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Monoclonal antibody therapeutics: history and future

Nicholas APS Buss, Simon J Henderson, Mary McFarlane, Jacintha M Shenton and Lolke de Haan

Over the last three decades, monoclonal antibodies have made a dramatic transformation from scientific tools to powerful human therapeutics. At present, approximately 30 therapeutic monoclonal antibodies are marketed in the United States and Europe in a variety of indications, with sales in the US alone reaching approximately $18.5 billion in 2010. This review describes how antibody engineering has revolutionized drug discovery and what are considered the key areas for future development in the monoclonal antibody therapy field.

Address
Biologics Safety Assessment, MedImmune, Granta Park, Cambridge CB21 6GH, UK

Corresponding author: Buss, Nicholas APS (bussn@medimmune.com)

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Introduction — a brief history of therapeutic monoclonal antibodies

Antibodies (Abs) are glycoproteins belonging to the immunoglobulin (Ig) superfamily that are secreted by B cells to identify and neutralize foreign organisms or antigens. Abs comprise two heavy and two light chains and are grouped into different isotypes dependent on which type of heavy chain they contain. Therapeutic monoclonal Abs (mAbs) are typically of the γ-immunoglobulin (or IgG) isotype (schematic representation in Figure 1). The hypervariable regions of each heavy and light chain combine to form the antigen binding site, referred to as the fragment antigen binding domain (Fab), whilst the fragment crystallizable (Fc) domain responsible for effector function is composed of two constant domains. This results in a bivalent IgG molecule that has a long serum half-life due to pH-dependent Ab recycling via the neonatal Fc receptor (FcRn; Figure 2).

Whilst the immune response to an antigen or organism is usually polyclonal in nature, in 1975 Kohler and Milstein were the first to describe the *in vitro* production of murine mAbs from hybridomas [1]. This was the first important step towards the development of human mAbs as therapeutics. In the late 1980s, murine mAbs (suffix: -omab; Figure 3) were in clinical development; however, they had significant drawbacks. Murine mAbs are often associated with allergic reactions, and the induction of anti-drug antibodies (ADAs). They also exhibit a relatively short half-life in man compared to human IgG, as a consequence of relatively weak binding to the human FcRn (Figure 2) [2]. Finally, murine mAbs are relatively poor recruiters of effector function, antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which can be critical for their efficacy, especially in oncology indications [3].

In an attempt to overcome the inherent immunogenicity and reduced effector function of murine mAbs in man, chimeric mouse–human antibodies (suffix: -ximab; Figure 3) were developed. This was enabled by grafting the entire antigen-specific variable domain of a mouse Ab onto the constant domains of a human Ab using genetic engineering techniques, resulting in molecules that are approximately 65% human [4]. These chimeric mAbs exhibit an extended half-life in man and show reduced immunogenicity, but nevertheless, the propensity of chimeric mAbs to induce ADAs is still considerable [5]. To further improve mAb properties, humanized mAbs (suffix: -zumab; Figure 3) were developed by grafting just the murine hypervariable regions onto a human Ab framework, resulting in molecules that are approximately 95% human [6]. Whilst humanized mAbs appeared to overcome the inherent immunogenic problems of murine and chimeric mAbs, humanization does have limitations and can be a laborious process.

The advent of *in vitro* phage display technology [7••,8–12] and the generation of various transgenic mouse strains expressing human variable domains [13–15] enabled the generation of fully human mAbs (suffix: -umab; Figure 3). Both humanized and fully human mAbs have significantly reduced immunogenic potential and show properties similar to human endogenous IgGs [16•]. A better understanding of factors that influence mAb immunogenicity has led to the development of *in silico* and *in vitro* tools to reduce clinical immunogenicity through deselection or deimmunization [16•,17]. Whilst there is no evidence that mAbs isolated using phage display or generated in transgenic mice behave differently in the clinical setting, it would appear that the mAb discovery process involving phage display more frequently requires lead optimization; on the other hand, phage display offers the opportunity for

Glossary

IgG isotypes: there are four IgG isotypes (IgG1, IgG2, IgG3 and IgG4), each with structurally and/or functionally distinct constant domains. From a mAb drug development perspective, IgG1 is often the preferred isotype due to its ability to elicit effector function and high intrinsic stability. If effector function is not desirable, IgG2 and IgG4 have strongly reduced effector function; however, both of these are associated with intrinsic stability issues. IgG3 exhibits similar effector function to IgG1 but is rarely used in drug development due to its short serum half-life, intrinsic instability, and allotypic polymorphisms.

