

# Developments in therapy with monoclonal antibodies and related proteins

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## ABSTRACT

Monoclonal antibody therapeutics have been approved for over 30 targets and diseases, most commonly cancer. Antibodies have become the new backbone of the pharmaceutical industry, which previously relied on small molecules. Compared with small molecules, monoclonal antibodies (mAbs) have exquisite target selectivity and hence less toxicity as a result of binding other targets. The clinical value of both mAbs and ligand traps has been proven. New applications of mAbs are being tested and mAbs have now been designed to target two (bi-specific, eg TNF- $\alpha$  and IL-17) or more targets simultaneously, augmenting their therapeutic potential. Because of space limitations and the wide ranging scope of this review there are regrettably, but inevitably, omissions and missing citations. We have chosen to highlight the first successes in inflammatory diseases and cancer, but a broader overview of approved mAbs and related molecules can be found in Table 1.

**KEYWORDS:** Biologic therapy, cancer, inflammation, monoclonal antibodies, traps

## Introduction

There has been a revolution in therapeutics over the past 20 years, due to the introduction of protein therapeutics – often termed ‘biologics’ – into routine, mainstream medicine. Biologics are expensive and like all medicines have risks. Their advantage is exquisite specificity due to greater surface area binding, resulting in decreased ‘off-target’ effects as compared with most small molecule drugs.

New biologic therapies come in several basic forms, either growth factors and cytokines (such as erythropoietin, G-CSF, interferon, enzymes, factors that regulate coagulation) or, more commonly, monoclonal antibodies (mAbs) and related proteins such as ‘traps’ in which cytokine receptors are made soluble and fused with antibody constant regions. The latter group (mAbs and traps) have dramatically advanced the therapy of chronic inflammatory diseases and cancer. In this review, we

will focus on mAbs and relatives, describing these therapeutics. The tumour necrosis factor (TNF)-blockers for autoimmune/inflammatory diseases are the most broadly deployed (with multiple products) and have engendered a revolution in therapeutic research/development, along with rather remarkable revenues. This therapeutic revolution is based on the synergy of three scientific disciplines: immunology, molecular biology and protein engineering.

## Brief history

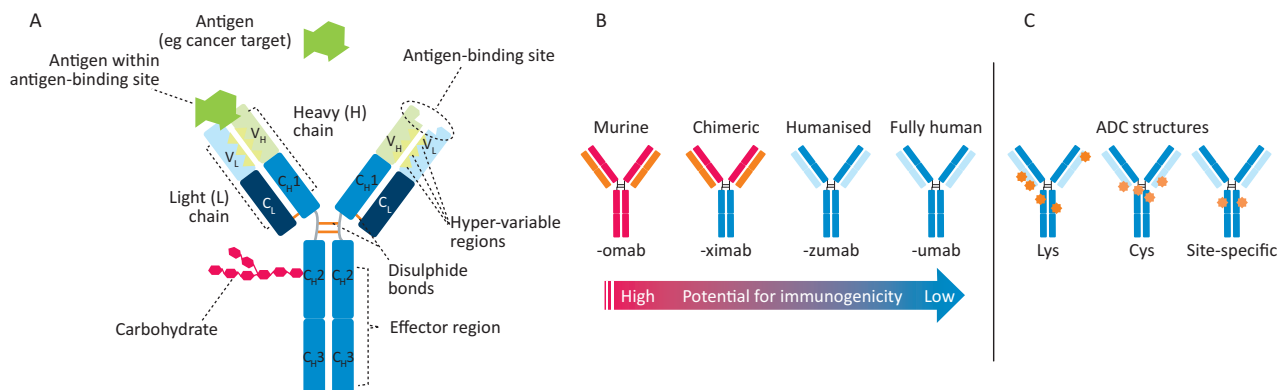
The first mAbs were produced by Milstein and Köhler (Cambridge University),<sup>1</sup> who won the Nobel Prize in Physiology or Medicine in 1984. mAbs were initially murine proteins and hence were immunogenic in humans and not suited for chronic therapy. This limitation was solved using molecular biology and protein engineering to create more human-like mAbs with little immunogenicity. There are several versions of mAbs and traps in clinical use and trials (Fig 1, Table 1). Initial attempts replaced most murine Fc sequences with human Fc, a process referred to as ‘chimerisation’.<sup>2</sup> This involves grafting the murine antigen binding Fab regions onto a human immunoglobulin (IgG) backbone. In ‘humanisation’, the mouse hyper-variable peptide binding loops<sup>3</sup> are grafted onto the human IgG backbone. More recent technologies allow for generation of fully human antibodies.<sup>4</sup>

## Improved therapy for inflammatory/autoimmune disease

The concept of autoimmune diseases, pioneered by Sir Frank McFarlane Burnet<sup>5</sup> (Nobel Laureate 1960), was validated by the detection of serum autoantibodies in many diseases in the 1960s. This suggested a role for B-lymphocytes, and human leukocyte antigen (HLA) associations documented from the 1970s indicated the role of T-cells when the peptide presenting function of HLA was understood. However, these advances did not lead to new treatments at the time because of the lack of specific therapeutics.

