Through the barricades: Overcoming the barriers to effective antibody-based cancer therapeutics

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Abstract

Since the turn of the century, cancer therapy has undergone a transformation in terms of new treatment modalities and renewed optimism in achieving long-lived tumour control and even cure. This is, in large part, thanks to the widespread incorporation of monoclonal antibodies (mAbs) into standard treatment regimens. These new therapies have, across many settings, significantly contributed to improved clinical responses, patient quality of life and survival. Moreover, the flexibility of the antibody platform has led to the development of a wide range of innovative and combinatorial therapies that continue to augment the clinician's armoury. Despite these successes, there is a growing awareness that in many cases mAb therapy remains suboptimal, primarily due to inherent limitations imposed by the immune system's own homeostatic controls and the immunosuppressive tumour microenvironment. Here, we discuss the principal barriers that act to constrain the tumour-killing activity of antibody-based therapeutics, particularly those involving antibody glycans, using illustrative examples from both pre-clinical and market approved mAbs. We also discuss strategies that have been, or are in development to overcome these obstacles. Finally, we outline how the growing understanding of the biological terrain in which mAbs function is shaping innovation and regulation in cancer therapeutics.

# Introduction

In recent decades, monoclonal antibodies (mAbs) have emerged as a major new class of cancer therapeutics ([Ayyar et al. 2016](#_ENREF_8); [Elvin et al. 2013](#_ENREF_49); [Farkona et al. 2016](#_ENREF_54); [Glassman and Balthasar 2014](#_ENREF_71); [Reichert and Dhimolea 2012](#_ENREF_141); [Weiner 2015](#_ENREF_180)). So widespread is the success of mAbs that most oncologists now view them as a vital component of contemporary cancer care ([Weiner 2015](#_ENREF_180)). Moreover, as intensive research continues to illuminate key aspects of their mechanism of action, we are now witnessing the appearance of the next generation of therapeutic mAbs, with high hopes for even greater clinical efficacy. Key advances are likely to arise through an ever-deepening understanding of the biological factors limiting current antibody-based therapies.

The principle advantages of mAbs is their high selectivity and fidelity in target binding, coupled to their stability, long serum half-lives and low immunogenicity ([Glassman and Balthasar 2014](#_ENREF_71); [Weiner 2015](#_ENREF_180)). These properties together with the fact that they are highly amenable to engineering and customisation, provides mAbs with significant advantages as biotherapeutics which is further boosted by their inherent ability to elicit cytotoxic effects. mAbs can elicit anti-cancer effects by either direct or immune-mediated mechanisms. Direct effects include growth arrest (e.g. blocking of growth receptor dimerization or ligand binding), anti-angiogenesis (by targeting growth factors such as vascular endothelial growth factor; VEGF, responsible for neovascularisation), or induction of various forms of programmed cell death through direct binding to receptors at the apex of these pathways ([Glassman and Balthasar 2014](#_ENREF_71); [Weiner 2015](#_ENREF_180)). More recently, some mAbs have even been suggested, under specific circumstances, to participate in non-receptor mediated immunogenic cell death (ICD) ([Garg et al. 2017](#_ENREF_67)).

Classically, antibody-mediated mechanisms can be transmitted either directly through the IgG Fc region or as a result of their Fab binding specificities. Immune-effects mediated by the Fc domain include antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) ([Nimmerjahn and Ravetch 2010](#_ENREF_125)). Fab directed binding can be used to directly activate apoptotic receptors ([Naoum et al. 2017](#_ENREF_120)) or agonise co-stimulatory molecules such as CD40 ([Wilson et al. 2017](#_ENREF_182)). Moreover, indirect immune stimulation can result from targeting of immune checkpoint receptors such as PD1 and CTLA-4 on key immune cells ([Farkona et al. 2016](#_ENREF_54)). Alternatively, recruitment and activation of these cellular effectors can be delivered by multi-specifics (mAbs and mAb-like molecules that can simultaneously bind two or more epitopes [intra or inter antigen], one per Fab arm) ([Kontermann and Brinkmann 2015](#_ENREF_99)). Finally, a third potential mode of operation is through the delivery of toxic payloads (chemical or radioactive) directly to tumour cells ([McCombs and Owen 2015](#_ENREF_115)).

Numerous features of the therapeutic target and fundamental principles of the human immune system can curtail the clinical efficacy of many mAbs, particularly with respect to the effectiveness of their effector functions. By understanding these limitations, therapeutic strategies can be developed to surmount these barriers. Here, we review some of the key principles that limit antibody therapy against cancer and the strategies that are being developed to overcome them, particularly those most relevant to the field of Glycobiology.

# **Beyond affinity**

Therapeutic mAbs have classically utilised the immunoglobulin G (IgG) format, including the majority of currently approved cancer mAbs ([Kaplon and Reichert 2018](#_ENREF_94); [Reichert 2017](#_ENREF_140)), and we largely restrict our discussions here to the biology and therapeutic use of this antibody class and its engineered derivatives. To understand the nature of the endogenous barriers that limit the efficacy of IgG-based therapeutic mAbs we must first consider their principle mechanisms of action (MOA) when applied as anti-cancer agents.

Antibodies predominantly derive their cytotoxic activity via the recruitment of immune cells and proteins to tissues expressing the target antigen([Weiner 2015](#_ENREF_180)). The immune cells recruited by mAbs typically include: natural killer (NK) cells which elicit perforin and granzyme mediated target destruction; neutrophils and monocytes/macrophages which predominantly eliminate cells via phagocytosis; and dendritic cells (DCs) which take up protein antigens and present derived peptides to promote adaptive immune responses, typically resulting in the generation of tumour specific cytotoxic T lymphocytes (CTLs). These diverse effector functions operate through a common mechanism whereby the Fc domain of an antibody binds to an Fc gamma receptor (FcγR) on the surface of the immune cell.

With the advent of technologies such as mice expressing human antibody repertoires, refined hybridoma technologies, high throughput paired H and L chain sequencing from human B-cells, and increasingly diverse recombinant antibody fragment libraries for selection, high affinity, low-immunogenicity mAbs can be developed to specific antigens. Although some caveats remain, these advances mean that contemporary considerations have to a great extent shifted to other aspects of mAb therapy, such as the clinical (and patient) pertinence of antigen targets, combination therapy, immune-stimulation, multi-specific modalities and improving IgG effector function in the face of known immunological and molecular barriers (Figure 1).

# **Keeping it human**

Whilst a great deal of progress has been made in minimising mAb immunogenicity, primarily through developing human antibody-expressing mice, recombinant humanisation of murine mAb and fully human cloning strategies, control of post-translational modification such as glycosylation is also an important consideration ([Ghaderi et al. 2012](#_ENREF_70); [Reusch and Tejada 2015](#_ENREF_142)). Pathogen carbohydrates are potent immunogens and a common focus for endogenous and vaccine based protective humoral immune responses ([Cuccui and Wren 2015](#_ENREF_33); [Dalziel et al. 2014](#_ENREF_37)). Given that the majority of mAbs are bulk-produced in non-human cell lines, it is desirable to avoid, or at least minimise the impact of non-human glycans such as Gal1,3Gal, Neu5Gc, 1,2 Xylose, core 1,3 fucose or high-mannose glycans, such as those found in yeast, that contain residues and linkages not present in the human oligomannose-type precursor glycans ([Dalziel et al. 2014](#_ENREF_37)). Expression of these glycans can result in rapid clearance or the generation of neutralising antibodies, inevitably detrimental to mAb efficacy. In more extreme cases, these structures can even lead to undesirable safety issues (see below) ([Chung et al. 2008](#_ENREF_28); [Pointreau et al. 2012](#_ENREF_133)). Importantly, even antibodies bearing human oligomannose-type glycans can be subject to significant interaction with the innate immune system, leading to rapid clearance ([Goetze et al. 2011](#_ENREF_73); [Gorovits and Krinos-Fiorotti 2013](#_ENREF_75)).

