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Surrogate approaches in development of monoclonal antibodies

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When cross-reactivity of a lead antibody across species is limited, antibody development programs require the generation of surrogate molecules or surrogate animal models necessary for the conduct of preclinical pharmacology and safety studies. When surrogate approaches are employed, the complexities and challenges for translation of preclinical safety and efficacy results to the clinic are undoubtedly enhanced. Because there are no currently established criteria or regulatory guidance regarding the application of surrogate approaches, a science-based strategy for translation of preclinical information to the clinic is vital for effective development of the lead antibody.

Introduction

One of the greatest challenges in the development of a novel monoclonal antibody therapeutic is identification of the most pharmacologically relevant species. When cross-reactivity of the lead antibody across species is limited, alternate approaches – such as surrogate molecules or surrogate animal models – are necessary for the conduct of preclinical safety studies. Although the surrogate approach enables examination of pharmacokinetic (PK) and pharmacodynamic (PD) properties in a potentially relevant species, it undoubtedly increases the complexities and challenges encountered during the course of antibody development [1–4]. Because successful translation of preclinical information through various development stages requires particular understanding of both antibody and antigen properties, application of surrogate approaches will necessitate comparative investigations of the relevant pharmacology (i.e. epitope and signaling/functional comparability), antibody affinity, and the sequence and structural properties of the antigen, as well as an understanding of the factors impacting antibody exposure–response relationships across various species [1,3–5]. This review addresses the application of surrogate approaches for the development of therapeutic monoclonal antibodies.

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Considerations for application of surrogate molecules

The biological activity and safety profiles of human and surrogate IgG antibodies are frequently evaluated in mouse and monkey models. Several caveats must be considered; when interpreting results from *in vivo* studies in these model systems. Because binding to various Fc receptors will impact antibody PK and *in vivo* potency, evaluation of species-specific differences in the engagement of IgG antibodies to the cellular immune system is of crucial importance. A great deal of interest in the field over the past 15 years has dealt with understanding the interaction of IgG antibodies with Fc receptors (i.e. FcRn and FcγR) [5–10].

Interaction with Fc receptors

In both humans and rodents, interactions of IgG antibodies with FcRn receptors regulate PK properties of the antibody *in vivo* [5,11–14]. FcRn is a member of the major histocompatability complex structural class that is involved in IgG transport and is one of the factors influencing the half-life of IgG antibodies in serum [5,10,11]. Because of a stronger affinity of human IgG antibodies for murine FcRns, human IgG antibodies exhibit a

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longer circulating half-life and a much lower clearance in mouse, in comparison with mouse IgG antibodies, which is not predictive of human clearance [2,5,11,12]. Undoubtedly, the differences in binding residues involved in interactions of mouse and human IgG antibodies with FcRn account for these differences [15,16]. It has been established that FcRn binds the Fc domain of IgG with high affinity at pH 6.0, and the complex dissociates at pH 7.2 [12–17]. FcRn binds to human and murine IgG in the CH_2 domain near the hinge region. The FcRn acts as a salvage receptor by binding to IgG after pinocytosis in the slightly acidified endosome; hence, the IgG is saved from a default degradation pathway by being recycled back to the cell surface, where the IgG is dissociated at neutral pH [5,12–17]. Crucial mouse Fc residues involved in the FcRn interaction are Ile^{235}, His^{310}, His^{433}, Asn^{434} and His^{435}; His^{310} and His^{435} are responsible for the pH-dependent binding because histidine residues have a $pK_a \sim 6$. For human, the crucial Fc residues involved with FcRn interactions are Ile^{235}, Ser^{254}, His^{435} and Tyr^{436} [10,12,15–17]. Measurement of the equilibrium dissociation constant (affinity) of the Fc domain/Fc receptor interaction is one of the key pieces of data needed to fully optimize antibody engineering for improved PK and/or PD properties (see next section).

Antibody effector function

The mode of action of antibodies *in vivo* is highly dependent on the antibody isotype. In the case of effector functions, including antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), different subclasses of antibodies elicit varying degrees of potency and efficiency. Mouse IgG2a, IgG2b and IgG2c are the most potent in mediating ADCC, and IgG2a and IgG2b can also efficiently activate the complement cascade [18,19]. In humans, IgG1 and IgG3 are the most effective in mediating ADCC and CDC [20–22]. When surrogate molecules are employed for conducting safety studies, it is crucial that the appropriate human comparable antibody isoforms are also selected. For example, the mouse equivalent to human IgG1 is IgG2a, not IgG1 [2,23]. This consideration is of crucial importance because PD and safety profiles of IgG antibodies *in vivo* can be partly regulated by their interactions with Fcγ receptors [1,3–5,23]. FcγRs bind to human and mouse IgG in the CH_2 domain near the hinge region. In humans, six FcγR family members have been identified, all having a similar protein structure belonging to the immunoglobulin superfamily [24]. These members include FcγRI (CD64), FcγRIIa,b,c (CD32a,b,c) and FcγRIIIa,b (CD16a,b) [24]. In mouse, the human equivalents of FcγRI, FcγRIIb and FcγRIIIa have been described. Mouse and human FcγRI and FcγRIIIa exhibit equivalent activating properties; mouse FcγRIIb exhibits inhibitory functions that dampen the cellular response, similar to the human homolog [24–26].