With the advent of mAbs, the role of T-cells was tested in rheumatoid arthritis patients using lytic antibodies to CD4, without clinical benefit.<sup>6</sup> Nor did small trials of several other anti-T-cell antibodies, such as anti-CD7. OKT3, a mouse mAb recognising CD3<sup>7</sup> given to reduce acute rejection in patients

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**Fig 1. Monoclonal antibodies.** A – antibody structure showing light (L) and heavy (H) chains with variable (V<sub>L</sub>, V<sub>H</sub>) and constant (C<sub>L</sub>, C<sub>H</sub>) regions, which are connected by inter-chain disulphide bonds. Antigen binding (green symbol) occurs at V<sub>H</sub>/V<sub>L</sub> domains, while effector functions are mediated via the Fc (C<sub>H</sub>) portion. B – increasing the amount of human sequences in a murine antibody decreases immunogenic potential. C – on an antibody-drug conjugate (ADC, cytotoxic drugs (orange, red stars) can be linked stochastically to lysine (Lys) residues or on cysteine residues either through reduction of inter-chain disulphides (Cys) or by engineering in cysteines at select conjugation sites (site-specific).

with organ transplants was the first monoclonal antibody approved for use in humans although it was later withdrawn.

### The success of anti-TNF therapy

The targeting of a single pro-inflammatory cytokine, TNF, to treat a complex disease in rheumatoid arthritis (RA) where multiple pro-inflammatory cytokines were upregulated was based on work using human disease tissue by Feldmann and Maini<sup>8</sup> and Brennan *et al.*<sup>9</sup> They analysed cytokine production from joints and cytokine regulation in cultures of rheumatoid synovium in which the majority of the cells survived, producing the mediators generated *in vivo*. In these cultures, blocking TNF- $\alpha$  reduced the production of many other inflammatory cytokines (IL-1, IL-6, GM-CSF, IL-8 etc),<sup>9</sup> thus defining a ‘TNF-dependent cytokine cascade’. The dramatic clinical success of TNF blockade, demonstrated first in late stage RA then in earlier stage disease, also validated this concept.<sup>8</sup> Noteworthy was the fact that tissue (bone and cartilage) damage was controlled. But also striking was the heterogeneity of the clinical response, with some individuals close to a cure and others virtually unimproved. The reasons for this are not yet clear, despite much work to try to elucidate the reasons. Genetic differences were an obvious possibility although never established, and recent clinical data demonstrating that non-responders may respond subsequently to anti-TNF has excluded it. Although anti-TNF in humans is relatively safe,<sup>10</sup> more infections in patients occur, eg with intracellular organisms, especially tuberculosis. Many large patient registers have documented the long-term benefits of anti-TNF therapy, reducing some complications and maintaining a favourable benefit/risk ratio.<sup>11</sup>

Currently, anti-TNF is used in RA, Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis, ankylosing spondylitis and juvenile RA; its use is now being explored in other indications (Table 1). Anti-TNF antibodies are the most successful and widely used antibody-based therapeutic. It is noteworthy that if used together with methotrexate early in the course of RA, over 50%<sup>12</sup> of patients can be taken off infliximab and remain virtually disease-free, even with reduced dosage

of methotrexate,<sup>12</sup> and some patients can be taken off all medication.<sup>13</sup>

In the wake of anti-TNF's success, other mAbs have also proven useful in inflammatory diseases. The antibody to the IL-6 receptor, tocilizumab (Actemra®),<sup>14</sup> pioneered by Professor Tadimitsu Kishimoto, has been approved for RA and for juvenile idiopathic arthritis. Currently, other anti-cytokine antibodies to IL-6, GM-CSF and the GM-CSF receptor have been approved (siltuximab (Sylvant®) – anti-IL-6) or are in late stage clinical trials after successful phase II studies.

Rituximab, a chimeric mAb to CD20,<sup>15</sup> (an antigen in most B-cells although not plasma cells), was first to treat B-cell-driven cancers such as non-Hodgkin lymphoma. It was pioneered by Jo Edwards for RA and subsequently approved, but was not successful in systemic lupus erythematosus (SLE) trials. Anti-CD52 (alemtuzumab) is a first generation humanised antibody, now used in multiple sclerosis. There are other antibodies approved, eg belimumab (also known as Benlysta) is an anti-BLys mAb approved for SLE,<sup>16</sup> ustekinumab (also known as Stelara) is an antibody to the shared p40 subunit of IL-12 and IL-23 approved for psoriasis and psoriatic arthritis and potentially for Crohn's disease,<sup>17</sup> and secukinumab (Cosentyx) is an anti-IL17A mAb approved for severe psoriasis and ankylosing spondylitis.<sup>18</sup>

Receptor IgG fusion proteins (‘ligand traps’) have also been generated. TNF receptor 2-Fc (etanercept, Enbrel®) is effective and has been approved.<sup>19</sup> Other ligand traps include riloncept (Arcalyst – an IL-1 Trap) and the anti-angiogenesis vascular endothelial growth factor trap (aflibercept (Eylea®) for retinopathies and ziv-aflibercept (Zaltrap®) for colorectal cancer) (Table 1). Abatacept (Orencia®) and belatacept (Nolojix®) are CTLA4-Fc fusion proteins that inhibit antigen presentation to T-cells and are effective in RA.

### Monoclonal antibodies in cancer therapy

mAb discovery: a case study of trastuzumab (Herceptin)

Modern mAb therapy of solid tumours was initiated by the humanised human epidermal growth factor receptor 2 (HER2)