Perhaps the best-known example of an immunogenic glycan epitope is the Gal1,3Gal motif on the anti-epidermal growth factor (EGFR) mAb cetuximab that, in some patients, resulted in anaphylaxis so severe it precluded subsequent use of the mAb and even proved fatal in a few cases ([Chung et al. 2008](#_ENREF_28); [Pointreau et al. 2012](#_ENREF_133)). Almost all of these patients had pre-existing anti-Gal1,3Gal IgE antibodies linked to prior exposure of the Gal1,3Gal epitope through lone-star tick bites (at least in the USA) which consequently recognised the Gal1,3Gal containing Fab region of cetuximab (average 30% terminal Gal1,3Gal/antibody) ([Berg et al. 2014](#_ENREF_15); [Weiss et al. 2016](#_ENREF_181)). Work is now in progress aimed at predicting severe cetuximab-induced hypersensitivity reactions prior to cetuximab exposure ([Iwamoto et al. 2016](#_ENREF_84); [Mariotte et al. 2011](#_ENREF_114)). This strongly suggests that high levels of circulating anti-Gal1,3Gal, antibodies, although an obvious hindrance to mAb half-life, do not pose a significant safety risk unless class-switched to IgE through prior sensitization.

However, these examples, although dramatic, are becoming increasingly isolated as current industry approaches employ greater regulation and sophistication of analysis. For example, it is now standard practice to carefully monitor mAb glycan profiles ([Batra and Rathore 2016](#_ENREF_12)), employ post-production modification techniques or address the problem at the source through media supplementation ([Hossler et al. 2017](#_ENREF_82)) or engineered glyco-biosynthetic pathways ([Dicker and Strasser 2015](#_ENREF_43)). Together, these have largely eliminated this impediment, although cases of ‘glycan drift’ in some commercialised mAbs have been reported ([Kim et al. 2017](#_ENREF_97)). Highlighting the importance of consistent glycosylation, mAb Fc glycosylation is now considered as a critical quality attribute (CQA) by market authorization agencies ([European Medicines Agency](#_ENREF_50); [Reusch and Tejada 2015](#_ENREF_142)) i.e. ‘*a physical, chemical, biological or microbiological property or characteristic that must be within an appropriate limit, range or distribution to ensure the desired product quality, safety and efficacy*.’

Finally, it is worth noting two novel developments that have sought to actually exploit glycan immunogenicity for therapeutic benefit. Racotumomab (VaxiraTM, Recombio) is an anti-idiotypic mouse mAb that mimics the non-human form of sialic acid, Neu5Gc, triggering a specific immune response against the tumour associated antigen NGcGM3, through a vaccine-like strategy ([Vazquez et al. 2012](#_ENREF_176)). Racotumomab is currently being tested in a phase 3 randomized controlled trial (RCT) of patients with advanced lung cancer (NCT01460472). In contrast, the mAb MABp1 (XilonixTM, XBiotech Inc.) has been cloned from B-cells taken from an individual with an immune response to interleukin-1 (IL-1). This has been described as a first-in-class ‘True HumanTM’ mAb and data from a phase 3 trial of patients with colorectal carcinoma (where IL-1 is a key player in inflammatory mediated morbidity [[O'Sullivan Coyne and Burotto 2017](#_ENREF_127)]) suggest it is very well-tolerated with a safety profile indistinguishable from placebo ([Hickish et al. 2017](#_ENREF_79)).

# Access all Areas: Enhancing tumour penetration

Antibodies show sufficient tumour penetration for clinical use primarily due to their long circulatory half-life ([Xenaki et al. 2017](#_ENREF_184)). Nonetheless, antibodies are large ~150 kDa molecular entities with a relatively high polarity that results in very slow rates of extravasation and tissue distribution that can present a barrier to their efficient penetration of solid tumours ([Glassman and Balthasar 2014](#_ENREF_71)) and metastatic masses ([Saga et al. 1995](#_ENREF_149)). Whilst large regions of untargeted cells are problematic for effective disease eradication, a potentially more serious issue is that of selecting for drug resistance in regions of marginally toxic mAb concentration ([Thurber et al. 2008](#_ENREF_172)).

Two of the key mechanisms governing drug transport into most biomass targets are convection (flow from pressure) and diffusion (along a concentration gradient). In solid tumours, the former is commonly reduced by the high interstitial fluid pressure of the tumour itself, thus, diffusion is the dominant transport mechanism for most mAbs ([Thurber et al. 2008](#_ENREF_172); [Xenaki et al. 2017](#_ENREF_184)). Diffusion can be hampered by factors such as an unusually dense extracellular matrix associated with many tumours and the ‘binding site barrier’ (BSB) where mAbs are immobilised near their site of entry when the mAb affinity is high, especially when there is a high antigen concentration ([Fujimori et al. 1990](#_ENREF_63); [Glassman and Balthasar 2014](#_ENREF_71); [Rudnick and Adams 2009](#_ENREF_146); [Rudnick et al. 2011](#_ENREF_147)). Transport of mAbs into tumours is also influenced by mAb clearance, both systemic (target-dependent and independent) and local (endocytosis). Thus, the relative rate of mAb transport, driven primarily by diffusion (and thus circulating mAb concentration), minus the sum of systemic/local clearance rate regulates the efficiency of mAb tumour penetration ([Thurber, et al. 2008](#_ENREF_156)).

Current innovations to promote transport over clearance include size reduction to either a Fab or single chain Fv (scFv) fragment which, for mAbs that are not dependent on Fc effector functions, have faster diffusion rates ([Kholodenko et al. 2017](#_ENREF_96); [Xenaki et al. 2017](#_ENREF_184)). However, this must be balanced against a higher clearance rate for these smaller molecules, especially those without a functional Fc that are not recycled via FcRn. Interestingly, scFv fragments are also subject to a form of BSB as their tumour diffusion rates reach a restriction threshold when their affinity surpasses around 10-9M ([Adams et al. 2001](#_ENREF_1); [Suksanpaisan et al. 2014](#_ENREF_166)). Lowering mAb affinity to minimise the BSB ([Fujimori et al. 1990](#_ENREF_63); [Glassman and Balthasar 2014](#_ENREF_71); [Rudnick et al. 2011](#_ENREF_147)) may also be useful, although not to the extent of promoting local clearance through greater off rates. A variety of other co-administrative/conjugation methodologies are proving successful in altering the transport/clearance balance include technologies such as nanoparticles ([Forero-Torres et al. 2015](#_ENREF_62)), tumour-penetrating peptides ([Y. Zhang et al. 2016b](#_ENREF_190)) and targeted liposomes ([Sofou and Sgouros 2008](#_ENREF_162)).

# Exploiting Fc effector functions

The Fc region of IgG contains a range of recognition motifs for binding innate immune effectors; these include Fc gamma receptors (FcγRs), the first component of the classical complement cascade C1q, and the neonatal Fc receptor (FcRn) and thus is responsible for mediating immune effector functions and *in vivo* IgG stability and long half-life([Nimmerjahn and Ravetch 2010](#_ENREF_125)). Antibodies known to utilise ADCC/P (or CDC) as part of their anti-tumoural efficacy can be engineered to augment such abilities through mutations to selected residues within the Fc region without affecting the overall pharmacokinetic profile.([Nimmerjahn and Ravetch 2012](#_ENREF_126))

IgG ADCC effector functionality is realised through the Fc's engagement with an array of FcRs, whose expression is predominantly restricted to immune cells. Such receptors are usually classified as either activatory or inhibitory, depending on their intracellular signalling motif. Humans express five activatory receptors, composed of the high affinity receptor FcR1 (CD64) and four low-affinity receptors FcRIIa (CD32A), FcRIIc (CD32C), FcRIIIa (CD16A) and FcRIIIb (CD16B), as well as a low-affinity inhibitory receptor, FcRIIb (CD32B). NK cell mediated ADCC appears to be dependent on the expression of FcRIIIa (partly by default as NK cells lack FcRI and FcRIIa) whilst FcRIIc is ablated by a nonsense mutation in most people ([Hargreaves et al. 2015](#_ENREF_77)).

Whilst the relative expression of each receptor is important and indeed their polymorphisms can occasionally confound mAb therapy ([Dahal et al. 2015](#_ENREF_34)), our ability to modulate the nature of FcR engagement by manipulating the Fc represents a key therapeutic opportunity for improving outcomes. Fc engineering seeks to replace or modify key amino acid residues (or post-translational modifications), guided by a robust body of mechanistic/structural data, with the goal of improved anti-tumour potency. Most work in this area has focused on the IgG polypeptide itself, with several notable successes in improving FcR engagement ([Bang et al. 2017](#_ENREF_10); [Kellner et al. 2013](#_ENREF_95)), as exemplified by margetuximab (Merck, Macrogenetics) which is currently being studied in phase 3 clinical trials ([Strohl 2018](#_ENREF_164)). Moreover, interest in glycan Fc engineering has been kindled by the demonstration of enhanced Fc effector functionality through modification of specific residues within the Fc N-glycan at N297 ([Jefferis 2009](#_ENREF_87); [W. Li et al. 2017b](#_ENREF_110); [Yu et al. 2017](#_ENREF_186)).