Effector function: the ability of an antibody to trigger cell lysis, either through engagement of activating Fcγ receptor on effector cells (referred to as antibody-dependent cytotoxicity, ADCC), or by fixing complement and activation of the complement cascade (complement-dependent cytotoxicity, CDC).

Phage display: laboratory technique for the study of protein–protein or protein–peptide interactions that uses bacteriophages to connect proteins with the genetic information that encodes them. Phage display of antibody fragments has been exploited for the *in vitro* isolation of therapeutic antibodies.

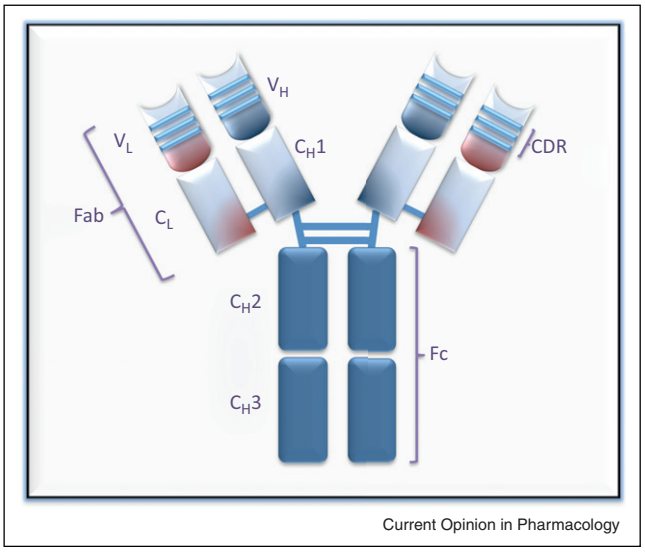
Anti-drug antibodies (ADAs): the immune system can develop an antibody response to protein drugs, including mAbs, which are referred to as anti-drug antibodies. ADAs may cause or contribute to drug-induced hypersensitivity and serum sickness, and can alter the pharmacokinetic profile and reduce the efficacy of a protein drug.

Anaphylatoxins: small pro-inflammatory polypeptides that are produced during activation of the complement system and consequent cleavage of complement factor C3, C4 or C5. This results in the generation of anaphylatoxins C3a, C4a and C5a, of the C3 and C5 convertase and membrane attack complex.

Antibody glycosylation: an enzymatic post-translational modification process that results in the attachment of glycans to antibody (heavy) chains. Antibody glycosylation typically affects Ab effector function and half-life.

Half-life: or elimination half-life in pharmacokinetics, this is the time required for the plasma/serum concentration of a drug to decrease by half its steady state concentration.

Figure 1



Antibody structure. Immunoglobulin G (IgG) Abs are large (approximately 150 kDa), proteins comprising pairs of heavy and light chains connected by disulphide bonds. The heavy chains contain a variable domain (V_H), a hinge region and three constant (C_H1, C_H2 and C_H3) domains. The light chains contain one variable (V_L) and one constant (C_L) domain. IgG structure can also be divided into the Fragment antigen binding (Fab) region that is composed of one constant and one variable domain of both the light (V_L and C_L) and the heavy (V_H and C_H1) chain and the fragment crystallizable (Fc) domain that is composed of two constant domains (C_H2 and C_H3). The specificity of Abs is mediated by their variable domains and represented by the Fab region. The variable domains can be further subdivided into hypervariable regions (or complementarity-determining regions [CDR]) which bind to the antigen directly and framework regions which serve as a scaffold for the CDR to contact the antigen.

more directed lead isolation and control over the specificity and affinity of the mAb [18]. They should therefore be considered as complementary technologies that have made therapeutic mAbs more accessible than ever before, with approximately 30 mAbs being marketed in the US and/or Europe (Table 1) and a record number of mAbs in clinical development [19].