**Table 1. Targets and approvals for monoclonal antibodies and derivatives (asterisks denote approvals in USA only)**

Target	Function	FDA-approved mAbs, Traps, Bi-specific mAbsB	Autoimmune	Malignancy	Cardiovascular	Other/comments
CD52	Marker of T- and B-lymphocyte subsets	Alemtuzumab (Lemtrada)	Multiple sclerosis	Chronic lymphocytic leukemia		Trade name changed from Campath-1H (cancer) to Lemtrada (multiple sclerosis) Humanised IgG1k
CD41 (glycoprotein IIb/IIIa)	Platelet aggregation inhibitor	Abciximab (ReoPro)			Perisurgical anti-thrombosis	Mouse/human IgG1
CD25	Alpha-chain of the IL-2 receptor	Basiliximab (Simulect)	Multiple sclerosis			Fab fragment
CD19/CD3	Bi-specific T-cell directed killing of CD19	Daclizumab (Zenapax) Blinatumomab (Blincyto)	Transplant rejection	B-cell precursor acute lymphoblastic leukemia		Basiliximab: chimeric mouse/human IgG1k. Daclizumab: humanised IgG1k.
CD20	Participates in B-cell differentiation	Obinutuzumab (Gazyva) Ibritumomab tiuxetan (Zevalin) Tositumumab (Bexxar)* Ofatumumab (Arzerra) Rituximab (Rituxan)	Rheumatoid arthritis	B-cell malignancies		Obinutuzumab is the first approved glycoengineered IgG1 mAb with enhanced ADCC. Rituximab is chimeric mouse/human IgG1k Ibritumomab tiuxetan and tositumumab are radio-conjugates that can be used when tumours stop responding to the anti-CD20 mAbs. Ibritumumab and tositumumab are mouse IgG2a.
CD30	Member of the TNF receptor family	Brentuximab vedotin (Adcetris)		Hodgkin lymphoma, anaplastic large cell lymphoma		Second approved antibody-drug conjugate
CD38	Cyclic ADP ribose synthetase leads to generation of adenosine in the tumour microenvironment (immunosuppression); co-stimulatory receptor; more?	Daratumumab (Darzalex)		Multiple myeloma		

**Table 1. (Continued)**

Target	Function	FDA-approved mAbs, Traps, Bi-specific mAbsB	Autoimmune	Malignancy	Cardiovascular	Other/comments
CD80/CD86	Provide co-stimulatory signals necessary for T-cell activation and survival. Ligand trap prevents activation of CD28 immune checkpoint resulting in immune suppression	Noljix Orencia (both CTLA4-Fc fusion proteins)	Rheumatoid arthritis Transplant rejection			
CD279/PD-1	Immune 'checkpoint' receptor that suppresses immune response after binding to PDL-1 or 2	Nivolumab (Opdivo) Pembrolizumab (Keytruda)		Melanoma, non-small cell lung cancer, renal cell carcinoma, Hodgkin lymphoma, head and neck squamous carcinoma		These mAbs designed not to be immune active (eg no ADCC): IgG4. This allows the mAbs to increase activity of T-cells without inducing apoptosis PDL-1 is a biomarker for pembrolizumab
CD274/PDL-1	Tumour-expressed protein that activates the PD-1 immune suppression pathway	Atezolizumab (Tecentria) Avelumab (Bavencio)*		Urothelial (bladder) carcinoma, non-small cell lung cancer merkel cell carcinoma		Atezolizumab: IgG1k mAb engineered to lack ADCC activity PDL-1 is biomarker (Avelumab: intact IgG1k)* *Active IgG1 targets tumour cells. Approval likely
CTLA4/CD152	When activated through binding CD80/86 this receptor turns down cytotoxic T-cells	Ipilimumab (Yervoy)		Metastatic melanoma, mesothelioma		This is the first of the immuno-oncology mAbs to be approved (IgG1k). Largely due to efforts of J Allison (ref PMID 16730267)
Glycolipid GD2	Disialoganglioside preferentially expressed on tumours of neuroectodermal origin	Dinutuximab (Unituxin)		Neuroblastoma		Mouse/human chimeric IgG1k. Induces severe pain managed by morphine
HER2	A growth factor-like receptor tyrosine kinase without a known ligand	Trastuzumab (Herceptin) Pertuzumab (Perjeta) Ado-trastuzumab emtansine (Kadcyla)		Breast, gastric adenocarcinoma		Two monoclonal antibodies (T,P) and one ADC (ado-trastuzumab) Overexpression of HER2 leads to spontaneous signaling Crosslinking with mAb leads to internalization First commercially successful ADC (Lewis et al, PMID 19010901) Biomarker driven therapy

Table 1. (Continued)

Target	Function	FDA-approved mAbs, Traps, Bi-specific mAbsB	Autoimmune	Malignancy	Cardiovascular	Other/comments
EGFR (receptor for multiple growth factors)	Many tumours are addicted to the EGFR signalling pathway	Cetuximab (Erbix) Panitumumab (Vectibix)		Squamous cell carcinoma of the head and neck, colorectal cancer, non-small cell lung cancer		Biomarker: usually requires EGFR IHC positivity; always requires test for normal ras oncogene Cetuximab is mouse/human chimeric IgG1; Vectibix is human IgG2 from recombinant mice
TNF-alpha	Inflammatory cytokine that drives multiple autoimmune diseases*	Adalimumab (Humira) Certalizumab pegol (Cimzia) Golimumab (Simponi) Infliximab (Remicade) Etanercept (Enbrel) There are more than 20 anti-TNF biosimilars in various stages of development. Already approved are infliximab biosimilars (Remsina, Inflectra, Flexiabi), etanercept biosimilars (Erezi, Benepali), adalimumab biosimilars (Amjevita). This field will change very rapidly as many dossiers are now under regulatory scrutiny. Biosimilars are given a suffix, eg etanercept-szsz (Erezi)	Crohn's disease, ulcerative colitis, RA, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis, hidradentitis suppurativa, uveitis			Not all TNF blockers are approved for all indications Drugs in italics are biosimilars: adalimumab, infliximab, infliximab, inflectra, adalimumab-atto are IgG1k mAbs; certalizumab pegol is a pegylated Fab fragment; etanercept is a soluble Fc: TNF receptor 'trap' that binds TNF (there is one biosimilar) At least four biosimilar TNF-blockers have been approved in the EU (two each for infliximab and etanercept)
VEGF	Cytokine that stimulates vasculogenesis and angiogenesis. Overproduced in some inflammatory disorders and tumours to induce increased blood supply.	Bevacizumab (Avastin) Ramucirumab (Cyramza) Aflibercept (Eylea/Zaltrap) Ramizumab (Lucentis)	Age-related macular degeneration, macular oedema, diabetic macular oedema, diabetic retinopathy	Colorectal cancer, Non-squamous NSCLC, breast cancer, glioblastoma, renal cell carcinoma, gastric cancer or gastro-esophageal junction adenocarcinoma		Bevacizumab, ramucirumab are IgG1 mAbs for cancer therapy (ramucirumab was derived from phage display); ranizumab is a Fab fragment (single arm binder). It has a short half-life if administered intravenously, but it is stable and effective when locally injected into the eye Aflibercept is a ligand trap with optical (Eylea) and cancer (Zaltrap) applications

