



Research review paper

Therapeutic monoclonal antibodies and derivatives: Historical perspectives and future directions

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ARTICLE INFO

Article history:

Received 27 May 2016

Received in revised form 21 July 2016

Accepted 22 July 2016

Available online 25 July 2016

Keywords:

Biologics

Monoclonal antibodies

Fusion proteins

Immunotherapy

Clinical immunology

ABSTRACT

Biologics, both monoclonal antibodies (mAbs) and fusion proteins, have revolutionized the practice of medicine. This year marks the 30th anniversary of the Food and Drug Administration approval of the first mAb for human use. In this review, we examine the biotechnological breakthroughs that spurred the explosive development of the biopharmaceutical mAb industry, as well as how critical lessons learned about human immunology informed the development of improved biologics. We also discuss the most common mechanisms of action of currently approved biologics and the indications for which they have been approved to date.

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1. Introduction

This year marks the 30th anniversary of the Food and Drug Administration (FDA) approval of the first monoclonal antibody (mAb) for clinical use. Biologics have revolutionized the pharmaceutical industry and the practice of medicine, providing new hope for patients for whom traditional therapies have failed or treatment options did not

Abbreviations: mAb, monoclonal antibody; HAMA, human anti-mouse antibody; HACA, human anti-chimeric antibody; HAHA, human anti-humanized antibody; ADC, antibody-drug conjugates.

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previously exist. Although the use of biologics has become a standard and well-characterized part of clinical practice, their development has traveled a long and tortuous road. Each phase of development has been met with new challenges, yet they have catalyzed some of the most prolific and exciting advances in biotechnology. In this review, we will describe how advances in biotechnology and important lessons learned from clinical practice shaped the development of biologics.

2. History of biologics

2.1. Foundational technology and first-generation biologics

Two technologies were paramount in the development of biologics: plasmid engineering and hybridoma technology. In 1973, Cohen and collaborators reported that plasmids constructed *in vitro* could be introduced into *E. coli* to express genes for antibiotic resistance (Cohen et al., 1973). Cohen et al. mused that this discovery could be used to express specific sequences in bacterial plasmids. *E. coli* was soon harnessed to produce the first recombinant therapeutic agent that was later approved by the FDA for clinical use: human insulin (Chance et al., 1981; Goeddel et al., 1979; Tibaldi, 2012). The ability to express human proteins in bacterial plasmids was a critical step for both basic science and the development of new therapeutics and was later used to overcome challenges in protein engineering.

Another critical advance was the development of hybridoma technology. A major technical challenge in the early 1970s was the production of mAbs of pre-defined specificity. In 1975, Köhler and Milstein established a cell line of fused murine myeloma cells and murine splenocytes from an immunized donor, which secreted anti-sheep red blood cell antibodies (Köhler and Milstein, 1975). The myelomas harbor a mutation in the gene for hypoxanthine-guanine phosphoribosyltransferase, an enzyme required for the nucleotide salvage pathway (Ribatti, 2014). Köhler and Milstein took advantage of this deficiency as a selection marker for successful hybridomas (Köhler and Milstein, 1975, 1976). If myelomas successfully fused with antibody-producing B-cells from the mouse spleen, they would survive in hypoxanthine-aminopterin-thymidine medium, which blocks *de novo* nucleotide synthesis (Ribatti, 2014). The survival of hybridomas depends on the functional salvage pathway provided by splenocytes, whereas unfused myeloma cells perish due to their inability to produce nucleotides; unfused B-cells survive only short-term in culture (Ribatti, 2014). This system became the foundation of modern monoclonal antibody production and led to FDA approval of the first therapeutic mAb after only a decade: a murine anti-CD3 mAb called muromonab-CD3 (OKT-3) for preventing acute kidney transplant rejection (Brekke and Sandlie, 2003).

Although antibody-based therapy was anticipated to revolutionize medicine, its premiere fell short of expectations. First-generation mAbs were fully murine in origin (Fig. 1A), which was problematic for

two major reasons: 1) human complement and Fc receptors do not bind well to the Fc region of murine immunoglobulins (Bruhns, 2012), resulting in a failure to direct human immune components to the target intended for elimination; and 2) patients develop human anti-mouse antibodies (HAMAs) (Clark, 2000), leading to rapid clearance of murine antibodies and truncating their therapeutic window. Although problematic for drugs meant to be long-acting, the high clearance rate of murine mAbs is beneficial for agents designed for short-term action, such as radioisotope-antibody conjugates utilized in the diagnosis of tumors (e.g., ^{111}In -cabromab pendetide). However, HAMAs can cause a dramatic, widespread immune response that may be fatal (Clark, 2000; Hwang and Foote, 2005). As a result of these complications, most first-generation therapeutic mAbs have been withdrawn; currently, only one unconjugated murine mAb maintains FDA approval (muromonab-CD3; Table 1). The shortcomings of first-generation biologics highlighted the importance of making mAbs less immunogenic, teeing off the race to make mAbs more human in origin.

2.2. Second-generation biologics

Second-generation mAbs are characterized by their partial human origin; the two major classes are chimeric (Fig. 1B) and humanized (Fig. 1C) mAbs. The enhanced complexity of these molecules required an equally elaborate advance in biotechnology, combining genetic engineering and hybridoma techniques. In 1984, Morrison et al. joined the exon for the heavy chain variable region of an anti-phosphocholine antibody gene with those for human IgG1 or IgG2 heavy chain constant (Fc) regions (Morrison et al., 1984). They used this construct to produce chimeric anti-phosphocholine antibodies bearing human Fc regions and murine variable regions in a well-characterized mouse myeloma (Morrison et al., 1984). Nearly simultaneously, Boulianne and colleagues reported a similar technique to create chimeric IgM antibodies (Boulianne et al., 1984). Just a decade later, the first chimeric therapeutic mAb, abciximab, received FDA approval for peri-surgical prevention of thrombosis for coronary artery interventions (Lefkovits and Topol, 1995); there are currently eight unconjugated chimeric mAbs and one chimeric biosimilar (discussed later) that are FDA approved for clinical use (Table 1). Although chimeric mAbs are approximately 75% human in origin and markedly less immunogenic than murine mAbs, their administration nevertheless induces human anti-chimeric antibodies (Clark, 2000; Lee et al., 2010).

In 1986, Jones et al. reported that the hypervariable, complementarity-determining region (CDR), which determines antigen specificity, could be replaced using the same genetic engineering strategy as that used for chimeric antibodies (Jones et al., 1986). They replaced the CDR of a human myeloma protein with that of a murine antibody against hapten NP-cap, resulting in a product that maintained the antigen specificity of the murine component but was approximately 95%

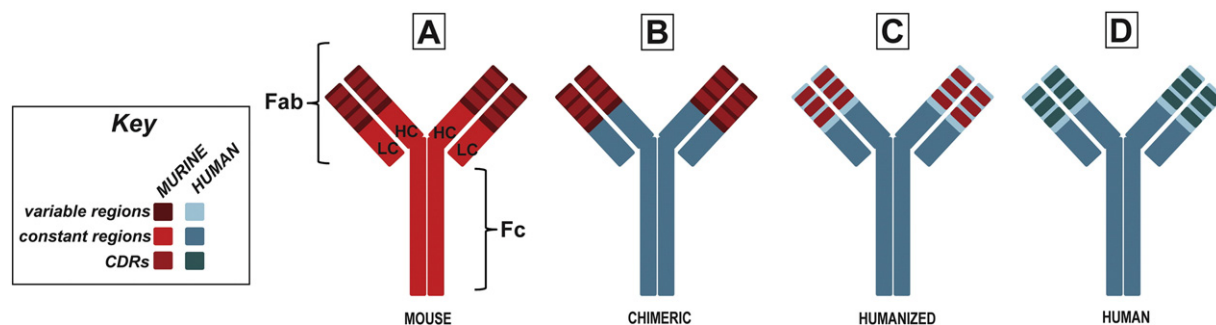


Fig. 1. The evolution of monoclonal antibodies. A) The initial wave of FDA approved monoclonal antibodies were produced by murine hybridomas, and were fully murine in origin. Advances in recombinant DNA technology allowed for the creation of B) chimeric antibodies, which consisted of murine variable regions and human constant regions and C) humanized antibodies, which replaced hypervariable regions of human antibodies with their murine counterparts. The advent of transgenic and phage display technologies allowed for the creation of D) fully human antibodies. Abbreviations: CDR: complementarity determining region; Fab: fragment, antigen-binding; Fc: fragment, crystallizable; HC: heavy chain; LC: light chain.