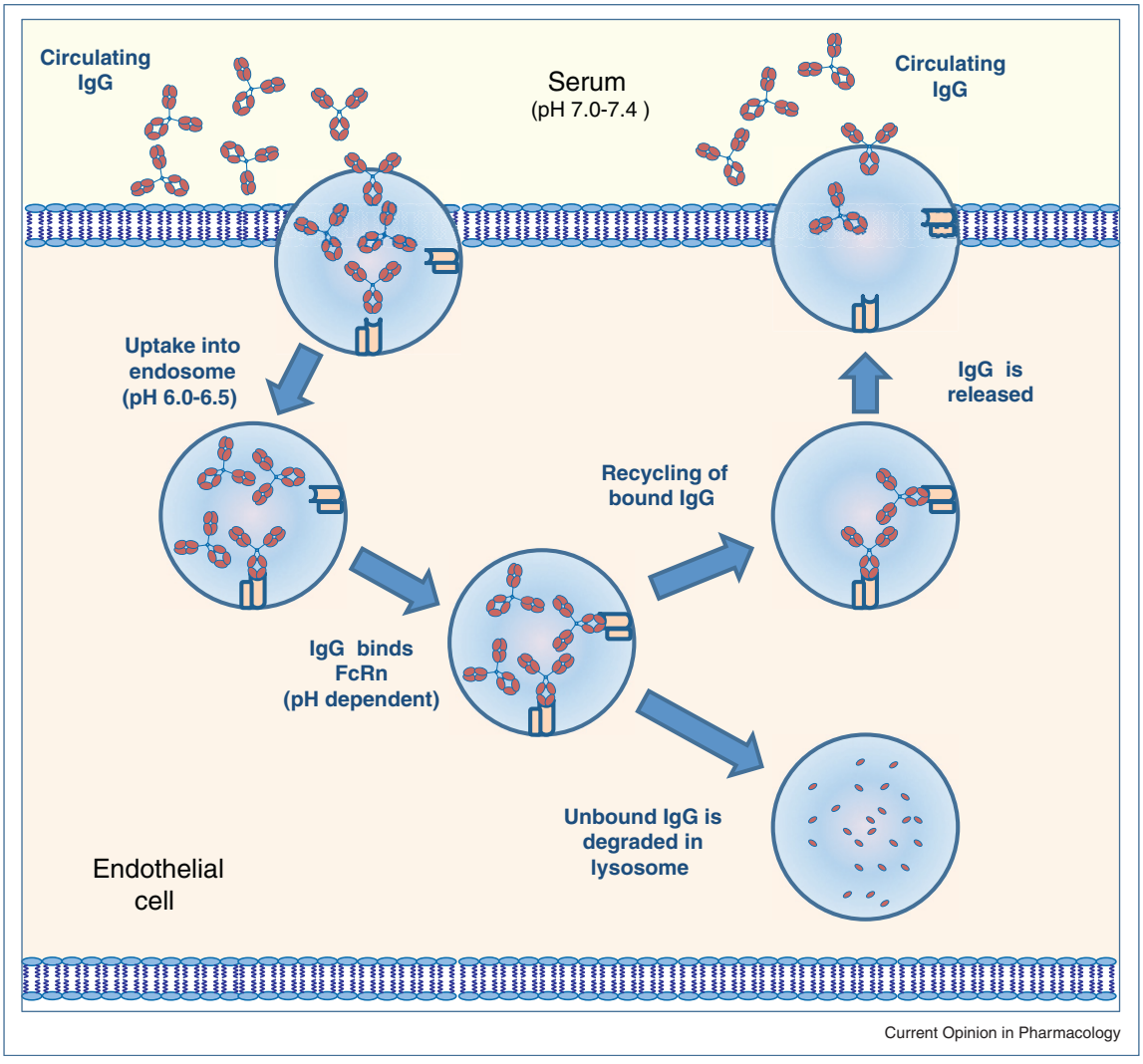
For this review, four key areas in mAb research were identified that have either seen substantial recent advancement, or are anticipated to represent areas of significant advancement and application going forward. These are: Fc engineering; Ab drug conjugates; bispecifics and brain delivery of mAbs, all of which are briefly discussed below.