**Table 1. (Continued)**

Target	Function	FDA-approved mAbs, Traps, Bi-specific mAbsB	Autoimmune	Malignancy	Cardiovascular	Other/comments
IgE	Binds to mast cells, basophils, other cells that express Fc-epsilon receptor, and induces release of inflammatory cytokines	Omalizumab (Xolair)	Asthma			IgG1k mAb also used off-label to treat IgE-related conditions (allergic rhinitis, drug allergies, other)
Alpha-4 integrin	Alpha-4 integrin facilitates exit of inflammatory cells from blood into intestine or across the blood brain barrier	Vedolizumab (Entyvio) Natalizumab (Tysabri)	Multiple sclerosis, Crohn's disease and ulcerative colitis			IgG4 natalizumab therapy has been associated with PML caused by John Cunningham virus in immunocompromised patients. IgG1k vedolizumab MAY not be associated with PML
Complement C5	Inhibits complement cascade	Eculizumab (Solaris)			Prevents destruction of red blood cells by activated complement (paroxysmal nocturnal hemoglobinuria)	IgG2/4 mAb Most expensive drug in the world (\$409,500 annually)
Interleukin-1	Mutations in cryopyrin lead to overproduction of IL-1 and inflammatory disease; IL-1 also drives other inflammatory diseases	Canakinumab (Ilaris) Rilonacept (Arcalyst)*	Rare inflammatory syndromes, active juvenile arthritis, gouty arthritis			Canakinumab is an IgG1k mAb; rilonacept is an IL-1 'trap' designed from the IL-1R fused with human mAb Fc region.
P40 subunit of IL-12 and IL-23	Prevents cytokine (inflammatory) activation of cellular receptors	Ustekinumab (Stelara)	Plaque and psoriatic arthritis, Crohn's disease			IgG1k human mAb from recombinant mice
Receptor activator of NF kappa-B ligand (RANKL)	Activates osteoclastic bone resorption	Denosumab (Xgeva)		Can be used to prevent bone mass loss in cancer patients		Human IgG2 mAb inhibits bone resorption and enhances bone mass (due to multiple causes)
Interleukin-6	Overexpression of IL-6 is associated with multiple malignancies	Siltuximab (Sylvant)		Pseudo-malignancy: Castleman's disease (similar to lymphoma)		Murine/human chimeric IgG1k

**Table 1. (Continued)**

Target	Function	FDA-approved mAbs, Traps, Bi-specific mAbsB	Autoimmune	Malignancy	Cardiovascular	Other/comments
IL-6R (Interleukin 6 receptor)	Current approvals based upon role of IL-6 in promoting inflammatory autoimmune disease	Tocilizumab (Actemra)	Rheumatoid arthritis, polyarticular juvenile arthritis, juvenile idiopathic arthritis			Human/mouse chimeric mAb with initial approval for efficacy vs RA after failure of TNF blocker
BAFF (tumour necrosis factor superfamily member 13b)	Role in proliferation and differentiation of B-cells	Belimumab (Benlysta)	Systemic lupus erythematosus			IgG1-gamma/lambda
Bacillus anthracis (anthrax) toxin	Recognises the protective antigen portion of the toxin produced by Bacillus anthracis and prevents the toxin from entering cells and killing them	Obiltoximab (Anthem)*				Chimeric human/mouse chimeric mAb to anthrax toxin for prevention and treatment
PCSK9	PCSK9 binds to LDLR, causing it to be degraded. With the result of higher blood levels of LDL mAb vs PCSK9 prevents degradation of LDLR and thereby increases LDL removal from blood. Therefore, blocking PCSK9 can lower blood cholesterol levels	Evolocumab (Repatha) Alirocumab (Praluent)			Second line treatment for high cholesterol for adults whose cholesterol is not controlled by diet and statin treatment. Perhaps most important for hereditary high cholesterol	Evolocumab: IgG2 for low ADCC Alirocumab is IgG1k
SLAMF7/CD319	SLAMF7 triggers the activation and differentiation of a wide variety of immune cells (innate and adaptive immune response) perhaps primarily mediated by natural killer cells and myeloma cells	Elotuzumab (Empliciti)		Multiple myeloma		This IgG1k mAb is thought to activate SLAMF7 receptor and to have a secondary mechanism of mediating ADCC vs multiple myeloma cells
Dabigatran	Anti-coagulant	Idarucizumab (Praxbind)				This Fab fragment sequesters dabigatran and clears it from blood, reversing anti-coagulation

**Table 1. (Continued)**

Target	Function	FDA-approved mAbs, Traps, Bi-specific mAbsB	Autoimmune	Malignancy	Cardiovascular	Other/comments
IL-5	Induces differentiation and survival of eosinophils	Reslizumab (Cinqair) Mepolizumab (Nucala)	Asthma			Both IgG1k mAbs
IL-17A	Inflammatory cytokine	Ixekizumab (Taltz) Secukinumab (Cosentyx)	Plaque psoriasis, ankylosing spondylitis			Ixekizumab is an IgG4; secukinumab is an IgG1k
Clostridium	Reduce recurrence of Clostridium difficile recurrence	Bezlotoxumab (Zinplava) Actoxumab				Meant to be used for the prevention of recurring clostridial disease. Actoxumab is only approved in the UK.
PDGF	Inhibit association of the PDGF with its receptor	Olaratumab (Lartruvo)		Soft tissue sarcoma		Human IgG1k that prevents interaction of PDGF with its receptor(s)

An actively updated summary of MAb approvals can be found at [www.antibodysociety.org](http://www.antibodysociety.org).