The Fc is composed of a homodimer of heavy chain C2 and C3 domains ([Davies and Metzger 1983](#_ENREF_38); [Padlan 1994](#_ENREF_129)). The conserved N297 N-linked glycans that sit within the interstitial space of opposing C2 domains are critical to Fc-mediated effector molecules through their specific interactions with FcRs and C1q ([Nimmerjahn and Ravetch 2010](#_ENREF_125); [Radaev and Sun 2001](#_ENREF_137); [Raju 2008](#_ENREF_139)). However, these interactions are not necessarily optimal and may benefit from glycan-engineering. The core 1,6 fucose, more than any other glycan residue, was identified as a central molecular player in Fc-FcR interactions, specifically in relation to FcRIII. Core 1,6 fucose is added by the enzyme fucosyltransferase 8 (FUT8, EC 2.4.1.68) during secretion through the Golgi apparatus and is attached to the first Asn-linked GlcNAc. It has been observed that IgG generated with little or no core fucose result in enhanced ADCC and even ADCP ([Castilho et al. 2015](#_ENREF_23); [Golay et al. 2013](#_ENREF_74); [Grugan et al. 2017](#_ENREF_76); [Herter et al. 2014](#_ENREF_78); [Luo et al. 2017](#_ENREF_113); [Shields et al. 2001](#_ENREF_159); [Shields et al. 2002](#_ENREF_158); [Shinkawa et al. 2003](#_ENREF_160)). Crystal structures of fucosylated/non-fucosylated Fc in complex with the human FcRIIIa have provided a molecular rationale underlying this phenomenon ([Ferrara et al. 2006](#_ENREF_56); [Ferrara et al. 2011](#_ENREF_57); [Mizushima et al. 2011](#_ENREF_116)). It would appear that a key Asparagine (N)-glycan expressed on the receptor itself, located at residue N162, directly interacts with the Fc N297 glycans (although there are also protein-glycan interactions) to facilitate a stable complex (Figure 2). The presence of an 1,6-fucose residue on the Fc glycan sterically hinders this process, probably by reducing the flexibility of the key Fc T296 residue ([Isoda et al. 2015](#_ENREF_83)), negating the formation of a stable complex. Accumulating evidence confirms that enhanced ADCC effect is restricted to signalling through FcRIII via this intermolecular glycan-glycan interface ([Bruggeman et al. 2017](#_ENREF_20); [Dekkers et al. 2017](#_ENREF_40); [Ferrara et al. 2011](#_ENREF_57); [Luo et al. 2017](#_ENREF_113); [Sakae et al. 2017](#_ENREF_150); [Subedi and Barb 2016](#_ENREF_165)). More recent observations have illuminated further specifics including the requirement of only one of the N-glycans to be afucosylated ([Shatz et al. 2013](#_ENREF_157)) and that the processing state of the receptor can also influence binding affinity([Patel et al. 2018](#_ENREF_131)).

To date, two low/no fucose mAbs have been licensed for oncology indications ([Table I](#Table_I)) ([Strohl 2018](#_ENREF_164)) and more are set to join them in the near future. As yet no industry platform standards have emerged to remove or minimise Fc fucose, with a plethora of competing technologies available, with genetically modified cell lines, metabolic interference and post-translational enzymatic modification technologies all staking an early claim in what is set to be a rapidly expanding niche industry.

The success of hypofucosylation in promoting clinically useful IgG effector function has perhaps overshadowed some other emerging strategies in this field, although many of these observations still lack the consistency of effect associated with fucose. A good illustration of this can be found in the ongoing debate that surrounds the impact of sialic acid on Fc effector functions. The sialylation of pertuzumab (anti-HER2, human IgG1 used mostly in breast cancer treatment) has been shown to hinder ADCC ([Luo et al. 2017](#_ENREF_113)), consistent with earlier reports ([Boyd et al. 1995](#_ENREF_18); [Naso et al. 2010](#_ENREF_121); [Scallon et al. 2007](#_ENREF_151)), including rituximab (anti-CD20, chimeric IgG1, used in haematological malignancies)([C. L. Chen et al. 2017a](#_ENREF_25); [Lin et al. 2015](#_ENREF_111)) but is at odds with studies that do not observe any significant impact of sialylation on ADCC, again using rituximab ([Quast et al. 2015](#_ENREF_136)), and a study using a generic monoclonal IgG1 (Roche, in house) ([Thomann et al. 2015](#_ENREF_171)). Similarly, the impact of sialylation on CDC is equally unclear with seemingly contradictory reports that it can either promote([Luo et al. 2017](#_ENREF_113)) or inhibit CDC ([Quast et al. 2015](#_ENREF_136)). Similar conflicting data surrounds the effect of sialic acid on the anti-inflammatory activity of intravenous immunoglobulin (IVIg). Early work linking IVIg Fc 2,6 sialylation with anti-inflammatory properties ([Anthony et al. 2008](#_ENREF_5); [Schwab and Nimmerjahn 2013](#_ENREF_154); [Schwab et al. 2014](#_ENREF_155); [Tjon et al. 2015](#_ENREF_173)) has since come under considerable scrutiny with in relation to the proposed mechanism involving DC-SIGN as the key IVIg receptor ([Bayry et al. 2009](#_ENREF_13); [Campbell et al. 2014](#_ENREF_22); [Nagelkerke et al. 2014](#_ENREF_118); [Yu et al. 2013a](#_ENREF_187)). While there are a growing number of examples where sialic-acid dependent anti-inflammatory properties of antibodies can be detected, there is considerable room for closer testing of the proposed mechanisms and for the evaluation of IVIg glycoforms in more physiologically relevant experiments.

Therefore, the influence of sialylation on Fc effector functions is either more complex, possibly reflecting an interdependence with fucose ([T. Li et al. 2017a](#_ENREF_109)) or even mAb specific. Recently, Dekkers et al ([Dekkers et al. 2017](#_ENREF_40)) attempted to explore this complexity by combining six glyco-engineering methods to generate the 20 major human IgG1-glycoforms, followed by screening of their functional capacity. Consistent with earlier reports ([Yu et al. 2013a](#_ENREF_187); [Yu et al. 2013b](#_ENREF_188)), they show that sialylation had little-to-no effect on FcγR binding, but confirmed that hypo-fucosylation of IgG1 increased binding to FcγRIIIa and FcγRIIIb and could be further (albeit modestly) increased with subsequent hyper-galactosylation, resulting in enhanced NK cell-mediated ADCC.

In an oncology setting, similar ambiguity surrounds the impact on effector functions of other Fc glycan residues such as galactosylation, the presence of which has been argued to promote FcRIIIa mediated ADCC, in an auxiliary role to hypofucosylation, as well as CDC ([Dekkers et al. 2017](#_ENREF_40); [Thomann et al. 2016](#_ENREF_170)). Moreover, it has been reported that elevated galactosylation can increase (independent of fucosylation) C1q-binding, downstream complement deposition, and complement dependent cytotoxicity ([Dekkers et al. 2017](#_ENREF_40); [Peschke et al. 2017](#_ENREF_132)). However, current evidence suggests that galactosylation has a much weaker influence on FcγR binding than fucosylation.