Optimizing monoclonal antibodies: Fc engineering

Whilst the variable regions broadly determine the specificity and selectivity of a mAb, the Fc region adds considerable functionality to the molecule. The Fc region can interact with the FcRn, thus mediating an extended half-life (Figure 2) and, dependent on isotype, by mediating effector function (ADCC and/or CDC). ADCC and CDC are predominantly triggered by IgG1 and IgG3 mAbs with other isotypes showing much reduced effector function [20]. ADCC is triggered by the Ab through engagement of Fcγ receptors (FcγRs) expressed on immune effector cells, eventually resulting in killing of the target cell. CDC is triggered by C1q binding to an Ab, which leads to release of anaphylatoxins, the formation of the membrane attack complex, activation of C1q receptors on effector cells, and ultimately death of the target cell. Depending on the therapeutic goal, triggering effector function can either be desirable or undesirable.

The amino acid residues in the Fc domain and Ab hinge region that interact with FcγRs, C1q and FcRn have been identified [21]. In addition, it is known that glycosylation of the Fc domain impacts on effector function [22]. All of these offer opportunities for Fc engineering and mAb optimization. Mutation of key amino acid residues and techniques to modify glycosylation of the Fc domain have been employed to either increase or decrease binding of therapeutic mAbs to FcγRs or C1q, thereby modulating effector function without impacting binding to FcRn. These modifications have been extensively reviewed elsewhere [23,24•].

Figure 2

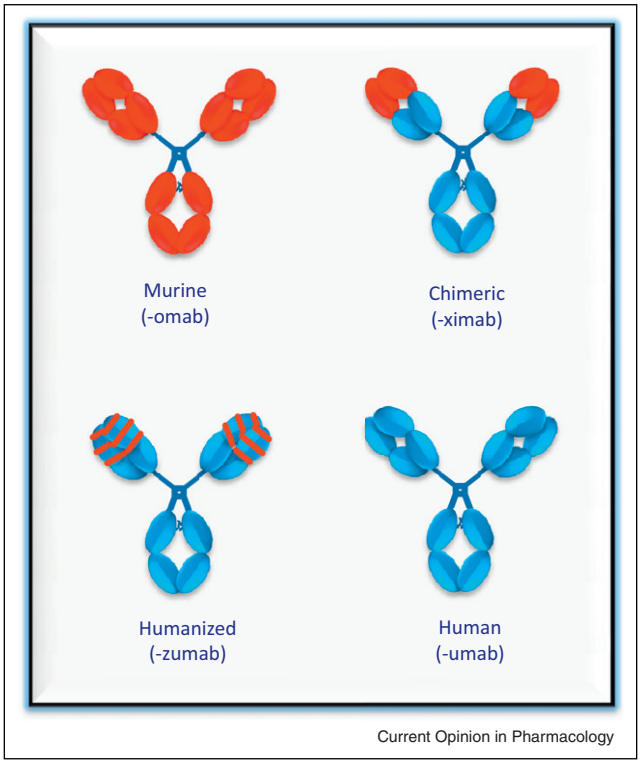


Antibody recycling via the neonatal fc receptor (FcRn). The long half-life of Abs is the consequence of Ab salvage from the lysosomal pathway by FcRn as originally proposed by Brambell *et al*. in 1964 [58]. The Fc region binds to FcRn in endosomes (pH 6.0–6.5) and is diverted away from the degradative lysosomal pathway. Recycled IgG is released at the cell surface and this interaction with the FcRn is responsible for the long half-life of Abs.

In addition to modulation of effector function, Fc engineering has been employed to further extend mAb half-life. For example, a twofold to fourfold improvement of the half-life for IgG1 mAbs has been achieved by introducing mutations that increase binding to FcRn under acidic endosomal pH conditions allowing for less frequent dosing [25–27]. Furthermore, whilst IgG3s mediate both ADCC and CDC, they exhibit a short half-life and are therefore not considered a suitable isotype for therapeutic mAbs [28]. However, it has recently been shown that replacing arginine at position 435 (a key contact residue with FcRn) with a histidine, results in improved binding of IgG3 to FcRn under acidic pH conditions, and a serum half-life that is comparable to that of an IgG1 molecule [29••]. This opens up the possibility of engineering therapeutic IgG3 mAbs.