MMAbs can be murine, chimeric (human Fc region), humanised or human; Traps are derived from receptors and compete with natural receptor for binding target; Bbi-specific mAbs are engineered to bind to two different targets simultaneously (usually to bring immune cell into contact with target cell, thereby triggering target cell killing). Antibody drug conjugate (X): toxin or radioisotope attached to mAb to increase efficacy. Asterisks denote approvals in USA only; others are approved in both USA and UK.

ADC = antibody-drug conjugate; ADCC = antibody-dependent cell-mediated cytotoxicity; ADP = adenosine diphosphate; BAFF = B-cell activating factor; CTLA-4 = cytotoxic T-cell lymphocyte associated protein-4; EGFR = epidermal growth factor receptor; HER2 = human EGFR-2; IHC = immunohistochemistry; Ig = immunoglobulin; IL = interleukin; LDL = low density lipoprotein; LDR = low density lipoprotein receptor; NSCLC = non-small cell lung cancer; PCSK9 = proprotein convertase subtilisin/kexin type 9; PDGF = platelet-derived growth factor; PD-1 = programmed cell death protein 1; PDL-1 = programmed cell death ligand 1; PML = progressive multifocal leukoencephalopathy; RA = rheumatoid arthritis; SLAMF7 = signalling lymphocyte activation molecule; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor



mAb trastuzumab (Herceptin®).<sup>20</sup> The rigorous science that laid the foundation for this breakthrough mAb also initiated personalised/biomarker driven drug discovery and treatment in oncology.

Trastuzumab, the first successful monoclonal anti-cancer antibody to be successful against solid tumours, is well tolerated in patients. The pathway leading to TNF-resistance of most tumour cell lines<sup>21</sup> was unraveled by collaboration between the Shepard (Genentech) and Schreiber laboratories (Chicago), which revealed that macrophages kill tumour cells largely by secreting TNF.<sup>22</sup> They hypothesised that if tumour resistance to macrophages could be reversed, the tumour would become sensitive to killing by host defence. Macrophage (or TNF)-resistant tumour cells implanted into syngeneic mice formed aggressive tumours, while their TNF-sensitive parental cells regressed.<sup>21</sup>

The mechanism of TNF-resistance of tumours needed to be widespread since most tumour cell lines were resistant. Sporn and Todaro's autocrine growth factor hypothesis<sup>23</sup> of malignant transformation involving autocrine stimulation by transforming growth factors seemed plausible. Various growth factors were combined with TNF on TNF-sensitive tumour cell lines and growth factors that activated receptor tyrosine kinases converted TNF-sensitive tumour cells to TNF-resistant cells.<sup>24</sup> Host defence was completely subverted and the growth inhibitor (TNF) even became a growth factor.<sup>25</sup>

Slamon *et al*<sup>26,27</sup> and Zhou *et al*<sup>28</sup> reported that overexpression of the receptor tyrosine kinase HER2 in breast and ovarian cancers was associated with more aggressive disease contemporaneously with Shepard's demonstration that overexpression of HER2 induced resistance to TNF<sup>29</sup> and that mAbs to HER2 inhibited growth of HER2-overexpressing tumours *in vitro* and *in vivo*.<sup>30–32</sup> The lead antibody, MuMab4D5 – the murine parent of trastuzumab,<sup>24</sup> was ineffective on normal cells or tumour cells not overexpressing HER2.<sup>26,33</sup> Anti-HER2 also validated the importance of stability of the target itself as HER2 is indispensable in driving disease in subsets of breast, gastric and ovarian cancers. These findings laid the foundation for the success of trastuzumab and subsequently for pertuzumab,<sup>34</sup> which binds a separate epitope on HER2, and then trastuzumab emtansine, an antibody drug conjugate.<sup>35</sup> The clinical success of trastuzumab required the co-development of two novel companion diagnostic approaches: immunohistochemistry and fluorescence *in situ* hybridisation for selecting patients most likely to respond.<sup>36</sup> This was a new idea at the time, but is now a routine part of most cancer drug discovery and therapeutic programmes. Finally, this mAb showed that antibody therapy was feasible in solid tumours, overcoming problems such as high internal tumour pressure, which causes vascular collapse and reverse flow of fluid in solid tumours.<sup>37</sup>

MuMab4D5 was humanised using a consensus human variable domain sequence from the Kabat database, together with human immunoglobulin constant domain (IgG1, kappa) sequences.<sup>38</sup> This resulted in the synthesis of a recombinant antibody, non-existent in nature, with almost 95% human sequence, reducing concerns related to immunogenicity. The humanised antibody has three mechanisms of action dependent upon upregulated HER2:

- 1 growth inhibition
- 2 antibody-dependent cell-mediated cytotoxicity (ADCC)
- 3 induction of TNF sensitivity.<sup>20,30</sup>

The strategy employed in the development of trastuzumab is now widely employed in cancer drug discovery (Fig 2). Very recently in clinical trials, a combination of trastuzumab and another anti-HER2 mAb (pertuzumab<sup>30</sup>) generated a complete pathological response in approximately 50% of HER2-positive patients,<sup>39</sup> which is a previously unheard of therapeutic advance in the treatment of breast cancer. The HER2 programme proved that mAbs have a place in therapy of solid tumours and that tyrosine kinase oncogenes were viable targets in cancer.