Table 1
Biologic agents by year of FDA approval.[†]

Year of FDA Approval	Biologic agent	Trade name	Target	Classification
1986	Muromonab-CD3	Orthoclone OKT3	CD3	Mouse
1994	Abciximab	ReoPro	IIb/IIIa	Chimeric
1996	¹¹¹ In-capromab pendetide	Prostascint	PSMA	Mouse, radioisotope conjugate
	^{99m} Tc-nofetumomab	Verluma	40 kDa GP	Mouse, radioisotope conjugate
1997	Daclizumab	Zenapax [Zinbryta; 2016]	CD25	Humanized
	Rituximab	Rituxan	CD20	Chimeric
1998	Basiliximab	Simulect	CD25	Chimeric
	Etanercept	Enbrel	TNF- α	Fusion protein
	Infliximab	Remicade	TNF- α	Chimeric
	Palivizumab	Synagis	F protein	Humanized
	Trastuzumab	Herceptin	HER2	Humanized
1999	^{99m} Tc-arcitumomab	CEA-Scan	CEA	Mouse, radioisotope conjugate
2001	Alemtuzumab	Campath-1H, Lemtrada	CD52	Humanized
2002	Adalimumab	Humira	TNF- α	Human
	⁹⁰ Y-ibritumomab tiuxetan	Zevalin	CD20	Mouse, radioisotope conjugate
2003	Alefacept	Amevive	CD2	Fusion protein
	¹³¹ I-tositumomab	Bexxar	CD20	Mouse, radioisotope conjugate
2004	Bevacizumab	Avastin	VEGF	Humanized
	Cetuximab	Erbitux	EGFR	Chimeric
	Omalizumab	Xolair	IgE	Humanized
2005	Abatacept	Orencia	CD80/CD86	Fusion protein
2006	Natalizumab	Tysabri	α_4 integrin	Humanized
	Panitumumab	Vectibix	EGFR	Human
	Ranibizumab	Lucentis	VEGF	Humanized
2007	Eculizumab	Soliris	C5	Humanized
2008	Certolizumab pegol	Cimzia	TNF- α	Humanized, PEGylated
	Rilonacept	Arcalyst	IL-1	Fusion protein
	Ustekinumab	Stelara	IL-12/IL-23	Human
2009	Canakinumab	Ilaris	IL-1	Human
	Golimimumab	Simponi	TNF- α	Human
	Ofatumumab	Arzerra	CD0	Human
2010	Denosumab	Prolia; Xgeva	RANKL	Human
	Tocilizumab	Actemra; RoActemra	IL-6R	Humanized
2011	Aflibercept	Eylea	VEGF/PIGF	Fusion protein
	Brentuximab vedotin	Adcetris	CD30	Chimeric, drug conjugate
	Belatacept	Nulojix	CD80/CD86	Fusion protein
	Belimumab	Benlysta	BAFF	Human
	Ipilimumab	Yervoy	CTLA-4	Human
2012	Pertuzumab	Perjeta	HER2	Humanized
	Raxibacumab		PA	Human
2013	Obinutuzumab/afutuzumab	Gazyva/Gazyvaro	CD20	Humanized
	Ado-trastuzumab emtansine	Kadcyla	HER2	Humanized, drug conjugate
2014	Blinatumomab	Blinicyto	CD19/CD3	Murine, bispecific T-cell engager
	Nivolumab	Opdivo	PD-1	Human
	Pembrolizumab/lambrolizumab	Keytruda	PD-1	Humanized
	Ramucirumab	Cyramza	VEGFR2	Human
	Siltuximab	Sylvant	IL-6	Chimeric
	Vedolizumab	Entyvio	$\alpha_4\beta_7$	Humanized
2015	Alirocumab	Praluent	PCSK9	Human
	Daratumumab	Darzalex	CD38	Human
	Dinutuximab	Unituxin	GD2	Chimeric
	Elotuzumab	Empliciti	SLAMF7	Humanized
	Evolocumab	Repatha	PCSK9	Human
	Idarucizumab	Praxbind	Dabigatran	Humanized
	Mepolizumab	Nucala	IL-5	Humanized
	Necitumumab	Portrazza	EGFR	Recombinant human IgG1
	Secukinumab	Coxentix	IL-17A	Human
2016	Atezolizumab	Tecentriq	PD-L1	Humanized
	Infliximab-dybb	Inflectra	TNF- α	Chimeric biosimilar
	Ixekizumab	Talzi	IL-17A	Humanized
	Obiltoxaximab	Anthim	PA	Chimeric
	Reslizumab	Cinqair	IL-5	Humanized

[†] Biologics are listed in alphabetical order by year of approval.

human in origin (Jones et al., 1986). Antibodies produced with this strategy are referred to as 'humanized'; there are 20 presently FDA-approved unconjugated humanized mAbs (Table 1). Although it was hoped that humanized mAbs would avoid anti-antibody responses, patients treated with humanized mAbs can produce human anti-humanized antibodies (HAHAs). However, it is widely accepted that increased human content is associated with decreased immunogenicity (Hwang and Foote, 2005). Second-generation mAbs have significantly longer clearance times and lower immunogenic responses than murine

mAbs, but immunogenicity can still be elicited by the presence of foreign CDRs (Harding et al., 2010). Thus, the next great challenge was to generate a fully human mAb (Fig. 1D).

Fusion proteins are another biologic of the second-generation era, usually produced with a construct consisting of the human gene for a receptor for the antigen of interest and the gene for the Fc region of human IgG1 (Boulianne et al., 1984). When expressed in *E. coli*, the products of the two genes form a single molecule. Although fusion proteins contain the Fc region of immunoglobulins, they are a class of