Finally, Fc engineering can be employed to stabilize IgG molecules. The most striking example is with the IgG4 isotype, which is infrequently used for therapeutic mAbs as IgG4s can undergo half Ab formation due to Fab arm exchange with endogenous IgG4 [30,31]. This may impact on mAb pharmacokinetics, result in monovalency, and affect the avidity and activity of the mAb. However, it has been shown that mutations in the hinge region of IgG4 mAbs stabilize the molecule and reduce Fab-arm exchange, which may increase the therapeutic use of this IgG subclass [32,33]. Thus, Fc engineering has opened up opportunities for creating a much wider range of differentiated mAb-based molecules, whose activity can be tailored to specific therapeutic indications.

Figure 3



Monoclonal antibody types and nomenclature. Therapeutic mAbs can be murine (suffix: -omab), chimeric (suffix: -ximab), humanized (suffix: e.g. -zumab) or human (e.g. -umab) and are named accordingly.

Antibody drug conjugates

The ideal antibody drug conjugate (ADC) combines a mAb with specificity for — typically — a tumour-specific antigen with no, or low, expression in normal tissues, with a highly potent cytotoxic chemical. The cytototoxic chemical is attached to the mAb using a linker that maintains stability in the systemic circulation, yet enables release of the cytotoxin when the mAb is bound to or is internalized by a target cancer cell. Thus far, only two ADCs have been approved for use in humans, with one of these (gemtuzumab ozogamicin (Mylotarg^{TM})) being voluntarily withdrawn as its efficacy did not differentiate from chemotherapy alone [34]. Nevertheless, the recent approval of brentuximab vedotin (Adcetris^{TM}) [35] and the therapeutic potential of trastuzumab emantisine (T-DM1; microtubule polymerization inhibitor mertansine conjugated to trastuzumab [36]) has signalled a new era in the interest and development of ADCs.

The synthetic linkers used for conjugation are generally either cleavable (disulphide based, hydrazone or peptide linkers) or non-cleavable (e.g. non-reducible thioether bond) and can impact significantly on the efficacy of the ADC. Gemtuzumab ozogamicin contains an acid-labile hydrazone linker that is hydrolyzed in acidic endosomes or lysosomes [37], and the lack of differentiating efficacy with this ADC is possibly linked to a lack of *in vivo* linker stability. In contrast, brentuximab vedotin has a dipeptide linker that is cleaved by lysosomal enzymes after internalization and is highly stable *in vivo* [38].

More recently, amide bond-based linkers have been developed that remain attached covalently to the cytotoxin after lysosomal degradation of the mAb [39]. Furthermore, Shen *et al.* have shown that the site of conjugation of the reactive cysteine thiol group in the mAb used for coupling maleimide linkers can differentially influence the stability, efficacy and safety of the ADC, depending on accessibility to reactive thiols in albumin, free cysteine or glutathione in plasma [40•].

Optimization of the ratio of cytotoxin molecules per mAb is another key consideration for ADC design. A three to four payload:mAb ratio is considered optimal, with higher ratios being associated with ADC aggregation, loss of affinity for the target, and rapid systemic clearance which is exacerbated if the drug and linker are hydrophobic [41]. The relatively limited success of mAb monotherapy in oncology has fuelled interest in ADCs, and it is likely that this molecule class will only gain further interest in pharmaceutical development in the years ahead.

Bispecifics

Bispecifics comprise a diverse group of mAb-based therapeutics that can have multiple, functionally different, binding domains within the same construct that allow for interaction with two target antigens. These should not be confused with mAb mixtures, which have recently emerged as a novel and exciting way to target multiple antigens, or multiple epitopes within the same antigen [42••]. Bispecifics can exist in many different formats, from tandem monovalent binding fragments to IgG-based Abs onto which multiple additional antigen-binding domains are attached [43,44,24•]. Full-length antibody structures offer advantages over fragment-based formats because they benefit from the pharmacokinetic properties and, potentially, effector function of the Fc domain.