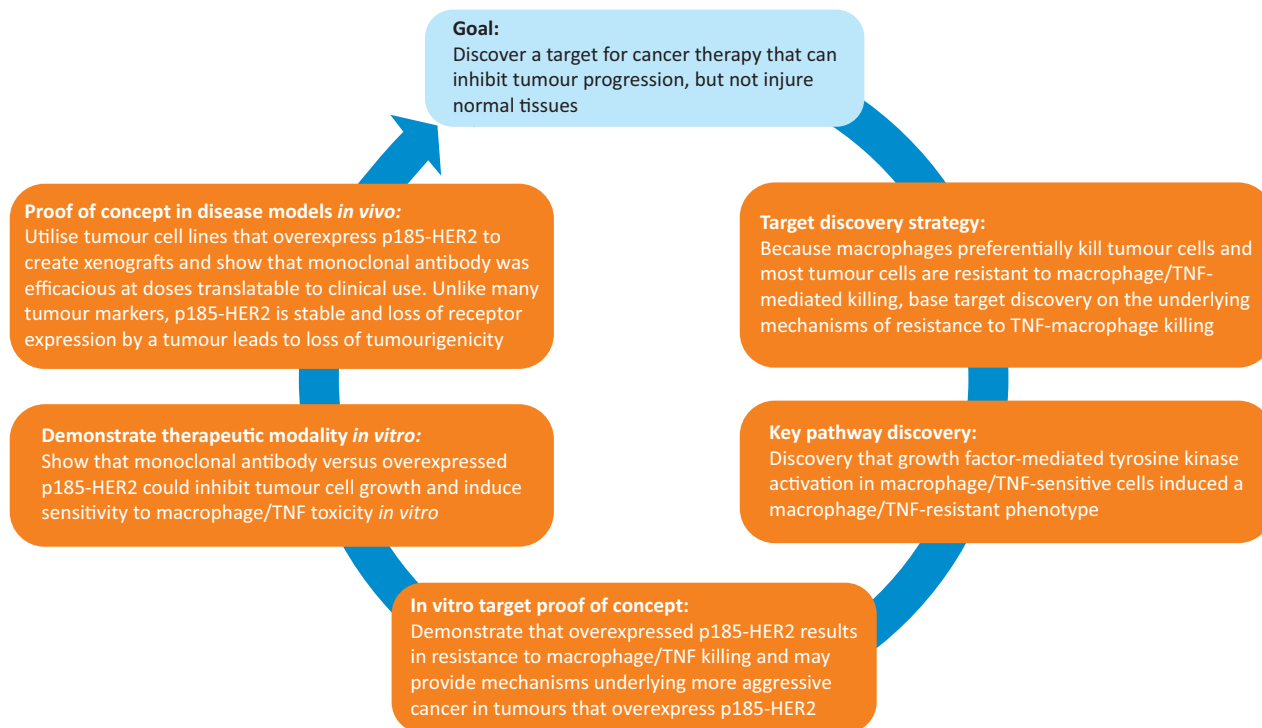
Not all successful mAb therapeutics followed this pathway of discovery. The first approved mAbs targeted membrane proteins shared between haematologic malignancies and their precursor immune cell subsets (eg alemtuzumab (Campath®) – an anti-CD52, rituximab (Rituxan®) – an anti-CD20). These mAbs ablate both normal and cancer cells. Alemtuzumab was the first humanised mAb therapeutic, depleting lymphocytes, monocytes and dendritic cells, useful in diseases such as chronic lymphocytic leukemia. Similarly, rituximab depletes CD20-expressing B-cells. Resistance to these mAb therapeutics occurs with the loss of the target as neither CD52 nor CD20 is essential for malignant growth. These mAbs have toxicity issues due to massive depletion of immune cells, potentially causing 'cytokine release syndrome' or 'tumour lysis syndrome' – a 'cytokine storm' resulting from aberrant immune activation attributable to cellular debris activating 'danger receptors'. Despite these issues, both rituximab and alemtuzumab are successful drugs and often lead to disease regression.

More recently, Jim Allison and other cancer researchers discovered the utility of mAbs targeting 'immune checkpoints'. By inhibiting these pathways (eg programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) or cytotoxic T-cell lymphocyte associated protein-4), tumour-associated immune suppression can be reversed with clinically effective immune cell attack on tumours in about 20% cases. Some patients have had dramatic improvements, notably in metastatic melanoma, and there is no doubt that this is a major step forward. Not unexpectedly there are immune-mediated diseases in a large fraction (about 30%) of treated patients, but this field of mAb therapy is growing very rapidly. CTLA-4 expressed on regulatory T-cells exerts immune suppressive activity. Anti-CTLA-4 prevents CTLA-4 from inhibiting immunity.<sup>40</sup> PD-1 is present on 'exhausted' T-cells; anti-PD-1 reactivates them. The first approvals of this new class of anti-cancer mAbs were in melanoma with ipilimumab (anti-CTLA-4, Yervoy®) and pembrolizumab (anti-PD1, Keytruda®) in 2015, followed by atezolizumab (anti-PD-L1, Tecentriq®) for non-small cell lung cancer and bladder cancer. Checkpoint inhibitor mAbs, such as anti-OX40, can also be agonistic.<sup>40</sup>

## Other antibody-related approaches and mechanisms

Therapeutic antibodies: structural marvels that mediate multiple functional effects

Antibodies have evolved over the past 400 million years to provide broad ranging host defence in all vertebrates. Thus, it is not surprising that engineered antibodies have emerged as one of the main sources of new medicines. Antibodies evolved to possess several important functional properties exploited by recombinant mAb therapeutics, such as exquisite specificity



**Fig 2. Strategy and key steps for development of trastuzumab, from target discovery to *in vitro* and *in vivo* proof of concept studies.** HER2 = human epidermal growth factor receptor 2; TNF = tumour necrosis factor

and affinity toward their antigen targets, recruiting immune system effector components and a long serum half-life.