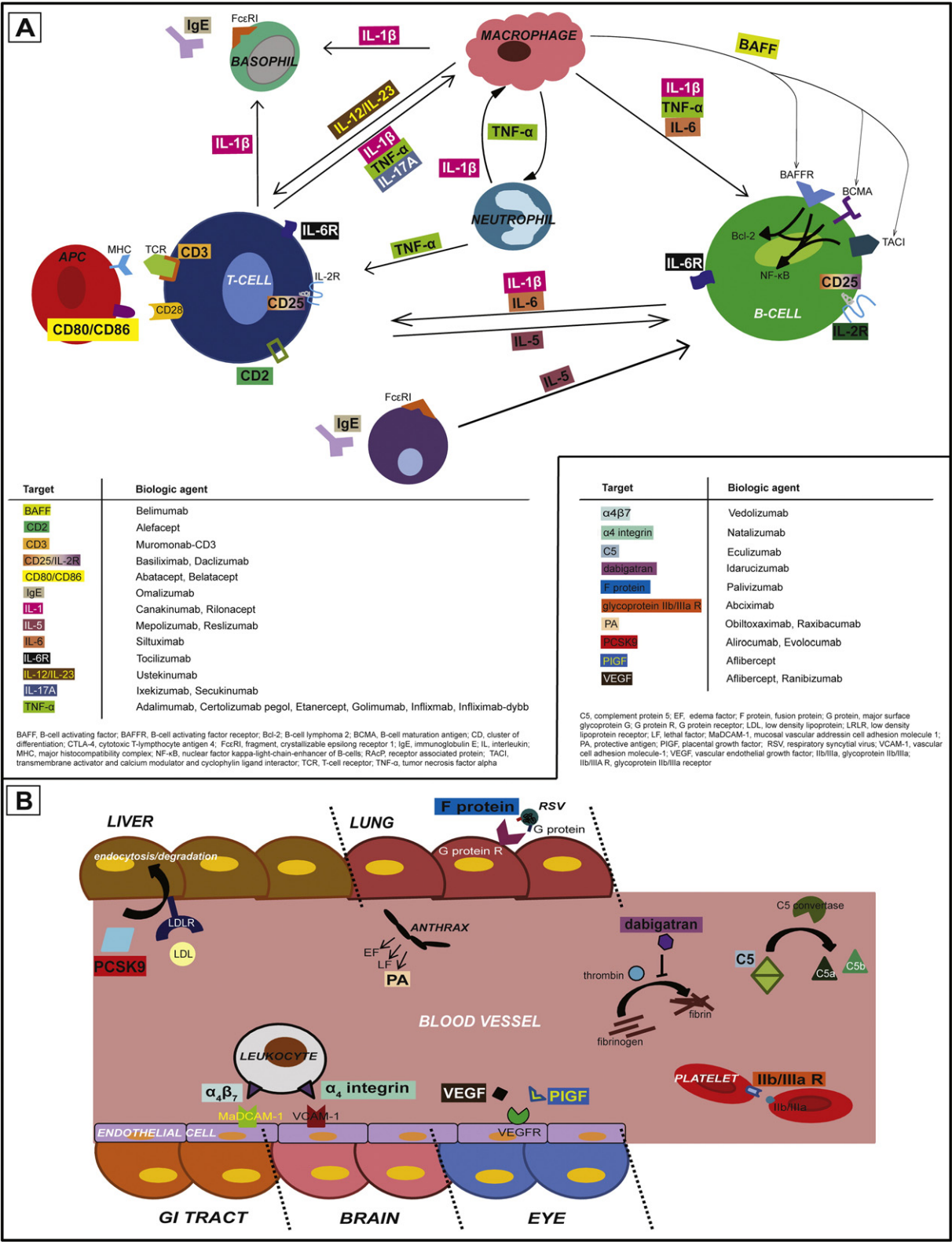


Fig. 2. Pathways targeted by all biologics approved to date. A) Immune pathways regulated by biologic therapies; B) miscellaneous diseases targeted by biologic therapies, including inflammatory conditions, infections, and drug neutralization; C) oncologic pathways targeted by biologic therapies, including tumor-specific markers and immune system activation pathways utilized to combat cancers.

biologics distinct from mAbs due to their unique production process and the use of a receptor rather than a Fab region to bind the antigen of interest. There are currently six fusion proteins that are FDA approved for clinical use (Table 1).

2.3. Third-generation biologics

An ethical quandary posed the biggest roadblock to creating fully human antibodies: how could they be produced without using

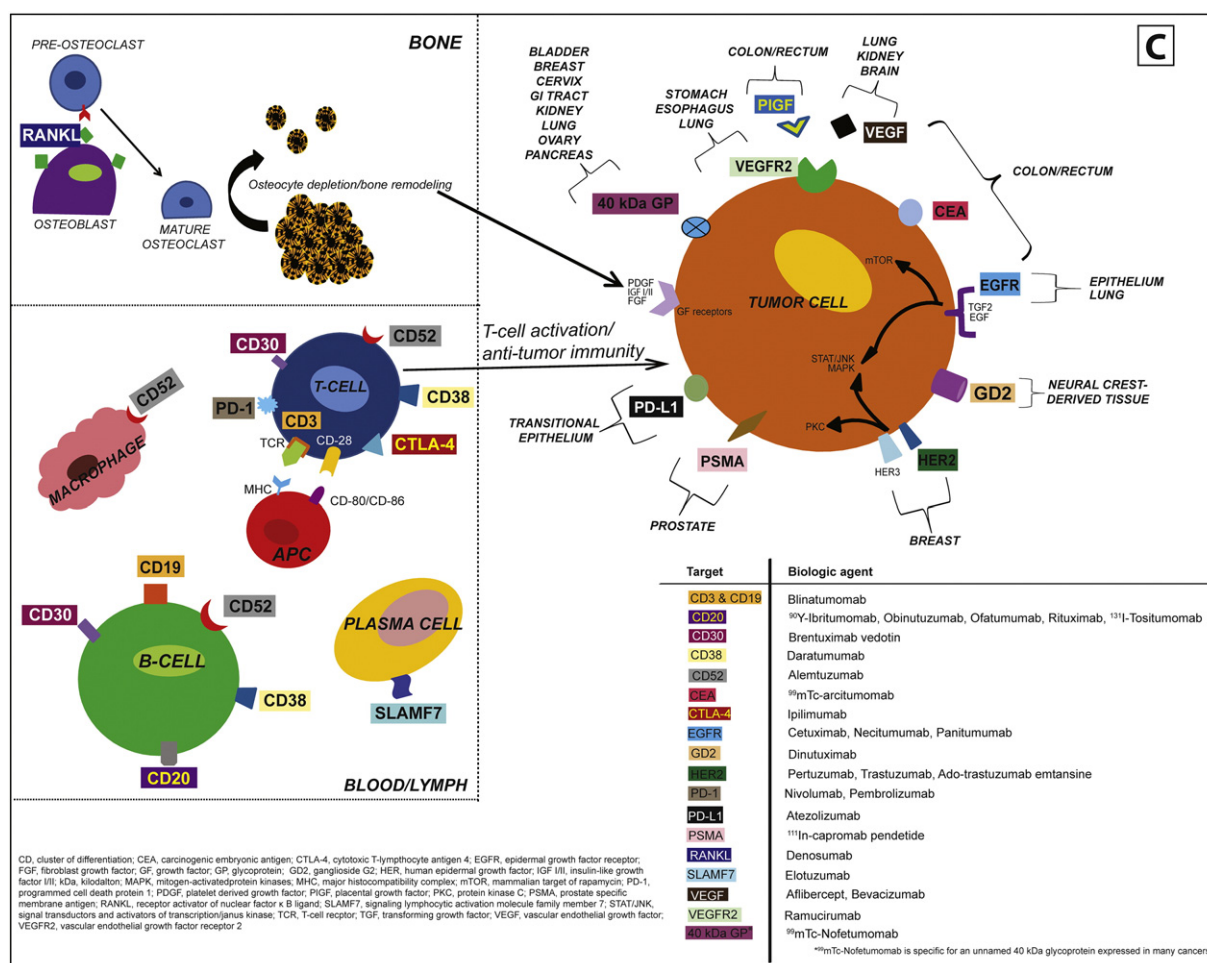


Fig. 2 (continued).

humans? The breakthrough of phage display technology allowed scientists to circumvent this issue. First described by Smith, foreign genes can be inserted into Gene III of filamentous bacteriophages, which codes for a minor coat protein called pIII (Smith, 1985). When a foreign gene is inserted between the regions coding for the amino and carboxyl domains of pIII, it is expressed on the surface of phages without corrupting the functional integrity of the native protein (Smith, 1985); several other bacteriophage strains have since been successfully adapted for the same purpose (Bazan et al., 2012). In 1990, McCafferty et al. expressed human immunoglobulin variable (V) genes in this manner (McCafferty et al., 1990), revolutionizing the antibody engineering process. Now, rather than amplifying V genes from hybridomas, cloning them, and screening antibody fragments secreted by bacteria for binding capacity, scientists can express V genes on bacteriophages and select rare (i.e., high binding capacity) phages for the antigen of interest via affinity chromatography (McCafferty et al., 1990). One advantage of this technique is that it allows for random mutations that can then be selected for based on binding affinity, mimicking the natural immune system (Winter et al., 1994). The authors of this seminal paper used this technique to develop the first fully human mAb: adalimumab (Fig. 2A), an anti-tumor necrosis factor (TNF) agent that gained FDA approval in 2002 for the treatment of several autoimmune and inflammatory conditions, including rheumatoid arthritis and Crohn's disease (Rau, 2002); it has since become the highest grossing biopharmaceutical product (Ecker et al., 2015). There are now 17 unconjugated human antibodies that are approved for clinical use (Table 1).