Despite the need for greater clarity surrounding the biology of many glycoforms, there does seem to be opportunities to simultaneously enhance ADCC and CDC activities of mAbs by combining glyco-engineering and protein-engineering technologies. On the protein side, Fc modifications have been identified that can enhance either ADCC/ADCP ([Horton et al. 2008](#_ENREF_81); [Lazar et al. 2006](#_ENREF_103)) or CDC ([C. H. Lee et al. 2017](#_ENREF_104); [Moore et al. 2010](#_ENREF_117); [Tammen et al. 2017](#_ENREF_167)) and some mAbs currently in clinical trials such as ocaratuzumab (anti-CD20, Mentrik Biotech, LLC), ([Ganjoo et al. 2015](#_ENREF_66)), margetuximab (anti-HER2, Merck, Macrogenics) ([Bang et al. 2017](#_ENREF_10)) and MOR208 (anti-CD19, Morphosys, Xencor)([Jurczak et al. 2018](#_ENREF_92)) contain such modifications. However, combining mutations with the aim of simultaneously enhancing ADCC and CDC has proven challenging ([Radaev et al. 2001](#_ENREF_138); [Schneider and Zacharias 2012](#_ENREF_153); [Sondermann et al. 2000](#_ENREF_163)). Recent work by Wirt et al. ([Wirt et al. 2017](#_ENREF_183)), has demonstrated the feasibility of combining the Fc protein modification EFTAE (S267E/H268F/S324T/G236A/I332E), known to enhance CDC (without compromising ADCC) ([Moore et al. 2010](#_ENREF_117)), with a glycoengineered, non-fucosylated phenotype resulting in a ritixumab variant with improved CDC and ADCC efficacy without any loss in CD20 affinity. Of note, a similar pattern of enhanced ADCC/CDC efficacy has been achieved by crossing the afucosylated format with a mixed isotype IgG1/IgG3 Fc ([Natsume et al. 2008](#_ENREF_123)). Overall, it seems that the effects on receptor recognition arising from glycan and protein engineering are largely independent and can be readily combined to create new properties.([Yu et al. 2013b](#_ENREF_188)) However, it is important to recognise that these two effector mechanisms may be competitive, as suggested previously ([Wang et al. 2008](#_ENREF_178)), and not result in more active reagents in vivo.

Clearance rate directly impacts upon the efficacy of any therapeutic antibody. Although IgG antibodies are protected from rapid lysosomal degradation thanks to the FcRn recycling mechanism (FcRn interacts with the Fc independently of the Fc glycan) ([Roopenian and Akilesh 2007](#_ENREF_145)), there exists a variety of secondary glycan specific clearance mechanisms open to modulation through glycan engineering. Prominent among these is the mannose receptor that recognises terminal mannose or GlcNAc ([Taylor et al. 2005](#_ENREF_169)). IgG produced in non-human cells frequently express high mannose glycans ([Goetze et al. 2011](#_ENREF_73); [P. Zhang et al. 2016a](#_ENREF_189)) and such antibodies have shorter half-lives ([Goetze et al. 2011](#_ENREF_73); [Kanda et al. 2007](#_ENREF_93); [Liu et al. 2011](#_ENREF_112)). Given that IgG glycosylation can be extremely heterogeneous, the impact of high mannose glycans on overall efficacy will depend on their relative abundance within the glycan population and may be diluted out by non-mannose variants. Similarly, the asialoglycoprotein receptor may play a similar role in IgG clearance, despite the very low levels of sialic acid found in the Fc. Studies with pertuzumab have shown that desialylation of the Fc results in rapid hepatocyte mediated clearance ([Luo et al. 2017](#_ENREF_113)). It will be interesting to see if antibody therapies can be developed that exploit this clearance mechanism for tissue targeting, perhaps for therapies targeting the liver. Precedence for the effectiveness of such glycan-dependent targeting has been the clinical success of mannose-terminating β-glucocerebrosidase for the treatment of glycolipid storage disorders ([Elstein 2011](#_ENREF_48)).

A further critical player dictating anti-cancer mAb efficacy is the immunosuppressive tumour microenvironment (TME) produced by the developing cancer itself. Numerous reports have detailed the many mechanisms and axes through which tumours are able to suppress both innate and adaptive immunity to avoid elimination ([Gajewski et al. 2013](#_ENREF_65)). Notable amongst those cells affected and themselves central to many of these immunosuppressive processes are tumour associated macrophages (TAM) ([Gabrilovich et al. 2012](#_ENREF_64)). These phenotypically plastic cells are particularly pertinent to anti-cancer mAb efficacy as they are capable of expressing the full repertoire of FcγR, are able to modulate their expression in response to their environment and the repertoire they express is closely linked to their effector capacity. Also, the tissue location of the tumour itself seems to influence what role TAMs play in the activity of cytotoxic mAbs ([Lehmann et al. 2017](#_ENREF_107)). The activatory to inhibitory FcγR binding ratio of mAb has been known for a number of years to determine mAb efficacy in vivo ([Gabrilovich et al. 2012](#_ENREF_64)). It has now become clear that the activatory to inhibitory receptor expression ratio on TAM can similarly determine efficacy with recent reports demonstrating how the inhibitory FcγRIIb is upregulated on TAM in both solid and haematological tumours and that this can directly reduce mAb activity in the TME ([Arce Vargas et al. 2017](#_ENREF_7); [Dahal et al. 2017](#_ENREF_35)). Importantly, these inhibitory effects can be reduced through appropriate mAb isotype selection and through immunomodulatory approaches which effectively correct the TAM FcγR repertoire to a productive, high activatory to inhibitory FcγR expression ratio ([Arce Vargas et al. 2017](#_ENREF_7); [Dahal et al. 2017](#_ENREF_35)). Glycoengineering mAbs is another strategy that could further increase efficacy in the suppressive TME by enhancing appropriate FcγR engagement.

# Beating the House

The efficient interaction of a therapeutic mAb with relevant FcRs is central to ADCC/P mediated efficacy. Yet, under physiological conditions, the high concentration of endogenous serum IgG (adults have on average 10 g/L intravascular polyclonal IgG) and more importantly, various immune complexes, which display far higher avidity for FcR, will likely impair the binding of the therapeutic antibody to the FcRs, and may increase the required dosing levels of the therapeutic mAb ([Preithner et al. 2006](#_ENREF_134)), potentially lowering its therapeutic index. This is particularly the case for FcRI ([Dekkers et al. 2017](#_ENREF_40)), the high affinity receptor, which binds monomeric IgG with high affinity and so is presumed to be fully saturated under normal homeostatic conditions.

Defucosylation may be sufficient to partially overcome this barrier, at least for FcRIII dependent activation ([Nechansky et al. 2007](#_ENREF_124); [Preithner et al. 2006](#_ENREF_134)). However, this effect is dependent on key parameters such as target density and mAb concentration, with lower antigen densities particularly vulnerable to endogenous IgG suppression ([Preithner et al. 2006](#_ENREF_134)). Furthermore, IgG hypofucosylation has little or no impact on IgG binding to FcRI or FcgRIIA/C ([Dekkers et al. 2017](#_ENREF_40)).

One strategy to overcome this issue may lie with IgG inactivating enzymes such as EndoS ([Collin and Olsen 2001](#_ENREF_31)) and IdeS ([Johansson et al. 2008](#_ENREF_89); [von Pawel-Rammingen et al. 2002](#_ENREF_177)) which are currently undergoing clinical trial in patients with autoimmune conditions or those undergoing organ transplantation ([Collin and Bjorck 2017](#_ENREF_32); [Sethi et al. 2017](#_ENREF_156)). EndoS is an IgG specific endoglycosidase ([Collin and Olsen 2001](#_ENREF_31)) whilst IdeS is an IgG specific endopeptidase ([von Pawel-Rammingen et al. 2002](#_ENREF_177)), both produced by the pathogen *Streptococcus pyrogenes*, a highly virulent pathogen responsible for a range of human diseases. By destroying or inactivating endogenous IgG, these agents can theoretically create a therapeutic window in which all activatory FcRs are available for mAb engagement ([Baruah et al. 2012](#_ENREF_11); [Jarnum et al. 2017](#_ENREF_86)). IdeS is of particular interest as it is rapidly cleared from the body enabling a staged dosing regimen without the requirement for the therapeutic antibody to be resistant to the enzyme ([Baruah et al. 2012](#_ENREF_11); [Jarnum et al. 2017](#_ENREF_86)). It remains to be seen if these observations can be fully translated to the clinic. One particular feature requiring attention is the inevitable immunogenicity of the bacterial enzymes which potentially limits their use to a single prime prior to antibody therapy. However, pre-clinical work using several established therapeutic mAbs is encouraging and there is scope to use a series of antigenically distinct enzymes to lengthen or reopen the therapeutic window ([Jarnum et al. 2017](#_ENREF_86)).

# The more the merrier: multi-specifics

Multispecific antibodies bring together the specificities of two or more antibodies into one molecule thus simultaneously targeting different antigens or epitopes ([Kontermann and Brinkmann 2015](#_ENREF_99)) and may well represent the next generation of targeted mAbs for cancer therapy ([Weidle et al. 2014](#_ENREF_179)). Multi-target functionality can interfere with multiple surface receptors or ligands associated with proliferation and/or inflammatory processes or recruit/trigger contacts between tumour cells and key immunological effector cells. The simplest exponents of this technology are bispecifics such as the bi-specific T-cell engagers [BiTEs] ([Baeuerle et al. 2009](#_ENREF_9)).