Antibodies possess a unique tertiary domain structure, folding into a complex quaternary structure. Human serum immunoglobulin is approximately 80% IgG, as are most approved therapeutic antibodies. These complex proteins (approximately 150 kDa) comprise four polypeptide chains – 2 heavy and 2 light chains – with disulphide bonds providing significant rigidity. The heavy chain consists of a variable domain (VH) and three constant domains (CH1, CH2 and CH3). The light chain consists of a variable domain (VL) and a constant domain (CL) (Fig 1A). IgG antibodies are bivalent and have post-translational modifications. Glycosylation in the Fc domain also helps to provide stability and modulates properties such as binding to Fc receptors.<sup>41</sup>

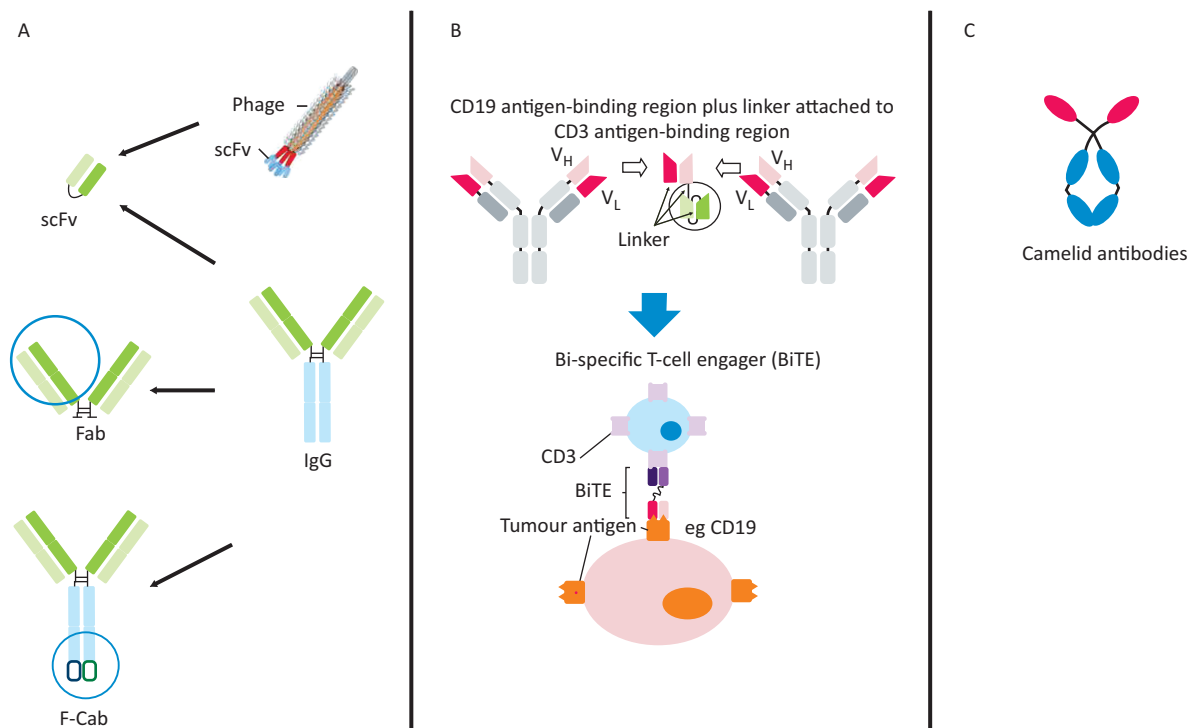
#### *The antibody/antigen interaction*

Several technologies are used to identify novel antibodies: hybridomas, genetically modified mice with human immunoglobulin sequences and phage or yeast display. B-cells from a mouse injected with an antigen are fused with immortalised B-cell myeloma cells to make hybridomas. To improve their efficacy in patients, protein engineers created chimeric antibodies containing human constant domains and murine variable domains, which reduce immunogenicity and activate effector functions (Fig 1). Approved chimeric antibodies include Remicade® (infliximab), Erbitux® (cetuximab) and Rituxan® (rituximab). As an example of a ‘humanised’ antibody Herceptin® (trastuzumab) was isolated from a murine hybridoma

and then ‘humanised’ where all but the binding site to the HER2 antigen was changed to a human sequence (Fig 1). Genetically engineered mice have been developed that can make fully human antibodies, eg Vectibix® (panitumumab) – anti-epidermal growth factor receptor. Another important approach employs synthetic (human) antibody libraries that are displayed on the surface of yeast or phage, which is useful for less immunogenic targets. There are advantages and limitations to each of these methods; therefore, antibody discovery groups will attempt multiple approaches in parallel against a particular target.

#### *Antibody effector function*

Immunoglobulins can stimulate immune defence mechanisms through ADCC, antibody-dependent cellular phagocytosis and complement-dependent cytotoxicity. During ADCC, immune cells – usually natural killer (NK) cells – lyse a target cell with an IgG bound to a cell surface target. CD16 Fc receptors expressed on NK cells bind to the Fc region of the IgG, forming a lytic synapse between the NK cell and the target cell, destroying the target cell. During complement-dependent cytotoxicity, complement is recruited to the IgG-bound surface pathogen through C1q binding to the antibody Fc domain, triggering proteolytic events, yielding a membrane attack complex that disrupts cellular membranes with cell death. Many therapeutic antibodies use ADCC as a key mechanism of action, eg trastuzumab and rituximab. Methods have been developed for increasing or decreasing antibody effector function, the number of epitope binding sites or attaching cytotoxic drugs. Some of these strategies are shown in Fig 1.