Transgenic approaches have also been used to create fully human mAbs. Although the specific protocols vary between research groups,

the general strategy is to silence endogenous immunoglobulin genes in mice and introduce their human counterparts at the germ line level, effectively 'humanizing' the murine immune system (Scott, 2007). Humanized mice are immunized against the antigen of interest, and antigen-specific B-cells are isolated and utilized in traditional hybridoma production systems. Whereas phage display offers the advantage of speed, transgenic systems capitalize on the natural immune selection of an intact organism, allowing for the production of high-affinity antibodies without need for further *in vitro* engineering (Jakobovits et al., 2007; Ribatti, 2014; Scott, 2007). In 2006, the FDA approved the first fully human mAb produced by transgenic technology: panitumumab (Fig. 2C), an epidermal growth factor receptor (EGFR)-specific mAb for wild-type KRAS colorectal cancer that was developed using the XenoMouse platform (Abgenix/Amgen) (Jakobovits et al., 2007). Several transgenic mice for producing human mAbs are currently available on the market (Mampo and Gonzalez-Fernandez, 2014); rat and bovine systems are also being investigated (Bruggemann et al., 2015).

Anti-antibody responses are also observed in patients treated with human antibodies (Hansel et al., 2010), demonstrating that immunogenicity cannot be predicted by the ratio of human-to-foreign material in the antibody alone. However, it is unlikely that any specific feat of bio-engineering will be able to fully eliminate all adverse reactions to mAbs and their derivatives. Many factors may contribute to the potential immunogenicity of mAbs of all classes, including variation in immune-related genes between populations, immunogenicity of the diseases under study, and interaction with other therapeutic agents (Nelson et al., 2010). Therefore, the issues of benefit-vs-

immunogenicity of individual fully human mAbs can only be addressed by using head-to-head comparison with similar non-fully human mAb and post-marketing surveillance. Despite the potential for immunogenic events, latest approved therapeutic mAbs of all classes are generally well tolerated, and low risk-to-benefit ratios favor their use (Hansel et al., 2010).

2.4. Modified biologics

As understanding of both the intricacies of human immunity and the finer points of mAb engineering grows, scientists are striving to increase the sophistication of mAbs using chemical modifications. Early pioneers saw the value of using mAbs to target specific cells or tissues, developing several radioisotope-conjugated murine mAbs for tumor diagnosis. These mAbs allow physicians to detect tumor size and location by single-photon emission computerized tomography scans. This same principle can be used to deliver chemotherapeutic agents specifically to malignant cells, minimizing collateral cytotoxicity to healthy tissue (de Goeij and Lambert, 2016). There are currently two such antibody-drug conjugates (ADCs) on the US market (de Goeij and Lambert, 2016). The first is brentuximab vedotin (Fig. 2C), a chimeric mAb against receptor CD30 that is associated with malignant lymphocytes in Hodgkin's and systemic anaplastic large cell lymphomas. Brentuximab vedotin carries anti-mitotic agent monomethyl auristatin E. The second ADC is ado-trastuzumab emtansine (Fig. 2C), a modified version of the previously approved HER2/neu receptor antibody trastuzumab. Whereas trastuzumab alone halts tumor growth in HER2-positive metastatic breast cancer, the drug conjugate exerts a cytotoxic effect by inhibiting microtubule assembly (de Goeij and Lambert, 2016).

Another form of mAb modification is PEGylation, which is the addition of polyethylene glycol to the Fc portion of a mAb to further reduce immunogenicity and widen the therapeutic window. PEG addition may block antigen processing (Sathish et al., 2013), effectively 'hiding' the modified drug from the recipient's immune system and thereby prolonging renal clearance (Lee et al., 2010). However, whether PEGylation actually minimizes immunogenicity is debatable, as anti-PEG antibodies have been found in patients treated with PEGylated therapeutics (Sathish et al., 2013). Additionally, PEGylated molecules cannot cross the placenta, opening up the future possibility of biologic therapy in pregnant women. Indeed, certolizumab pegol (Fig. 2A), the only currently FDA-approved PEGylated mAb, has been successfully used in a pregnant woman without untoward effects on the developing fetus (Goel and Stephens, 2010); however, large controlled studies are needed before practice recommendations can be altered. There are currently nine FDA-approved modified mAbs (Table 1).

3. Functional diversity of biologics

Hybridoma, recombinant DNA, phage display, and transgenic technologies have opened up the possibility of producing biologic agents for many ailments. Theoretically, their potential application is limited only by our knowledge of disease etiology. Since their debut, mAbs have been adapted to treat a wide array of illnesses, including autoimmune diseases (Fig. 2A), cancers (Fig. 2C), hyperlipidemia (Fig. 2B), and blood disorders (Fig. 2B), and their mechanisms of action are equally diverse. Due to the complicated and interconnected nature of various signaling pathways, many biologics operate using multiple mechanisms of action. However, they can be roughly categorized based on five characteristics: their cytotoxicity, modulation of cellular activation/interaction, prevention of growth and proliferation, modulation of immune signaling, and neutralization of foreign entities.

3.1. Cytotoxicity

A primary goal in both cancer and autoimmune disease therapy is to eliminate cells with aberrant function. mAbs can induce cell death

through a variety of mechanisms. As discussed earlier, they may be chemically modified to deliver a chemotherapeutic agent, such as the anti-mitotic agent monomethyl auristatin E (brentuximab vedotin), cytoskeleton destabilizing agent DM1 (ado-trastuzumab emtansine), or radioactive isotopes (^{131}I -tositumomab, ^{90}Y -ibritumomab) for targeted cell death (Fig. 2C). Additionally, mAbs can activate endogenous immune pathways to elicit cell death. MAbs of the IgG subclass interact with various Fc γ receptor types to elicit different types of antibody-mediated responses, depending on the antibody subclass and Fc receptor type. For example, IgG1 and IgG3 antibodies may interact with Fc γ R1 and Fc γ R3 receptors expressed on natural killer (NK) cells or granulocytes, prompting them to kill the cells to which the mAb is attached (i.e., antibody-dependent cell-mediated cytotoxicity (ADCC)) (Michaud et al., 2014). Similarly, IgG mAbs can promote phagocytosis (i.e., antibody-dependent cell-mediated phagocytosis (ADCP)) by interacting with various Fc γ receptors (R1, RIIa, RIIb2) on monocytes, macrophages or neutrophils (Michaud et al., 2014). Finally, IgG-type mAbs, especially IgG1 and 3, activate complement proteins, particularly C1q, and promote the formation of the membrane attack complex, leading to eventual cell death (i.e., complement-dependent cytotoxicity (CDC)) (Jiang et al., 2011). The mechanisms underlying the activation of ADCC, ADCP, and CDC are complex and myriad; the factors influencing the activation of each specific pathway are reviewed elsewhere (Hayes et al., 2014; Jefferis, 2012; Vidarsson et al., 2014). Agents designed to induce cell death, such as those targeting CD20 for B-cell depletion (i.e., ^{90}Y -ibritumomab, obinutuzumab, ofatumumab, rituximab, and ^{131}I -tositumomab, Fig. 2C), likely utilize all three known pathways. Furthermore, antibodies can induce apoptosis independent of the Fc region through mechanisms not yet fully elucidated (Ludwig et al., 2003).

3.2. Modulation of cellular activation/interaction

Another functional strategy of mAb therapy is the modulation of cellular activation and/or interaction. T-cells, which require input from multiple pathways to be activated, are a common target of this strategy (Martin et al., 2013). Blocking T-cell activation has been successful in treating autoimmune diseases, such as juvenile idiopathic arthritis and rheumatoid arthritis, and in the prevention of organ transplant rejection (Martin et al., 2013). T-cells require the co-stimulation of CD28 and the T-cell receptor (TCR) by CD80/CD86 and the major histocompatibility complex on antigen-presenting cells, respectively, to become activated. The first therapeutic mAb, muromonab-CD3, suppresses T-cell activation by binding to the TCR subunit (Fig. 2A) CD3 (Norman, 1995); additionally, two biologics (i.e., abatacept and belatacept, Fig. 2A) are directed against CD80/CD86 (Baldo, 2015). Each of these inhibits the interaction of T-cell co-receptors to prevent cellular activation (Baldo, 2015; Norman, 1995). Biologics have also been developed to block other co-stimulatory proteins on T-cells, such as CD2 (alefacept), and receptors for pro-inflammatory mediators, such as CD25 (basiliximab and daclizumab, Fig. 2A), likewise preventing T-cell activation. However, in treating cancer, one of the goals is to activate the endogenous immune system to attack neoplastic cells. Several mAbs are designed to block T-cell repressor pathways (Fig. 2C), such as cytotoxic T-lymphocyte-associated protein 4 (ipilimumab) and programmed cell death protein 1 (nivolumab and pembrolizumab) (Zhu et al., 2016). These so-called 'immune checkpoint inhibitors' effectively prime T-cells to exert anti-tumor effects (Zhu et al., 2016).