The first bispecific to reach the market in 2009 was catumaxomab (Removab^{TM}) for the treatment of malignant ascites in patients with human epithelial cell adhesion molecule (EpCAM)-positive carcinoma. This rat–mouse hybrid IgG2a/b bispecific simultaneously targets CD3 on T cells and EpCAM on tumour cells to facilitate killing of tumour cells and could be considered a trifunctional molecule, as the Fc region creates a third functional binding site to facilitate ADCC [45]. Currently, many bispecifics in development tend to work to similar principles of catumaxomab, and aim to bring effector cells into close contact with specific tumour-associated antigens to facilitate cell killing; a strategy that was first

Table 1

Licensed therapeutic mAbs

Scientific name	Trade name	Origin and isotype	Target	Licensed indication(s) and year of first approval-region
Capromab pendetide	Prostascint	Murine IgG1	PSA[a]	Diagnostic imaging (1996-US)
Muronomab-CD3	Orthoclone OKT3	Murine IgG2a	CD3	Transplant rejection (1992-US)
Tositumumab iodine 131	Bexxar	Murine IgG2	CD20	NHL[b] (2003-US)
Ibritumomab tiuxetan	Zevalin	Murine IgG1	CD23	NHL (2002-US, 2004-EU)
Basiliximab	Simulect	Chimeric IgG1	CD25	Prophylaxis for transplant rejection (1998-US)
Brentuximab vedotin	Adcetris	Chimeric IgG1	CD30 ADC[c]	ALCL[d] and Hodgkin lymphoma (2011-US)
Catumaxomab	Removab	Chimeric IgG2a/b	CD3, EpCAM[e]	Malignant ascites (2009-EU)
Cetuximab	Erbitux	Chimeric IgG1	EGFR[f]	Colorectal, head and neck cancer (2004-US, EU)
Infliximab	Remicade	Chimeric IgG1	TNF[g]	RA[h], ankylosing spondylitis, Crohn's disease, ulcerative colitis (1998-US, 1999-EU)
Rituximab	Rituxan, MabThera	Chimeric IgG1	CD20	B cell non-Hodgkin lymphoma (1997-US, 1998-EU)
Alemtuzumab	Campath, MabCampath	Humanized IgG1	CD52	B-CLL[i] (2001-US)
Bevacizumab	Avastin	Humanized IgG1	VEGF[j]	Colorectal, lung, breast cancer (2004-US, 2005-EU)
Daclizumab	Zenapax	Humanized IgG1	CD25	Prophylaxis for transplant rejection (1997-US, 1999-EU (withdrawn 2009))
Eculizumab	Soliris	Humanized IgG2/4	Complement factor 5	Paroxysmal nocturnal haemoglobinuria, atypical haemolytic-uremic syndrome (2007-US)
Efalizumab	Raptiva	Humanized IgG1	CD11a	Psoriasis (2003-US (withdrawn 2009))
Gentuzumab ozogamcin	Mylotarg	Humanized IgG4	CD33 ADC	Leukemia (2000-US)
Natalizumab	Tysabri	Humanized IgG4	$\alpha_4\beta_1$ integrin	Multiple sclerosis (2004-US, 2006-EU)
Omalizumab	Xolair	Humanized IgG1	IgE	Severe asthma (2003-US, 2005-EU)
Palivizumab	Synagis	Humanized IgG1	RSV[k] F protein	Prevention of RSV infection in neonates (1998-US)
Ranibizumab	Lucentis	Humanized IgG1	VEGF	Macular degeneration (2006-US, 2007-EU)
Tocilizumab	Actemra, Roactemra	Humanized IgG1	IL-6R[l]	Castleman's syndrome, RA (2010-US, 2009-EU)
Trastuzumab	Herceptin	Humanized IgG1	HER-2[m]	HER-2 positive breast cancer (1998-US)
Adalimumab	Humira	Human IgG1	TNF	RA, Crohn's disease, plaque psoriasis (2002-US, 2003-EU)
Belimumab	Benlysta	Human IgG1	BLys[n]	Systemic lupus erythematosus
Canakinumab	Ilaris	Human IgG1	IL-1β[o]	Muckle-Wells syndrome (US, EU-2009)
Denosumab	Prolia, Xgeva	Human IgG2	RANKL[p]	Osteoporosis, bone metastasis (2009-US, EU)
Golimumab	Simponi	Human IgG1	TNF	RA, psoriatic arthritis, ankylosing spondylitis (2009-US, EU)
Ipilimumab	Yervoy	Human IgG1	CTLA4[q]	Advanced melanoma (2011-US, EU)
Ofatumumab	Arzerra	Human IgG1	CD20	CLL (2009-US, 2010-EU)
Panitumumab	Vectibix	Human IgG2	EGFR	Colorectal cancer (2007-US, EU)
Ustekinumab	Stelara	Human IgG1	IL-12p40[r]	Plaque psoriasis (2009-US, EU)
Pertuzumab	Perjeta	Humanized IgG1	HER-2[m]	HER-2 positive breast cancer (2012-US)