Bi-specifics can be thought to fall into one of two classes; those that have an Fc (or structural and/or functional equivalent) and those that do not. Fc-containing molecules can be achieved by a variety of recombination formats (including classic bivalent IgG, disulphide stabilised scFvs or Fabs) have similar half-lives to IgG mAbs and can be bulk-produced in much the same way. The anti EpCAM x CD3 catumaxomab (RemovabTM, Fresenius Biotech/Trion Pharma), licensed in 2009 (European Medicines Agency [EMA] only) for treating malignant ascites ([Chelius et al. 2010](#_ENREF_24)) falls into this category (although catumaxomab no longer has EMA market authorization)([European Medicines Agency 2017a](#_ENREF_51)). Fc effector functionality can be fully or partly retained through genetic engineering and the general principles discussed above remain valid (depending on the degree of functionality), although, as yet, little has been published or translated into the clinic.

Unsurprisingly, bi-specifics that lack an Fc rely completely on their antigen-binding ability to exert their therapeutic efficacy and also half-life. This liberation from the constraints of the classical IgG format has led to innovations such as bi- and tri-specific killer engagers (BiTEs and TriKEs) that target NK cells to tumours ([Felices et al. 2016](#_ENREF_55); [Gleason et al. 2012](#_ENREF_72); [Tay et al. 2016](#_ENREF_168)). The first to achieve market authorization in this category was the anti-CD19/CD3 BiTE, blinatumomab (BLINCYTOTM, Amgen Inc.) for the treatment of a variant of acute lymphoblastic leukaemia. Not to be outdone by Fc-less variants, recent work in the field of broadly neutralising antibodies to HIV have shown that tri-specifics can be incorporated into a more classical antibody structural paradigm ([Xu et al. 2017](#_ENREF_185)), a trick that is likely to tested in a cancer context soon ([Schmohl et al. 2017](#_ENREF_152)).

# Release the brake, step on the gas: combination immunotherapy

Combination therapy is nothing new to oncology where doublet and triplet regimens, ideally with components containing different MOAs, are commonly used to enhance efficacy and combat the emergence of tumour resistance. Yet, the growing awareness that a pathologically altered immune system is an important component of the tumour microenvironment has added a new dimension to this strategy, combinational immunotherapy, and one where mAbs play a central role. As mAbs can selectively target components of the immune system, either by agonistic binding to stimulatory molecules or direct inhibition of immune checkpoints, they are being increasingly utilised to unblock pre-existing tumour directed immune responses that have been suppressed by the tumour micro-environment.

To date, most combination immunotherapy includes a mAb directed against one of the T-cell immune checkpoints mediated by PD-1 or CTLA-4 ([Farkona et al. 2016](#_ENREF_54)). Following the success of CTLA-4 monotherapy, providing effective cures (>10 year survival) in a modest proportion of patients, and the even more promising effects of PD-1/PDL1 blockade evoking responses in a larger proportion of patients, these mAb have been combined, with signs of even greater efficacy. Unfortunately this success comes at the anticipated cost of higher toxicity and so alternative combinations are being sought. A plethora of mAbs have been pressed into action, from classical monoclonal IgGs through to BiKEs and TriKeS ([Davis et al. 2017](#_ENREF_39)) and the scale of clinical trial activity, across a host of cancers, is enormous with hundreds of clinical studies currently ongoing with anti-PD1 combinations alone ([Dempke et al. 2017](#_ENREF_41); [Kourie et al. 2016](#_ENREF_100); [Kourie et al. 2017](#_ENREF_101); [Vanpouille-Box et al. 2017](#_ENREF_175)).

Checkpoint inhibition aside, it should be noted that combinational therapy is also being explored using more classical anti-tumour mAbs (so-called direct targeting mAbs) directed against different tumour antigens, as part of a wider effort to personalise patient medicine. This strategy, which has a significantly longer history than checkpoint inhibition, is now used in a tumour /patient biomarker specific manner. For example, rituximab (anti-CD20) is being combined with apratuzumab (anti-CD22) or varlilumab (anti-CD27) in non-Hodgkin’s lymphoma ([Leonard et al. 2005](#_ENREF_108)). Combination mAb therapy can also exploit other components of immune-signalling or tumour micro-environment known to be important for a tumour’s progression. A promising example is combining ritixumab with FcRIIb antagonists in lymphoid malignancies ([Roghanian et al. 2015](#_ENREF_144)). In B-cell malignancies FcRIIb negatively regulates both adaptive and innate immunity, inhibits tumour clearance in murine models and is a marker of patient response to ritixumab therapy ([C. S. Lee et al. 2015](#_ENREF_105); [Roghanian et al. 2015](#_ENREF_144); [Roghanian et al. 2016](#_ENREF_143)).

# Weaponized antibodies: ADCs

Antibody drug conjugates (ADC) bring together the tumour specificity and optimal pharmokinetics of mAbs with the potent cytotoxicity of established small molecule cytotoxics, to create a novel class of combinational anti-cancer therapy ([McCombs and Owen 2015](#_ENREF_115)). However, this synergistic benefit must be considered alongside a more demanding clinical development pathway, relative to unmodified mAbs ([Bornstein 2015](#_ENREF_17)). Four first generation ADCs have acquired market approval within the oncology space, gemtuzumab ozogamicin (anti-CD33, Mylotarg®, Pfizer/Wyeth), Inotuzumab ozogamicin (anti-CD22, BesponaTM, Pfizer/Wyeth), trastuzumab emtansine (anti-HER2, Kadcyla®, Genentech/Roche) and brentuximab vedotin (anti-CD30, Adcetris®, Seattle Genetics).

Parameters that impact on the pharmacokinetic and safety profile of any given ADC include the degree of tumour antigen specificity, the nature of the drug conjugate, the type of linker employed and the methodology used in the conjugation process itself. The target antigen must be abundantly expressed by the tumour cells, promoting internalisation/payload delivery coupled with a high degree of tumour specificity to avoid off target payload delivery and associated toxicity. Both of these considerations have been shown to impact on ADC efficacy and safety, respectively, in regulatory RCTs ([Bornstein 2015](#_ENREF_17); [McCombs and Owen 2015](#_ENREF_115)).

Target-independent uptake of ADC payload by normal cells is a primary hindrance to efficacy and thought to be a major component of off-target toxicity. This can be either through non-tumour antigen expression in normal tissues ([Hinrichs and Dixit 2015](#_ENREF_80)) or antigen-independent mechanisms such as uptake through the mannose-receptor ([Gorovits and Krinos-Fiorotti 2013](#_ENREF_75)), FcRn or FcR ([Hinrichs and Dixit 2015](#_ENREF_80)).

The ADC linker is a critical component which can impact the clinical efficacy and safety profile. Linkers should be stable enough to survive the rigours of circulation yet also be amenable to rapid and efficient disengagement from the toxic payload during the internalisation process. The three traditional linkers are hydrazones, disulphides and peptides, with a shift toward the latter two for reasons of stability. Alongside normal cell ADC uptake, early cleavage of the linker, releasing the payload off-target, can produce widespread toxicities ([Donaghy 2016](#_ENREF_45)). The experience of gemtuzumab ozogamicin, the first ever ADC approved by the US Food and Drug Administration (FDA) in 2000 (for patients with relapsed acute myeloid leukaemia [AML]) ([Bross et al. 2001](#_ENREF_19)), is illuminating. Early versions of ADCs, including gemtuzumab ozogamicin, employed hydrazone linkers. It is believed that the heterogeneous nature of the conjugate linker played a part in gemtuzumab ozogamicin’s post-authorization safety issues that led to its voluntary withdrawal from the US market in 2010 ([Bornstein 2015](#_ENREF_17); [Food and Drug Administration 2010](#_ENREF_58); [Jain et al. 2015](#_ENREF_85); [McCombs and Owen 2015](#_ENREF_115)). However, persistent efforts by clinicians have established that the simple expediency of an altered dosing regimen can overcome these clinical problems, resulting in gemtuzumab ozogamicin’s re-approval by the FDA in 2017 (for patients with newly diagnosed AML) ([Appelbaum and Bernstein 2017](#_ENREF_6)).