Modulating specific cellular interactions has also been a successful strategy for other cell types. For example, the mAb denosumab prevents osteoclast formation by binding receptor activator of nuclear factor kappa-B ligand expressed on osteoblasts, preventing binding to its receptor on pre-osteoclasts (Fig. 2C) (Burkiewicz et al., 2009; Cheng and Fong, 2014). This therapy prevents bone remodeling characteristic of osteoporosis and bone cancer (Burkiewicz et al., 2009; Cheng and Fong, 2014). Other biologics target proteins involved in recruiting circulating cells into tissue; for example, blocking integrins such as α_4

(natalizumab) and $\alpha_4\beta_7$ (vedolizumab) has been used to treat diseases in which abnormal leukocyte extravasation into the central nervous system (multiple sclerosis) or gastrointestinal tract (Crohn's disease) plays an important role (Fig. 2B) (McLean and Cross, 2016). Another integrin, $\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa), mediates platelet aggregation and activation; it is targeted by abciximab to prevent thrombosis complications caused by surgical interventions involving the coronary artery (Fig. 2B).

Most approved mAbs manipulate cell-to-cell interaction or activation by blocking a single known component of the interaction. However, as antibody engineering has become more sophisticated, a new class of biologics has appeared on the therapeutic horizon: bispecific antibodies, which are engineered such that each arm expresses a hypervariable region of different specificity. The idea is that one arm is specific to an immune cell and the other arm specific to something that the immune cell should eliminate, such as a neoplastic cell; the antibody, thus, acts as a scaffold to promote interaction (Garber, 2014). The FDA recently approved the first such mAb: blinatumomab (Fig. 2C), a murine-derived bispecific T-cell engager for Philadelphia chromosome-negative relapsed/refractory B-cell precursor acute lymphoblastic leukemia (Mullard, 2015; Sanford, 2015). This molecule is specific for CD3, a T-cell co-receptor, and CD19, which is expressed on lymphocytes of B-cell lineage (Fig. 2C). Blinatumomab brings the two cell types together, promoting T-cells to kill potentially neoplastic B-cells (Mullard, 2015; Sanford, 2015). Although currently the only antibody in its class to be approved in the US, blinatumomab will likely be followed by others, as the market is burgeoning with similar therapeutics for both oncological and autoimmune indications (Garber, 2014).

3.3. Prevention of growth and proliferation

Some biologics inhibit signaling pathways required for growth and proliferation, which is a favored design for cancer therapeutics (Scott et al., 2012). Growth factors and their respective receptors are common targets in this class (Scott et al., 2012). For example, EGFR is targeted by three different mAbs (i.e., cetuximab, necitumumab, and panitumumab; Fig. 2C) to treat KRAS wild-type colorectal cancer (CRC) and squamous cell cancers such as head, neck, and non-small cell lung cancer (NSCLC). Other mAbs such as pertuzumab, trastuzumab, and ado-trastuzumab emtansine are directed against the HER2 receptor, an EGFR family member overexpressed in some breast cancers (Fig. 2C) (Mitri et al., 2012). Blocking this receptor is thought to downregulate proliferative intracellular signaling pathways such as MAPK, STAT/JNK, and mTOR (Normanno et al., 2006).

Biologics against both vascular endothelial growth factor (VEGF) and one of its receptors, VEGFR2, have been approved for autoimmune disorders (Fig. 2A) and malignancies (Fig. 2C). The binding of VEGF to its receptors is critical for the growth of endothelial cells required for neovascularization, a process often hijacked by tumors to ensure their survival (Furuya et al., 2005). mAbs that inhibit angiogenesis such as aflibercept, ramucirumab, and bevacizumab have been used to treat cancers, including gastric/gastro-esophageal junction adenocarcinoma, NSCLC, CRC, renal cell cancer, and glioblastoma multiforme (Fig. 2C). Other anti-angiogenic biologics such as aflibercept and ranibizumab have been approved for wet age-related macular degeneration, which is caused by abnormal vascularization of the choriocapillaries (Fig. 2B) (Cernak and Nogova, 2016).

3.4. Modulation of immune signaling

An ever-increasing number of mAbs target proteins of the immune system, such as pro-inflammatory cytokines (Fig. 2A). Biologics targeting TNF- α are among the most successful biopharmaceutical products, with three of the six highest grossing biopharmaceutical products in 2013 being anti-TNF agents (Ecker et al., 2015). These agents function as a 'trap' or 'sponge' to bind secreted proteins,

preventing them from activating their receptors and downstream effector pathways. Many biologics target a wide array of cytokines besides TNF- α , including interleukin (IL)-1, IL-5, IL-6, IL-12, IL-17A, IL-23, and B-cell activating factor (Fig. 2A). Some biologics also target receptors, acting as a 'blockade' and preventing their interaction with cytokines. So far, the FDA has approved a mAb against IL-6R. Neutralizing pro-inflammatory cytokines and their downstream pathways suppresses immune cell activation, which is an effective strategy for treating many autoimmune and inflammatory diseases.

Despite being a critical component of both innate and adaptive immune systems, cytokines are hardly the only desirable therapeutic target for immunosuppression. The complement system may contribute to the pathogenesis of several autoimmune diseases, including systemic lupus erythematosus, systemic sclerosis, and dermatomyositis (Ballanti et al., 2013). Eculizumab, which is approved to treat paroxysmal nocturnal hemoglobinuria and hemolytic uremic syndrome, binds complement protein C5 (Fig. 2B), preventing its proteolysis by C5 convertase to its active cleavage products C5a and C5b (Rother et al., 2007). Although currently the only FDA-approved biologic targeting the complement cascade, eculizumab will surely be followed by others, as this component of immunity has been discussed as an area of therapeutic interest since the 1980s (Aguado et al., 1985).

Other indispensable aspects of the immune system are the endogenously produced antibodies that are the hallmark of the humoral immune system. The five major classes of antibodies (also known as immunoglobulins or Igs) are IgA, IgD, IgE, IgG, and IgM (Janeway, 2005). Each modulates the function of different immune cells, making them a desirable target for diseases with well-characterized etiology (Janeway, 2005). Omalizumab, the first mAb to target an endogenous immunoglobulin, binds IgE (Fig. 2A), which mediates mast cell and basophil activation in hypersensitivity responses, such as allergic asthma (Serrano-Candelas et al., 2016). As our understanding of the mechanisms underlying specific effector functions of each isotype grows, more biologics may be designed to target these pathways. Also, as discussed earlier, several biologics modulate cell-to-cell interactions or cellular activation target cells of the immune system and thus arguably could fall within this category of functionality.

3.5. Neutralization of foreign entities

Another type of mAbs target foreign bodies. The first FDA-approved member of this class was palivizumab, a monoclonal antibody that targets the fusion (F) protein of respiratory syncytial virus (Fig. 2B) and dismantles the machinery required for viral infection (Maeurer et al., 2016). In addition to this antiviral mAb, raxibacumab and obiltoxaximab are two other FDA-approved antitoxin mAbs (Reichert, 2016; Tsai and Morris, 2015). Both agents act as sponges, binding the protective antigen of *Bacillus anthracis* (Fig. 2B) and preventing cell entry of the exotoxins edema factor and lethal toxin. Both agents are approved for the prophylaxis of inhalation anthrax.

Finally, the FDA recently approved idarucizumab, a first-in-class mAb designed to neutralize another therapeutic agent, dabigatran (Fig. 2B). Dabigatran is an oral anticoagulant used to lower the risk of stroke and thrombus formation in patients with non-valvular atrial fibrillation, initially touted as a replacement for warfarin. Although superior at lowering rates of stroke and systemic embolism, some physicians were reluctant to prescribe dabigatran due to the fact that, unlike warfarin, its pharmacological effects cannot be quickly or easily reversed in the event of a major hemorrhage (Connolly et al., 2009; Eerenberg et al., 2011). However, treatment with idarucizumab rapidly and completely reversed the effect of dabigatran, offering an antidote to the unwanted side effects of an otherwise promising medication (Pollack et al., 2015).