**Fig 3. Emerging antibody technologies.** A – derivatives of classical mAbs: scFv, Fab, and F-Cab antibody formats; B – engineered bi-specific mAbs: structure and mechanism of a bi-specific T-cell engaging (BiTE) antibody; C – novel mAb frameworks: unique structure of camelid antibodies. mAb = monoclonal antibody

#### Antibody pharmacokinetics

Antibodies have a long serum half-life, typically lasting 2–4 weeks in circulation. Antibody binding the Fc domain of the neonatal Fc receptor, FcRn, recycles the antibody, protecting it from other clearance mechanisms. This long half-life with less frequent dosing is often more favourable for patients when extended drug function is needed. Antibody dosing is typically intravenous or subcutaneous. Oral administration is not feasible because of rapid degradation of the antibody in the gut. In addition, therapeutic antibodies cannot pass in appreciable quantities through the intact blood-brain barrier.

#### Antibody-drug conjugates: using antibodies for tumour-selective delivery of cytotoxics

The therapeutic use of antibody-drug conjugates (ADCs) is based on antibody-mediated tumour-selective delivery of potent cytotoxic compounds. This relies on antibody-induced receptor internalisation, followed by trafficking of the ADC to the lysosomes where the cytotoxic drug is released from the ADC. The free drug (inside the target cell) initiates its anti-tumour activity. Stability of the linker that joins the antibody and cytotoxic agent is key to both efficacy and safety. The linker must be stable in the circulation to prevent premature release of the cytotoxic agent, yet allow release of the cytotoxic drug within the tumour cell. The most common linkers are cleavable linkers such as peptides, cleaved by proteases, or disulphide linkers, which undergo reduction to release the drug within the lysosome. ADCs can also utilise a stable or non-cleavable linker, the free drug is released in the lysosome after lysosomal degradation of the entire ADC molecule.

Despite decades of intense research and development, only three ADCs have gained marketing approval. The first, gemtuzumab ozogamicin (Mylotarg<sup>®</sup>), was approved then taken off the market. Significant advances were subsequently made in ADC linker design and conjugation technologies, yielding two additional approved ADCs, one for CD30-positive Hodgkin lymphoma and anaplastic large cell lymphoma (brentuximab vedotin (Adcentris<sup>®</sup>), and one for metastatic breast tumours overexpressing HER2/*neu* trastuzumab emtansine (Kadcyla<sup>®</sup>). The anti-CD30 antibody component of brentuximab vedotin is linked to the tubulin polymerisation inhibitor mono-methyl auristatin E through a protease cleavable linker, valine-citrulline. Trastuzumab emtansine is comprised of trastuzumab covalently linked to the maytansinoid derivative, DM1, through a stable, non-cleavable linker. Current research suggests that more efficacious and better tolerated ADCs will be developed, which will provide greater clinical benefit to cancer patients.

#### Emerging antibody technologies

The Fab fragment (Fig 3A) used in ranibizumab (Lucentis)<sup>42</sup> can be manufactured in *Escherichia coli*. The short half-life of Fabs can be remedied by polyethylene glycol used in certilizumab pegol, Cimzia<sup>®</sup>.<sup>43</sup>

Until recently, it has been difficult to synthesise bi-specific mAbs. Now various types are possible. Bi-specific T-cell engaging (BiTE) antibodies consist only of the variable regions from distinct antibodies (Fig 3B) and are the format of the first approved bi-specific antibody, catumaxomab (Removab<sup>®</sup>), for malignant ascites.

## Antibody-based cellular immunotherapies

A new class of antibody-based therapeutics called ‘chimeric antigen receptor T-cells’ are emerging in cancer. An antibody-like antigen binding region is fused with signalling components of the T-cell receptor and the chimeric gene is inserted into the patient's own T-cells. When the T-cells recognise their target on tumour cells, the T-cell cytotoxic response is activated, lysing the cancer cells.<sup>43</sup> Using CTL019, a chimeric antigen receptor T-cell engineered to target cancer cells that express the CD19 protein present on acute lymphoblastic leukemia cells, 19 of 22 paediatric patients with lethal acute lymphoblastic leukaemia experienced remission.<sup>44</sup> However, this treatment upregulates inflammatory cytokines, with consequent toxicity.

## Conclusions

mAb therapeutics have been approved for 33 targets in more than 37 distinct diseases (Table 1), most commonly for cancer (27 approvals). The clinical value of both mAbs and ligand traps has been proven. The most targeted pathway is the TNF pathway, now employed to treat nine different indications and likely to expand as TNF is found to be a driver of additional diseases and conditions, including postoperative cognitive decline<sup>45</sup> and fibrosis.<sup>46</sup> New applications of mAbs are being tested, with approvals in bone metabolism<sup>47</sup> and hypercholesterolemia.<sup>48</sup>

Antibodies have become the new backbone of the pharmaceutical industry, which previously relied on huge libraries of small molecules. Compared with small molecules, mAbs have an exquisite target selectivity and, therefore, less toxicity attributable to binding to other targets. mAbs have now been designed to target two or more targets simultaneously, augmenting the therapeutic potential. We are only just at the beginning of the mAb therapeutic era. ■

## Conflicts of interest

GLP is an employee of Genentech and owns Roche stock. CT and HMS are employees of Halozyme and own Halozyme stock. MF is one of the inventors of anti-TNF therapy and receives royalties on combination therapy.

## Author contributions

All authors wrote part of the review, read all of it and approved the final version.

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