The traditional conjugation technology used in the first-generation ADCs comes with an inherent variability in that the toxic payload will link to random cysteine or lysines, leading to a heterogeneous mix of ADCs, both in terms of location and number of drug conjugates. This heterogeneity is thought to contribute to suboptimal efficacy and safety properties as well as limiting further optimisation strategies. To overcome this issue, next generation ADCs have been developed using strategies aimed at producing site-specific conjugate homogenous ADCs ([Agarwal and Bertozzi 2015](#_ENREF_2)). Several amino acid engineering techniques have been successfully employed ([Nanna et al. 2017](#_ENREF_119)), including addition of unnatural amino acids, enzymatic conjugation to specific amino acid sequences and transpeptidation. In parallel, exploiting the invariable N297 N-glycan itself, primarily through using chemoenzymatic (glycosyltransferase) conjugation, has shown great promise ([Dennler et al. 2014](#_ENREF_42); [Okeley et al. 2013](#_ENREF_128); [Qasba 2015](#_ENREF_135); [van Geel et al. 2015](#_ENREF_174); [Zhou et al. 2014](#_ENREF_191)). Many of these next generation methods have been commercialised and are now in development ([Beck et al. 2017](#_ENREF_14); [Lambert and Morris 2017](#_ENREF_102)).

Like other mAbs, ADCs are also being tested in combination ([Gerber et al. 2016](#_ENREF_69)). For example, early data from phase 1/2 trials of brentuximab vedotin with nivolumab (anti-PD-1) have demonstrated a greater than 90% response rate in patients with relapsed/refractory Hodgkin’s lymphoma ([Diefenbach et al. 2016](#_ENREF_44)).

# Me too, me new: biosimilars and biobetters

Biosimilars, officially approved versions of original products that can be manufactured once the former’s patent expires, is certain to be a rapid growth area in cancer mAb development, particularly as we move through the ‘patent cliff’ that affects so many currently licensed mAbs ([Calo-Fernandez and Martinez-Hurtado 2012](#_ENREF_21)). Biosimilar mAbs hold the promise of broader global patient access and significant payer monetary savings across a range of oncology indications.

A biosimilar normally establishes ‘high similarity’ to the original reference product that has already received market authorization. Demonstration of this form of bioequivalence is sufficient for small biological molecules; but this approach is not fully applicable to larger biologics such as mAbs which are very similar (in amino acid sequence and overall structure) but not identical to the reference due to post-translational modifications such as glycosylation which can impact on efficacy ([Pasina et al. 2016](#_ENREF_130)). Rather, a comparable therapeutic index is sought. Both the EMA and the FDA now have advanced biosimilar frameworks in place to deal with the non-clinical and clinical tests required to show bioequivalence [Table II](#Table_II), ([European Medicines Agency 2017c](#_ENREF_53); [Food and Drug Administration 2017b](#_ENREF_60)) although these guidelines are still evolving, particularly in relation to areas partly out of their control such as national [Europe] or state (US] prescription interchangeability (switching) legislation.

Yet, the regulatory hurdle to market authorization is not the final barrier for biosimilars. Understandably in such a high stakes financial market, originator/biosimilar manufacturer disputes are common. These include litigation in response to patent breaches (where multiple patents exist covering not only the branded product, but also key steps in the manufacturing process) and license extensions (e.g. to paediatric indications), both of which can delay biosimilar market entry ([Konara et al. 2016](#_ENREF_98)). Nevertheless, key developments such as the coming together of originator and biosimilar manufacturers in organizations such as the Biosimilars Forum ([Cohen et al. 2017](#_ENREF_30)), global originator/biosimilar *quid-pro-quo* settlements ([Generics and Biosimilars Initiative 2017](#_ENREF_68)) as well as ongoing strengthening of FDA and EMA guidelines will likely mean biosimilar uptake in the US and Europe will continue to gather pace in the near future, as seen in many developing countries. A good bench mark is trastuzumab (US patent expires in 2019 [[Chopra and Lopes 2017](#_ENREF_27)]) Europe patent expired in 2014). In 2017 the FDA approved its first two trastuzumab biosimilars (MYL-14010, Mylan and Ontruzant®, Samsung Bioepis) (American Association for Cancer Research [2017](#_ENREF_3); Food and Drug Administration [2017c](#_ENREF_61)), and the EMA its first (Ontruzant) ([European Medicines Agency 2017b](#_ENREF_52)), although in developing nations five versions were approved between 2013 and 2016 ([Elgundi et al. 2017](#_ENREF_47)).

A biobetter (or ‘biosuperior’) is considered to be a biological drug with superior efficacy, safety, stability and/or dosing regimen over the reference product ([Anour 2014](#_ENREF_4); [Elgundi et al. 2017](#_ENREF_47); [Konara et al. 2016](#_ENREF_98)). Although the FDA and the EMA have yet to provide a definitive definition(s) and/or guidance, past precedence would suggest that most biobetters will likely be considered as new products due to modifications to their amino acid sequence, epitope recognition, Fc glycosylation (e.g. obinituzumab [Gazyva®]) or delivery mechanism (e.g. switching from IV to subcutaneous versions of ritixumab and trastuzumab, co-formulated with hyaluronidase to increase antibody absorption and distribution) ([National Cancer Institute 2017](#_ENREF_122)).

However, it is unclear if a glycan-engineering alone of modified of a patent expired reference antibody will fall into the biosimilar, biobetter or some form of intermediate ‘related’ category. Although a glycan-engineered patent-expired mAb could fall under a narrow definition of a biosimilar, its enhanced therapeutic index could render clinical equivalence comparison with the reference mAb obsolete. The nearest example to date is that of obinituzumab, a variant of anti-CD20 with Fc engineered defucosylation, considered a novel product by the FDA (and later the EMA) when licensed for treating patients with Chronic Lymphocytic Leukaemia (CLL) after demonstrating superior phase 3 RCT efficacy compared to ritixumab ([H. Z. Lee et al. 2014](#_ENREF_106)). Nevertheless, obinituzumab has also been humanised and recognises a different, albeit overlapping, epitope within CD20 than ritixumab, making it difficult to know if its enhanced efficacy is entirely due to hypofucosyaltion or not. On the other hand, these cumulative changes make a new product definition relatively straight forward. Regulatory developments in response to glycan-engineered trastuzumab biosimilars like TrasGEX (Glycotope GmBH, Germany) ([Eisner et al. 2015](#_ENREF_46)) will likely be more informative.

# Is glycosylation here to stay?

The inherent heterogeneity of glycosylation within a mAb preparation is likely impossible to fully overcome using conventional approaches. Aglycosylated mAbs offer a radical solution to the issue of glycan quality control coupled with ease of production ([Jung et al. 2011](#_ENREF_90)). Yet, the growing awareness that mAbs lacking the ubiquitous N297 N-glycan are relatively stable without enhanced immunogenicity or detrimental pharmacokinetics ([Simmons et al. 2002](#_ENREF_161)) has not led to any appreciable uptake within oncology. A primary stumbling block is their impaired effector function with largely abrogated ADCC/P and CDC (see above). Of interest is the development of aglycosylated antibodies which show abrogated binding to the low-affinity receptors but which have been engineered to engage FcRI and exhibit profound monocyte-dendritic ADCC activity ([Jung et al. 2010](#_ENREF_91)). More recently, progress has also been made in identifying aglycosylated variants that bind FcRIIIa ([T. F. Chen et al. 2017b](#_ENREF_26); [Jo et al. 2017](#_ENREF_88)). Nevertheless, their potential for agonist/antagonist based efficacy remains an open therapeutic opportunity, particularly when effector functions are undesirable – for example in the case of certain checkpoint blockers – where receptor:ligand blockade (rather than effector mediated functions) is the principal mode of action, such as may be the case with anti-PD-1 ([Dahan et al. 2015](#_ENREF_36)).

# Perspectives

The application of first generation mAbs to modern cancer treatment regimens has undoubtedly had a significant and lasting impact on patient well-being and survival. This is a fact made all the more remarkable when one considers that many of these antibodies, particularly those dependent on effector functions like ADCC and ADCP, were likely operating under suboptimal conditions, *in vivo,* as outlined above.However, it still remains to be seen if enhanced Fc effector efficacy through glycan engineering (for now, principally afucosylation) actually translates into patient benefit in head-to-head trials relative to the reference mAb.