Although there are relatively few biologics against foreign entities, these examples highlight the promise of mAb therapy and suggest that their potential therapeutic application is only as limited as our understanding of disease pathology.

Table 2
Diagnostic biologic agents.

Agent	Target diagnosis
^{99m} Tc-arcitumomab	Colorectal cancer
¹¹¹ In-cabromab pendetide	Prostate cancer
^{99m} Tc-nofetumomab	Bladder carcinoma
	Breast cancer
	Cervical cancer
	Gastrointestinal cancer
	Kidney cancer
	Lung cancer
	Ovarian cancer
	Pancreas cancer

4. Indications for biologics

Over the past 30 years, the FDA has approved biologics for the diagnosis (Table 2) and treatment of a wide array of diseases. Many of the most successful biologic drugs are indicated for autoimmune diseases (Table 3); biologics for the treatment of rheumatoid arthritis make up nearly 13% of the biopharmaceutical market alone. Another large share of the market is biologics for the treatment of cancer (Table 4), including blood cancers (e.g., chronic lymphocyte leukemia, Non-Hodgkin's lymphoma), solid tumors (e.g., squamous cell carcinoma), primary tumors (e.g., glioblastoma multiforme), and metastatic events (e.g., skeletal metastases). Biologics for the treatment of miscellaneous diseases (Table 5), such as respiratory events (e.g., allergic asthma), central nervous system degenerative disorders (e.g., multiple sclerosis), and chronic gastrointestinal conditions (e.g., Crohn's disease), are perhaps among the fastest growing areas in the market.

5. Future of therapeutic biologics

Biologics have revolutionized medicine and are undoubtedly among the most promising and fastest growing niches in the pharmaceutical industry. To date, 30 biologics have been approved just since the

Table 3
Autoimmune diseases currently with biologic treatments.

Disease	Presentation	Agent
Ankylosing spondylitis	Active	Adalimumab, golimumab, infliximab, infliximab-dybb, secukinumab
Cryopyrin-associated periodic syndromes	FCAS and Muckle-Wells syndrome	Canakinumab, rilonacept
Juvenile idiopathic arthritis	NOMID	Anakinra
	Moderate to severe, polyarticular	Abatacept, adalimumab, etanercept
	Active, systemic	Canakinumab, tocilizumab
Microscopic polyangiitis	–	Rituximab
Plaque psoriasis	Moderate to severe	Adalimumab, alefacept, etanercept, ixekizumab, secukinumab, ustekumab
	Chronic severe	Infliximab, infliximab-dybb
Psoriatic arthritis	Active	Adalimumab, etanercept, golimumab, infliximab, infliximab-dybb, secukinumab
Systemic lupus erythematosus	Active	Belimumab
Rheumatoid arthritis	Moderate to severe	Abatacept, adalimumab, anakinra, certolizumab pegol, etanercept, golimumab, infliximab, infliximab-dybb, tocilizumab
Uveitis	noninfectious intermediate; posterior; panuveitis	Adalimumab
Wegner's granulomatosis	–	Rituximab

beginning of 2010. In 2016, there are reportedly 53 biologics in Phase III or Phase II/III clinical trials for a diverse array of indications (e.g., HIV, acquired thromboticthrombocytopenicpurpura, Alzheimer's disease, migraines, systemic lupus erythematosus, and cancers) (Reichert, 2016). These agents target 42 different epitopes, 29 of which are novel (Reichert, 2016). Several of these investigational biologics are further engineered for improved efficacy. For example, research continues to unravel the differential responses elicited by the interaction between each of the IgG subclasses and specific Fc receptors (Vidarsson et al., 2014). Additionally, it has been shown that altering antibody glycosylation or other posttranslational modifications can affect antibody specificity, effector functions, and/or half-life (Chung et al., 2014; Hayes et al., 2014; Jefferis, 2012). Antibodies against an established target that carry one of the aforementioned modifications to improve some aspect of already commercially available biologics are collectively known as “biobetters” (Beck, 2011). The burgeoning knowledge in this field may be used to help guide the engineering process for future mAbs in order to elicit the desired functional outcome while minimizing potential adverse events (Boesch et al., 2015; Jefferis, 2012, 2016).

Another major area of interest in the biopharmaceutical industry is the creation of “biosimilars”, so-called “generic” versions of biologics. These products are defined by the FDA as being “highly similar to an FDA-approved biological product, known as a reference product, and has no clinically meaningful differences in terms of safety and effectiveness from the reference product” (FDA, 2016). Biosimilars have been highly anticipated as a potential solution for the prohibitive cost of biologic therapy (Beck, 2011; Emmanouilides et al., 2016). However, due to the complicated structure of biologics, their production is inherently extraordinarily complex and expensive; thus, it remains to be seen whether biosimilars will be able to live to the hype as the cheaper alternative (Beck, 2011; Emmanouilides et al., 2016). With the April 2016 FDA approval of the first such mAb biosimilar, infliximab-dybb (FDA, 2016), the world may soon have an answer.

Yet another up-and-coming trend in biologic design is the so-called ‘nanobody’, a class of mAb fragments a fraction of the size of full-sized mAbs (e.g., caplacizumab) (Reichert, 2016; Revets et al., 2005). It is hoped that nanobodies will retain the efficacy of full-sized mAbs while addressing problems posed by their mass, such as limited tissue penetration and high background signal during imaging (Revets et al., 2005).

As researchers and clinicians tease apart disease mechanisms and improve biologic engineering strategies, the market for biopharmaceuticals will continue to expand. We anticipate that the future discovery of novel biomarkers and the rise of personalized medicine will further inform the development of biologics, both in target selection and in clinical practice, when choosing the best therapeutic agent for individual patients.

6. Conclusions

Although they had a somewhat disappointing debut, biologics have persevered, feeding off of and promoting the development of increasingly sophisticated biotechnological techniques. Biologics have unquestionably revolutionized both the pharmaceutical industry and the practice of medicine, offering new hope to patients for whom one conventional therapy after another has failed. mAbs and fusion proteins are now standard therapies in many specialties, including cardiology, cardiothoracic and transplant surgery, dermatology, gastroenterology, hematology, neurology, oncology, ophthalmology, and rheumatology. The spectrum of maladies that can be treated by biologics is theoretically limited only by our knowledge of disease etiology; dozens of new biopharmaceuticals have entered the drug development pipeline as mechanisms of disease continue to be dissected. These drugs could serve to treat a whole host of new indications, bringing biotechnology-based treatments to medical disciplines that are still reliant on conventional therapies.

Table 4

Malignancies currently with biologic treatment.

Type	Presentation	Agent
Bone	Metastases	Denosumab
	Giant cell tumor	Denosumab
Bladder	Urothelial carcinoma	Atezolizumab
Brain	Recurrent glioblastoma multiforme	Bevacizumab
	High-risk pediatric neuroblastoma	Dinutuximab
Breast	HER2 overexpressing	Pertuzumab
	HER2 ⁺ metastatic	Pertuzumab, trastuzumab, trastuzumab emtansine
Colorectal	Metastatic	Aflibercept, bevacizumab
	EGFR ⁺ , KRAS wild-type metastatic	Cetuximab
	Metastatic, EGFR ⁺ with progression	Panitumumab
Gastric	Adenocarcinoma	Ramucirumab
Gastro-esophageal junction	Adenocarcinoma	Ramucirumab
Head & neck	Squamous cell carcinoma	Cetuximab
Leukemia/blood	Relapsed/refractory Philadelphia chromosome negative acute lymphoblastic leukemia	Blinatumomab
	Chronic lymphocytic leukemia	Alemtuzumab, obinutuzumab, ofatumumab, rituximab
	Multiple myeloma	Daratumumab, denosumab, elotuzumab
Lung	Non-small cell	Ramucirumab
	Non-squamous	Bevacizumab
	Squamous	Nivolumab
	Untreated, metastatic	Necitumumab
	Metastatic	Nivolumab
Lymphoma	Systemic anaplastic large cell	Brentuximab vedotin
	Cutaneous T-cell lymphoma	Alemtuzumab
	Hodgkin's	Brentuximab vedotin, nivolumab
	T-cell lymphoma	Alemtuzumab
	Non-Hodgkin's	Rituximab, ¹³¹ I-tositumomab
	Follicular, CD20 ⁺	Rituximab
	Diffuse large B-cell, CD20 ⁺	⁹⁰ Y-ibritumomab tiuxetan
	Relapsed, refractory, low-grade, follicular or transformed B-cell	Pembrolizumab
Melanoma	Metastatic	Ipilimumab, nivolumab
	Late-stage, inoperable	Bevacizumab, nivolumab
Renal	Metastatic, renal cell carcinoma	

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 5

Miscellaneous diseases with biologic treatment.