[a] PSA, prostate antigen.
[b] NHL, non-Hodgkin lymphoma.
[c] ADC, antibody drug conjugate.
[d] ALCL, anaplastic large cell lymphoma.
[e] EpCAM, epithelial cell adhesion molecule.
[f] EGFR, epidermal growth factor receptor.
[g] TNF, tumour necrosis factor.
[h] RA, rheumatoid arthritis.
[i] B-CLL, B-cell chronic lymphocytic leukemia.
[j] VEGF, vascular endothelial growth factor.
[k] RSV, respiratory syncytial virus.
[l] IL-6R, interleukin 6 receptor.
[m] HER-2, human epidermal growth factor receptor 2.
[n] BLys, B lymphocyte stimulator.
[o] IL-1β, interleukin 1β.
[p] RANKL, receptor activator of nuclear factor kappa-B ligand.
[q] CTLA4, cytotoxic T-lymphocyte antigen 4.
[r] IL-12p40, interleukin 12 p40 subunit; CD, cluster of differentiation.

suggested in the 1980s [46,47•]. The promise of immune cell re-targeting and synergistic/enhanced efficacy through engagement of multiple targets gives bispecifics the potential to revolutionize Ab therapy in the next decade.

Targeting and delivery of mAbs to the brain

With respect to neuro-oncology and neurodegenerative disorders, one of the major disadvantages of therapeutic mAbs is their inability to cross the blood–brain barrier (BBB). In mice, less than 1% of a systemically administered mAb is detected in the brain [48], whilst in man cerebrospinal fluid (CSF) levels have been observed to be ~300-times lower than in serum [49,50]. However, for targeting diseases of the brain, increased brain penetration could be highly beneficial. Many approaches for delivering mAbs in the brain have been investigated, from intracranial injection of mAbs to disruption of the BBB [51]. In addition alternative routes of delivery to the brain such as intranasal, intrathecal or intraventricular delivery have been explored [52]. However, all of these approaches have significant practical issues. Newer, less tried and tested, delivery systems such as liposomes, microspheres, nanoparticles, nanogels, microchips, biodegradeable polymers and bionanocapsules are also under investigation [53].

Recent interest has shifted to Trojan horse-like approaches involving bispecific mAbs that bind to receptors located on the endothelial cells of the BBB to facilitate receptor-mediated transcytosis of the other arm of the bispecific into the brain. Yu *et al.* recently reported delivery of a bispecific anti-transferrin receptor/anti-beta secretase (BACE1) construct, which showed good brain exposure in the rat compared to the anti-BACE1 mAb alone [54••]. Alternatively, a mAb for the human insulin receptor, which mediates transfer of endogenous insulin through the BBB, could also be used [55]. Another exciting approach involves the utilization of the camelid single domain Ab FC5, which appears to be able to cross the BBB *in vitro* and *in vivo* by binding to an unidentified luminal alpha(2,3)-sialoglycoprotein receptor which triggers clathrin-mediated endocytosis [56,57]. These exciting approaches may, in the future, allow for the delivery of mAbs and other biologics into the brain. This would offer new hope for effective therapies targeting neurological diseases with high unmet medical need, such as Alzheimer's, multiple sclerosis and brain tumours.

Conclusions

From the regulatory approval of the first murine mAb for therapeutic use in man in 1986 to the first bispecific mAb in 2009, mAbs and their derivatives are now key drug modalities in the pharmaceutical industry. Advances in Ab engineering and mAb production techniques have enabled the clinical development of mAbs with tailored properties with respect to half-life, effector function and stability. In addition much more complex mAb-based therapies such as ADCs, bispecifics, mAb mixtures, and potentially brain penetrant mAbs are being developed. These novel mAb-based therapeutics will likely revolutionize drug therapy in a wide spectrum of disease areas, and will hopefully be able to address significant unmet medical needs.

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