Whilst the availability of next generation mAbs such as ADCs and multi-specifics, coupled with combination therapy and a greater awareness of tumour-mediated immune suppression, will undoubtedly continue to benefit patients, the strategies outlined above will both facilitate these developments as well as allow a resurgence of several first generation mAbs with enhanced therapeutic indices. Although there well may be some technical and possibly legal obstacles to come, the biological barriers to these new mAbs are being broken down.

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# FIGURES AND TABLES

FIGURE 1

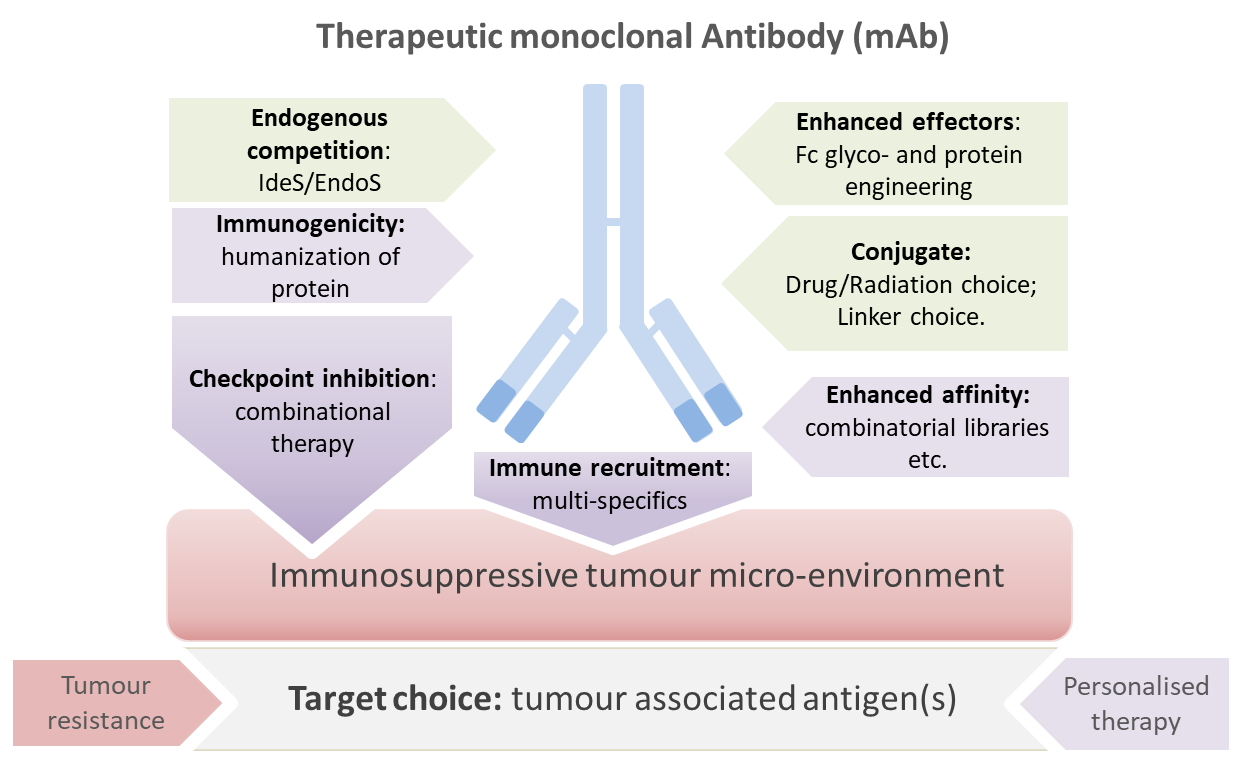


Fig.1. Illustration of the primary barriers (and key ongoing solutions) to effective mAb therapy. Antibody engineering and therapeutic strategies to overcome key barriers to effective monoclonal antibody therapy in cancer. Infographic illustration of the primary barriers (and key ongoing solutions) to effective mAb therapy. Parameters that can be modulated to improve mAb therapy are shown in green or purple (the former being the focus of this review). Modulated parameters aim to either directly enhance mAb functionality directly (antigen affinity, immunogenicity, enhanced effector function, and immune cell recruitment) or indirectly (removal of competing antibody FcR occupancy and removal of immunosuppressive barriers). A rational choice of tumour-associated antigen is also important, particularly in personalised therapy, as is an awareness of phenomenon of acquired tumour resistance.

FIGURE 2



Fig.2. Interaction of Fc receptor and antibody Fc glycans. (A) Overview of the interaction between IgG Fc (light blue) and FcRIIIa (orange) glycosylated at position N162 (yellow) as determined by X-ray crystallography (PDB accession code 3SGK). (B) A close-up view of the interaction between one of the IgG Fc glycans (blue) and the receptor N162 glycan (yellow). For clarity, the orientation differs slightly from that in Panel A. The protein-proximal GlcNAc and the site of potential α1,6-fucosylation has been annotated. The surface representation illustrates the close packing of the glycans.

Table I Fc glycan engineered monoclonal antibodies in clinical development for cancer treatment

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **mAb name, generic (brand)** | **Type** | **Target** | **MOA** | **Glycan modification/**  **conjugation** | **Approved indication, FDA (EMA)** | **Clinical data** |
| **Market authorized** | | | | | | |
| Mogamulizumab (Poteligeo®) | Humanized IgG1a | CCR4 | ADCC, CDC, direct cell-death signalling | Afucosylated | ATLL, CTCL (PTCL, CTCL) | Phase 3 |
| Obinutuzumab (GazyvaTM [US], GazyvaroTM (Europe]) | Humanized IgG1a | CD20 | ADCC, ADCP | Low fucose | CLL, FL (CLL, DLBL, FL) | Phase 3 |
| **Ongoing clinical trials** | | | | | **Potential Indication** |  |
| Ublituximab  (TG-1101) | Chimeric (mouse/human) IgG2a | CD20 | ADCC | Low fucose | CLL, B-cell malignancies | Phase 3 (+ ibrutinib), NCT02301156  Phase 2 (+ lenalidomide [Revlimid®]) |
| Inebilizumab (MEDI-551) | Humanized IgG1 | CD19 | ADCC, anti-proliferative | Afucosylated | DLBCL, CLL | Phase 2, NCT01453205  Phase 2, NCT01466153 |
| Gatipotuzumab (PankoMab-GEX) | Humanized IgG1 | TA-MUC1 | ADCC | Low fucose, modified galactose/branching | Ovarian cancer | Phase 2, NCT01899599 |
| CetuGEX | Chimeric (mouse/human) IgG1 (from cetuximab) | HER1 (EGFR) | ADCC | Fully human glycosylation, afucosylated | SCC (head and Neck) | Phase 2, NCT02052960 |
| Trastuzumab (TrasGEX®) | Humanized IgG1 | HER2 | anti-angiogenesis, cell cycle arrest, ADCC | Afucosylated | HER2 positive Breast cancer | Phase 1 (dose escalation) NCT01409343 |
| DI-B4 | Humanized IgG1 | CD19 | ADCC, anti-proliferative | Low fucose | B-cell malignancies | Phase 1 (NCT01805375) |
| ARGX-110 | Humanized IgG1b | CD70 | ADCC, CDC, ADCP, Treginhibition. | Afucosylated | AML, high-risk MDS, advanced malignancies, NPC | Phase 1/2, AML or High Risk MDS (NCT03030612)  Phase 1/2, advanced malignancies (NCT01813539)  Phase 1 NPC (NCT02759250) |
| ARGX-111 | Humanized IgG1 | c-Met | anti-proliferative, ADCC | Afucosylated | Advanced cancer (c-Met positive) | Phase 1b (NCT02055066) |
| MEDI-570 | Humanized IgG1 | ICOS | T-cell stimulation | Afucosylated | T-cell lymphomas | Phase 1 (NCT02520791) |
| SEA-CD40 | Humanized IgG1 | CD40 | ADCC, immune-activation | Afucosylated | Solid tumours/ some haematological malignancies | Phase 1 (+pembrolizumab NCT02376699) |
| KHK2823 | Fully human IgG1 | CD123 | anti-proliferative/ differentiation (IL-3R) | Afucosylated | AML, MDS | Phase 1 (NCT02181699) |
| JNJ-61186372 | Fully human IgG1c | EGFR/c-Met | Bispecific anti-proliferative | Low fucose | NSCLC | Phase 1 (NCT02609776) |
| GSK2857916 | Humanized  IgG1 | BCMA | Cytotoxic (ADC-MMAF) | Afucosylated | RRMM/advanced Hematologic Malignancies | Phase 1 (NCT02064387) |
| SEA-BCMA | Humanized IgG1 | BCMA | T-cell activation (ACTR) | Afucosylated | RRMM | Phase 1 (+autologous T-cell product ACTR087; NCT03266692) |

a From mouse

b From Llama

c Recombined derivative of two fully human mAbs

A more comprehensive list of engineered mAbs, including not only Fc glycan engineering, can be found in Stohl, 2018.([Strohl 2018](#_ENREF_164))