Disease	Presentation	Agent
Asthma	Severe, persistent allergic	Omalizumab
Blood disorders	Severe eosinophilic	Mepolizumab, reslizumab
	Atypical hemolytic uremic syndrome	Ecuzumab
	Paroxysmal nocturnal hemoglobinuria	Ecuzumab
Castleman's disease	Multicentric, HIV [−] and HHV8 [−]	Siltuximab
Crohn's disease	Moderate to severe	Adalimumab, certolizumab pegol, infliximab, infliximab-dybb, natalizumab, vedolizumab
Hyperlipidemia	Uncontrolled by diet or statins	Alirocumab, evolocumab
Inhalation anthrax	–	Obiltoximab, raxibacumab
Macular degeneration	Wet	Aflibercept, ranibizumab
Multiple sclerosis	Relapsing	Alemtuzumab, daclizumab, natalizumab
Organ transplant rejection	Kidney	Basiliximab, belatacept, daclizumab, muromomab-CD3
Osteoporosis	Heart and liver	Muromomab-CD3
Respiratory synovial virus	Postmenopausal	Denosumab
Thrombosis	–	Palivizumab
Ulcerative colitis	Percutaneous coronary intervention	Abciximab
	Moderate to severe	Infliximab, infliximab-dybb, vedolizumab

References

- Aguado, M.T., Lambris, J.D., Tsokos, G.C., Burger, R., Bitter-Suermann, D., Tamerius, J.D., Dixon, F.J., Theofilopoulos, A.N., 1985. Monoclonal antibodies against complement 3 neoantigens for detection of immune complexes and complement activation. Relationship between immune complex levels, state of C3, and numbers of receptors for C3b. *J. Clin. Invest.* 76 (4), 1418–1426.
- Baldo, B.A., 2015. Chimeric fusion proteins used for therapy: indications, mechanisms, and safety. *Drug Saf.* 38 (5), 455–479.
- Ballanti, E., Perricone, C., Greco, E., Ballanti, M., Di Muzio, G., Chimenti, M.S., Perricone, R., 2013. Complement and autoimmunity. *Immunol. Res.* 56 (2–3), 477–491.
- Bazan, J., Calkosinski, I., Gamian, A., 2012. Phage display—a powerful technique for immunotherapy: 1. Introduction and potential of therapeutic applications. *Human Vaccines & Immunotherapeutics* 8 (12), 1817–1828.
- Beck, A., 2011. Biosimilar, biobetter and next generation therapeutic antibodies. *mAbs* 3 (2), 107–110.
- Boesch, A.W., Alter, G., Ackerman, M.E., 2015. Prospects for engineering HIV-specific antibodies for enhanced effector function and half-life. *Curr. Opin. HIV AIDS* 10 (3), 160–169.
- Boulianne, G.L., Hozumi, N., Shulman, M.J., 1984. Production of functional chimeric mouse/human antibody. *Nature* 312 (5995), 643–646.
- Brekke, O.H., Sandlie, I., 2003. Therapeutic antibodies for human diseases at the dawn of the twenty-first century. *Nat. Rev. Drug Discov.* 2 (1), 52–62.
- Bruggemann, M., Osborn, M.J., Ma, B., Hayre, J., Avis, S., Lundstrom, B., Buelow, R., 2015. Human antibody production in transgenic animals. *Arch. Immunol. Ther. Exp.* 63 (2), 101–108.
- Bruhns, P., 2012. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* 119 (24), 5640–5649.
- Burkiewicz, J.S., Scarpace, S.L., Bruce, S.P., 2009. Denosumab in osteoporosis and oncology. *Ann. Pharmacother.* 43 (9), 1445–1455.
- Cernak, M., Nogova, L., 2016. Current antiangiogenic agents in oncology and ophthalmology. *Neoplasia* 63 (1), 10–17.
- Chance, R.E., Kroeff, E.P., Hoffmann, J.A., Frank, B.H., 1981. Chemical, physical, and biologic properties of biosynthetic human insulin. *Diabetes Care* 4 (2), 147–154.
- Cheng, M.L., Fong, L., 2014. Effects of RANKL-targeted therapy in immunity and cancer. *Frontiers in Oncology* 3, 329.
- Chung, A.W., Crispin, M., Pritchard, L., Robinson, H., Gorny, M.K., Yu, X., Bailey-Kellogg, C., Ackerman, M.E., Scanlan, C., Zolla-Pazner, S., Alter, G., 2014. Identification of antibody glycosylation structures that predict monoclonal antibody Fc-effector function. *AIDS* 28 (17), 2523–2530.
- Clark, M., 2000. Antibody humanization: a case of the 'Emperor's new clothes'? *Immunol. Today* 21 (8), 397–402.
- Cohen, S.N., Chang, A.C., Boyer, H.W., Helling, R.B., 1973. Construction of biologically functional bacterial plasmids in vitro. *Proc. Natl. Acad. Sci. U. S. A.* 70 (11), 3240–3244.
- Connolly, S.J., Ezekowitz, M.D., Yusuf, S., Eikelboom, J., Oldgren, J., Parekh, A., Pogue, J., Reilly, P.A., Themeles, E., Varrone, J., Wang, S., Alings, M., Xavier, D., Zhu, J., Diaz, R.,

