the design limits of an antibody drug. Most approved drugs are based on full-length IgG molecules close to the native structure of the antibodies in our bloodstream (a few on IgG Fab), sometimes conjugated to a

drug or radiolabel. Numerous other engineering approaches, however, are now being applied to this fascinating molecule. For example, by engineering the Fc glycosylation, dramatic improvements in efficiency can be obtained [12, 13]. As of mid-2013, numerous glycoengineered antibodies have entered clinical study, one is undergoing regulatory review, and one has already reached the market. Mogamulizumab, an afucosylated IgG1, was approved in Japan in 2012 [14], and the glycoengineered antibody obinutuzumab is undergoing regulatory review in the European Union and the United States. Substantial growth in the development of glycoengineered antibodies can be expected to follow on these successes (Chapter 8).

We should learn from nature by looking at the modular design it has used to create the highest diversity group of proteins from repeats of slightly changed domains with a single common basic structure (immunoglobulin fold). We can be inspired to utilize this modular approach for completely novel molecular designs. This has in fact already been done successfully since the early 1990s, and has led to a plethora of novel molecular designs. It allowed the creation of a quite diverse zoo of bispecific antibody designs (see Chapter 11) including the marketed bispecific product catumaxomab (see Chapter 51), the adjustment of the size for optimal pharmacokinetics, and the addition of functions that nature does not provide with an IgG at all. To date, clinical results with many of these new designs are not yet available or disappointing, but this may simply reflect the fact that the molecular design is still rather a result of trial and error than of an understanding of the underlying mechanisms – or it may be dictated mainly by the developer's patent portfolio. Nevertheless, there are clear signs that this will change in the future, if the history of therapeutic antibody development is any guide. In addition to the examples already discussed, ADCs and immunotoxins failed in many clinical studies over more than three decades before the vast body of knowledge collected throughout this time brought optimization and new ideas (see Chapters 13 adn 14). Even long-standing dogmas were put into perspective, for example, by the advantages seen for a combination of trastuzumab with mertansine, a microtubulin-inhibiting toxin. The resulting drug trastuzumab emtansine combines both effector functions requiring internalization and those where internalization is expected to be an impediment.

This illustrates one of the current problems of therapeutic antibody developments aiming beyond nature's IgG format. Fascinating concepts are under evaluation in hundreds of labs. Even then, success stories mainly originate from rather conservative and empirical approaches. Combination therapies are another example here. Put in a more positive perspective, there are so many ideas and so many parameters affecting therapeutic efficiency to be learned that the development of antibody therapeutics will not reach saturation any time soon. Furthermore, major technology patents that have blocked some developments in the past have expired or will expire in the near future (see Chapter 25).

On the other hand, the approved antibodies still target a rather limited set of antigens – clearly, the availability of additional validated targets is another major challenge for the development of new products at the moment. But, as the majority

of the human proteome is only sparsely characterized, there is good hope to still identify those novel targets.

Given the matured technologies to provide and produce human antibodies, the still growing number of targets, decreasing production cost, and the ongoing advent of novel therapeutic strategies, therapeutic antibodies have a long and golden future.

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