ACTR, antibody-coupled T-cell receptor; ADC, antibody-drug conjugate; ATLL, adult T-cell leukaemia; AML, acute myeloid leukaemia; BCMA, B-cell maturation antigen; c-Met, tyrosine-protein kinase Met; CDC, complement-dependent cytotoxicity; CLL, chronic lymphocytic leukaemia; CTCL, cutaneous T-cell lymphoma; DLBL, diffuse large B-cell lymphoma; EMA, European Medicines Agency; FL, follicular lymphoma; ICOS, anti-inducible T-cell co-stimulator; IL-3R, interleukin 3 receptor; MMAF, monomethyl auristatin phenylalanine; NSCLC, non-small cell lung carcinoma;PTCL, peripheral T-cell lymphoma; RRMM, relapsed/refractory multiple myeloma; Treg, regulatory T-cell.

Table II EMA and FDA definitions and regulatory requirements for market authorization of biosimilar and biobetter mAbs

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biosimilar | | Biobetter | |
| Definition | Regulatory requirements | Definition | Regulatory requirements |
| EMAa | ‘A biological medicinal product that contains a version of the active substance of an already authorized original biological medicinal product (reference medicinal product) in the EEA.’ *and* ‘Similarity to the reference product in terms of quality characteristics, biological activity, safety and efficacy based on a comprehensive comparability exercise needs to be established.’ | Stepwise approach to evaluate similarity of biosimilar to reference mAb.Data reviewed using a *totality-of-the-evidence* approach  **Non-clinical studies**  Performed *before* initiating clinical trials  **Step 1: *In vitro* non-clinical studies**   * Binding of target antigen(s) * Binding to representative isoforms of FcγRI, FcγRII and FcγRIII), FcRn and C1q * Fab-associated functions (e.g. neutralization of a soluble ligand, receptor activation or blockade) * Fc-associated functions (e.g. ADCC; CDC; complement activation) * Clinical studies to evaluate safety, PK, PD and efficacy endpoint data   **Step 2: Requirement for *in vivo* studies**   * Relevant *in vivo* model if full mAb effects cannot be elucidated in vitro   **Step 3: vivo studies**   * If required, PK and PD , dose toxicity * Impurities can increase toxicity * glycosylation variations can impact biological function   **Clinical studies**  Comparative clinical studies between biosimilar and reference mAb must be conducted  **Step 1: PK and PD study**  **Step 2 Clinical efficacy and safety**   * Preferred oncology endpoints, PFS/DFS and/or OS * If not feasible, ORR/CR in homogenous population can be considered * Severity and frequency of AEs, particularly those associated with reference mAb   **Other considerations:**   * Pharmacovigilance: Long-term immunogenicity and safety data may be required post-authorization * Extrapolation of indications is possible * Interchangeability of biosimilar and reference mAbs dependent on country clinical practicec | ‘An improved or optimized version of an existing biological drug, or a new biologic carefully designed to maximise clinical performance, i.e. safety and efficacy’.b | New product? |
| FDAd | ‘A biological product that is highly similar to a US-licensed reference biological product notwithstanding minor differences in clinically inactive components’ *and* ‘There are no clinically meaningful differences between the biological product and the reference product in terms of safety, purity and potency of the product.’ | Data reviewed using a *totality-of-the-evidence* approach.eA. Structural analysis:  * Primary amino acid sequence * Higher order structure (secondary, tertiary and quaternary (including aggregation) * Posttranslational modifications such as glycosylation and phosphorylation   **B. Functional Assays**   * Structural/functional characterization   **C. Animal Data**   * Required unless FDA determines unnecessary * Toxicity, PK/PD, immunogenicity   **D. Clinical Studies**   * Human pharmacology data (PK and PD) * Immunogenicity data required * Comparative clinical study required, safety and risk-benefit profile established * Appropriate endpoints (no specific oncology endpoints discussed) * Study population similar to original license for reference mAb * Non-inferiority design   **Other considerations:**   * Clinical evidence required for interchangeabilityf * Scientific justification required for extrapolation of clinical data allowed from one indication to another (addressing MOA, target/receptor, binding/dose concentration, location of target, PK/PD, immunogenicity, toxicities) * Postmarketing safety monitoring | Biobetters defined as engineered therapeutic proteins for better efficacy and safety g   * Resist chemical degradation: loss of activity, increased immunogenicity * Better tissue targeting: e.g. muscle uptake of therapeutic enzymes, penetration of the CNS * Diminish immunogenicity | New product? |

aEMA guidance for mAbs, <http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001382.jsp&mid=WC0b01ac058002958c>; general guidance on biosimilars, <http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000408.jsp&mid=WC0b01ac058002958c>

b Definition derived from ([Konara et al. 2016](#_ENREF_98)),(and references therein)

c Interchangeability is determined at a national level, currently only France requires this evidence.

d FDA guidance (covers mAbs, although not specific), <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf>

and general principles; <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291134.pdf>

e Example: MYL-14010 (Mylan N.V./Biocon Ltd.) demonstrated equivalent structural (Fc glycosylation occupancy, stability), functional (HER2 binding, FcRIIIa/FcRn binding, ADCC and cell proliferation inhibition), clinical pharmacological (healthy subject single dose trial, n=120, PK primary endpoint) and clinical efficacy/safety (phase 3 RCT of untreated metastatic HER2 positive breast cancer, n=458, loading and maintenance dosing primary endpoint overall response at week 24 [if within 90% CI, trial established +6% difference] and no clinically meaningful difference in adverse event rate) to its reference trastazumab.([Food and Drug Administration 2017a](#_ENREF_59); [Rugo et al. 2016](#_ENREF_148))

fU.S. state law can take precedence over FDA advice.

gDefinition derived from <http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2016/03/WC500203430.pdf> This is an FDA slide deck available from the EMA. It is unclear if the EMA endorses this definition or not.

n.b.1. The World Health Organization has also published biosimilar guidance in 2009 which may be taken into account by market authorization bodies outside the EU and the US. See <http://www.who.int/biologicals/areas/biological_therapeutics/BIOTHERAPEUTICS_FOR_WEB_22APRIL2010.pdf>

n.b.2. For a more comprehensive discussion of the regulatory issues surrounding biosimilar/biobetter mAbs, refer to ([Elgundi et al. 2017](#_ENREF_47)) (and references therein)([Elgundi et al. 2017](#_ENREF_47)) and [Konara et al. 2016](#_ENREF_91)) (and references therein)([Konara et al. 2016](#_ENREF_98))

ADCC, antibody-dependent cellular cytotoxicity; AE, adverse event; C1q, component 1q; CDC, complement-dependent cytotoxicity; CI, confidence interval; CNS, central nervous system; CR, complete response; DFS, disease-free survival; EMA, European Medicines Agency; FDA, Food and Drug Administration; FcR, PD, pharmacodynamics; PK, pharmacokinetics; mAb, monoclonal antibody; PFS, progression-free survival; OS, overall survival.

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# Potential conflicts of Interest

Martin Dalziel is currently employed by Oxford PharmaGenesis Ltd, a HealthScience communications consultancy that provides services to pharmaceutical companies. The views expressed in this article are his own and not necessarily representative of his current employer.

Stephen Beers has acted as a consultant and advisory board member for Astex Pharmaceuticals and has received research funding from Bioinvent International.

Mark Cragg is a retained consultant for BioInvent and has performed educational or advisory roles for Baxalta, Roche and Boehringer Ingleheim. He has received research funding from BioInvent International, Roche, Gilead, iTeos and GlaxoSmithKline.

Max Crispin is a named inventor on a patent application describing the combined therapeutic use of endoglycosidase and therapeutic antibodies (PCT/GB2013/050164). Rights were assigned to Immago Biosystems prior to acquisition by Hansa Medical. He has also provided testimony to JA Kemp patent attorneys who were acting on behalf of Hansa Medical. He has also acted as a consultant for LimmaTech Biologics.