- Lewis, B.S., Darius, H., Diener, H.C., Joyner, C.D., Wallentin, L., 2009. Dabigatran versus warfarin in patients with atrial fibrillation. *N. Engl. J. Med.* 361 (12), 1139–1151.
- de Goeij, B.E., Lambert, J.M., 2016. New developments for antibody-drug conjugate-based therapeutic approaches. *Curr. Opin. Immunol.* 40, 14–23.
- Ecker, D.M., Jones, S.D., Levine, H.L., 2015. The therapeutic monoclonal antibody market. *mAbs* 7 (1), 9–14.
- Eerenberg, E.S., Kamphuisen, P.W., Sijpkens, M.K., Meijers, J.C., Buller, H.R., Levi, M., 2011. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation* 124 (14), 1573–1579.
- Emmanouilides, C.E., Karampola, M.I., Beredima, M., 2016. Biosimilars: hope and concern. *J. Oncol. Pharm. Pract.* 22 (4), 618–624.
- FDA, 2016. FDA approves Inflectra, a biosimilar to Remicade. <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm494227.htm>.
- Furuya, M., Nishiyama, M., Kasuya, Y., Kimura, S., Ishikura, H., 2005. Pathophysiology of tumor neovascularization. *Vascular Health and Risk Management* 1 (4), 277–290.
- Garber, K., 2014. Bispecific antibodies rise again. *Nat. Rev. Drug Discov.* 13 (11), 799–801.
- Goeddel, D.V., Kleid, D.G., Bolivar, F., Heyneker, H.L., Yansura, D.G., Crea, R., Hirose, T., Kraszewski, A., Itakura, K., Riggs, A.D., 1979. Expression in *Escherichia coli* of chemically synthesized genes for human insulin. *Proc. Natl. Acad. Sci. U. S. A.* 76 (1), 106–110.
- Goel, N., Stephens, S., 2010. Certolizumab pegol. *mAbs* 2 (2), 137–147.
- Hansel, T.T., Kropshofer, H., Singer, T., Mitchell, J.A., George, A.J., 2010. The safety and side effects of monoclonal antibodies. *Nat. Revs. Drug Discov.* 9 (4), 325–338.
- Harding, F.A., Stickler, M.M., Razo, J., DuBridge, R.B., 2010. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *mAbs* 2 (3), 256–265.
- Hayes, J.M., Cosgrave, E.F., Struwe, W.B., Wormald, M., Davey, G.P., Jefferis, R., Rudd, P.M., 2014. Glycosylation and Fc receptors. *Curr. Top. Microbiol. Immunol.* 382, 165–199.
- Hwang, W.Y., Foote, J., 2005. Immunogenicity of engineered antibodies. *Methods* 36 (1), 3–10.
- Jakobovits, A., Amado, R.G., Yang, X., Roskos, L., Schwab, G., 2007. From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice. *Nat. Biotechnol.* 25 (10), 1134–1143.
- Janeway, C., 2005. *Immunobiology: The Immune System in Health and Disease*. sixth ed. Garland Science, New York.
- Jefferis, R., 2012. Isotype and glycoform selection for antibody therapeutics. *Arch. Biochem. Biophys.* 526 (2), 159–166.
- Jefferis, R., 2016. Posttranslational modifications and the immunogenicity of biotherapeutics. *Journal of Immunology Research* 2016, 5358272.
- Jiang, X.R., Song, A., Bergelson, S., Arroll, T., Parekh, B., May, K., Chung, S., Strouse, R., Mire-Sluis, A., Schenerman, M., 2011. Advances in the assessment and control of the effector functions of therapeutic antibodies. *Nat. Rev. Drug Discov.* 10 (2), 101–111.
- Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., 1986. Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* 321 (6069), 522–525.
- Köhler, G., Milstein, C., 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256 (5517), 495–497.
- Köhler, G., Milstein, C., 1976. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6 (7), 511–519.
- Lee, S.J., Chinen, J., Kavanaugh, A., 2010. Immunomodulator therapy: monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins. *J. Allergy Clin. Immunol.* 125 (2 Suppl 2), S314–S323.
- Lefkowitz, J., Topol, E.J., 1995. Platelet glycoprotein IIb/IIIa receptor inhibitors in ischemic heart disease. *Curr. Opin. Cardiol.* 10 (4), 420–426.
- Ludwig, D.L., Pereira, D.S., Zhu, Z., Hicklin, D.J., Bohlen, P., 2003. Monoclonal antibody therapeutics and apoptosis. *Oncogene* 22 (56), 9097–9106.
- Maeurer, M., Rao, M., Zumla, A., 2016. Host-directed therapies for antimicrobial resistant respiratory tract infections. *Curr. Opin. Pulm. Med.* 22 (3), 203–211.
- Martin, A., Tisch, R.M., Getts, D.R., 2013. Manipulating T cell-mediated pathology: targets and functions of monoclonal antibody immunotherapy. *Clin. Immunol.* 148 (1), 136–147.
- McCafferty, J., Griffiths, A.D., Winter, G., Chiswell, D.J., 1990. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* 348 (6301), 552–554.
- McLean, L.P., Cross, R.K., 2016. Integrin antagonists as potential therapeutic options for the treatment of Crohn's disease. *Expert Opinion on Investigational Drugs* 25 (3), 263–273.
- Michaud, H.A., Eliaou, J.F., Lafont, V., Bonnefoy, N., Gros, L., 2014. Tumor antigen-targeting monoclonal antibody-based immunotherapy: orchestrating combined strategies for the development of long-term antitumor immunity. *Oncoimmunology* 3 (9), e955684.
- Mitri, Z., Constantine, T., O'Regan, R., 2012. The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy. *Chemotherapy Research and Practice* 2012, 743193.
- Mompo, S.M., Gonzalez-Fernandez, A., 2014. Antigen-specific human monoclonal antibodies from transgenic mice. *Methods Mol. Biol.* 1060, 245–276.
- Morrison, S.L., Johnson, M.J., Herzenberg, L.A., Oi, V.T., 1984. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. U. S. A.* 81 (21), 6851–6855.
- Mullard, A., 2015. FDA approves first bispecific. *Nat. Rev. Drug Discov.* 14 (1), 7.
- Nelson, A.L., Dhimolea, E., Reichert, J.M., 2010. Development trends for human monoclonal antibody therapeutics. *Nat. Revs. Drug Discov.* 9 (10), 767–774.
- Norman, D.J., 1995. Mechanisms of action and overview of OKT3. *Ther. Drug Monit.* 17 (6), 615–620.
- Normanno, N., De Luca, A., Bianco, C., Strizzi, L., Mancino, M., Maiello, M.R., Carotenuto, A., De Feo, G., Caponigro, F., Salomon, D.S., 2006. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* 366 (1), 2–16.
- Pollack Jr., C.V., Reilly, P.A., Eikelboom, J., Glund, S., Verhamme, P., Bernstein, R.A., Dubiel, R., Huisman, M.V., Hylek, E.M., Kamphuisen, P.W., Kreuzer, J., Levy, J.H., Sellke, F.W., Stangier, J., Steiner, T., Wang, B., Kam, C.W., Weitz, J.L., 2015. Idarucizumab for dabigatran reversal. *N. Engl. J. Med.* 373 (6), 511–520.
- Rau, R., 2002. Adalimumab (a fully human anti-tumour necrosis factor alpha monoclonal antibody) in the treatment of active rheumatoid arthritis: the initial results of five trials. *Ann. Rheum. Dis.* 61 (Suppl. 2), ii70–ii73.
- Reichert, J.M., 2016. Antibodies to watch in 2016. *mAbs* 8 (2), 197–204.
- Revs, H., De Baetselier, P., Muyltermans, S., 2005. Nanobodies as novel agents for cancer therapy. *Expert. Opin. Biol. Ther.* 5 (1), 111–124.
- Ribatti, D., 2014. From the discovery of monoclonal antibodies to their therapeutic application: an historical reappraisal. *Immunol. Lett.* 161 (1), 96–99.
- Rother, R.P., Rollins, S.A., Mojcik, C.F., Brodsky, R.A., Bell, L., 2007. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. *Nat. Biotechnol.* 25 (11), 1256–1264.
- Sanford, M., 2015. Blinatumomab: first global approval. *Drugs* 75 (3), 321–327.
- Sathish, J.G., Sethu, S., Bielsky, M.C., de Haan, L., French, N.S., Govindappa, K., Green, J., Griffiths, C.E., Holgate, S., Jones, D., Kimber, I., Moggs, J., Naisbitt, D.J., Pirmohamed, M., Reichmann, G., Sims, J., Subramanyam, M., Todd, M.D., Van Der Laan, J.W., Weaver, R.J., Park, B.K., 2013. Challenges and approaches for the development of safer immunomodulatory biologics. *Nat. Rev. Drug Discov.* 12 (4), 306–324.
- Scott, C.T., 2007. Mice with a human touch. *Nat. Biotechnol.* 25 (10), 1075–1077.
- Scott, A.M., Wolchok, J.D., Old, L.J., 2012. Antibody therapy of cancer. *Nat. Rev. Cancer* 12 (4), 278–287.
- Serrano-Candelas, E., Martinez-Aranguren, R., Valero, A., Bartra, J., Gastaminza, G., Goikoetxea, M.J., Martin, M., Ferrer, M., 2016. Comparable actions of omalizumab on mast cells and basophils. *Clin. Exp. Allergy* 46 (1), 92–102.
- Smith, G.P., 1985. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228 (4705), 1315–1317.
- Tibaldi, J.M., 2012. Evolution of insulin development: focus on key parameters. *Adv. Ther.* 29 (7), 590–619.
- Tsai, C.W., Morris, S., 2015. Approval of raxibacumab for the treatment of inhalation anthrax under the US Food and Drug Administration “animal rule”. *Front. Microbiol.* 6, 1320.
- Vidarsson, G., Dekkers, G., Rispen, T., 2014. IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol.* 5, 520.
- Winter, G., Griffiths, A.D., Hawkins, R.E., Hoogenboom, H.R., 1994. Making antibodies by phage display technology. *Annu. Rev. Immunol.* 12, 433–455.
- Zhu, Z., Liu, W., Gotlieb, V., 2016. The rapidly evolving therapies for advanced melanoma: towards immunotherapy, molecular targeted therapy, and beyond. *Crit. Rev. Oncol. Hematol.* 99, 91–99.