Fc receptor expression profiles

The expression profile of Fc receptors in various tissues is an additional consideration for evaluation of a surrogate antibody candidate. Both human and mouse FcγRI are expressed on myeloid cells and mediate signals leading to phagocytosis [24–27]. Human FcγRIIIa is also responsible for IgG-initiated ADCC and is the only Fc receptor expressed on human NK cells. Mouse FcγRIII exhibits a similar expression profile to its human homolog and is the only FcγR expressed on mouse NK cells [24–28]. Recently, a new member of the mouse FcγR family has been identified: FcγRIV, an activating receptor that is expressed on neutrophils, monocytes, macrophages and dendritic cells and binds to IgG2a and IgG2b, but not IgG1 or IgG3 isotypes [29–31]. FcγRIV exhibits 63% overall amino acid identity to FcγRIIIa; however, mouse FcγRIV is not expressed on NK cells. Recent studies indicate that a majority of the effector function in mouse is attributable to this receptor [31]. In nonhuman primates, only one CD16 gene, homologous to human CD16a (FcγRIIIa), has been described [32]. In humans, CD16a is expressed on monocyte subpopulations, macrophages, NK cells and select γδ T cells. Similar to humans, CD16 expression was observed on a lymphocyte subpopulation, on monocytes and on neutrophils of sooty mangabeys [32]. CD16, however, was detected only on a lymphocyte subpopulation and on monocytes in macaques and baboons. The nonhuman primate ortholog also differed in its ability to bind IgG isotypes. Nonhuman primate CD16 interacts with human IgG1 and IgG2, but by contrast, human CD16 binds to IgG1 and IgG3 [21–23,32].

Antibody isotype and affinity

Knowledge of the affinity of IgG antibodies to various Fc receptors in any given animal species is vitally important for effective design of efficacy and safety studies when surrogate antibodies are employed (Table 1). Human IgG1 is believed to be the most effective of the human isotypes in mouse models, although IgG4 is comparable in activity, despite its low effector function with human cells [33,34]. The binding of human IgG1 to mouse FcγR, however, is of reduced affinity. Improved activity is generally

TABLE 1

Equilibrium dissociation constants (K_D) for Fc receptor/IgG complexes in mouse and humans

Receptor	Immunoglobulin	Equilibrium dissociation constant (nM)	Refs
Hu FcγRI	Hu IgG	0.5–10	[7,9]
Hu FcγRII	Hu IgG	>100	[7,9]
Hu FcγRIII	Hu IgG	5900	[7]
Hu FcRn[a]	Hu IgG1; Mu IgG1; Mu IgG2a and Mu IgG2b	630–2500; >50 000; >50 000; 18 100	[8,12]
Mu FcRn[a]	Hu IgG1; Mu IgG1; Mu IgG2a; Mu IgG2b	82–269; 750; 420; 500	[8,12]
Mu FcγRI	Mu IgG2a	6	[26]
Mu FcγRIIB	Mu IgG1, IgG2a, IgG2b	100–2500	[26]
Mu FcγRIII	Mu IgG1, IgG2a, IgG2b	670–3300	[26]
Mu FcγRIV	Mu IgG2a, IgG2b	3–59	[26]

[a] K_D value quoted is for binding at pH 6.0.; Mu: mouse; Hu: human.

observed when the antibody is in a format that is compatible with the species' immune system. For example, when the variable domains of adecatumumab, an antibody targeted against human EpCAM, were genetically fused to a mouse IgG2a backbone, ADCC with mouse effector cells was markedly improved, yet significantly reduced with human effector cells [35]. The genetically engineered antibody with the murine IgG2a backbone also exhibited improved activity in a B16 melanoma mouse model, in contrast to the fully human version of the antibody [35]. Therefore, these results underscore the importance of the species origin of the Fc portion of a monoclonal antibody.

It is also worth noting that human FcγR polymorphisms might obscure the predictive value of surrogate antibodies. For example, several reports describe that a polymorphism of FcγRIIIa is associated with tumor response in follicular lymphoma patients treated with rituximab as first-line therapy [36–38]. Importantly, equivalent polymorphisms are not recapitulated in mouse or nonhuman primate models; the impact of these polymorphisms on interactions between Fc domains and FcγRs, therefore, might not be predictive with a surrogate antibody in these model settings. In the case of CDC, current data do not indicate species dependence. For example, human adecatumumab (as described above) and mouse edrecolomab, two antibodies targeted against human EpCAM with similar affinity, exhibited identical CDC activity with human complement *in vitro* [39].

Considerations for application of surrogate animal models

Transgenic mouse models

Often the greatest challenge in the development of novel monoclonal antibody therapeutics is finding the most pharmacologically relevant species for safety and pharmacology testing [1,3]. It is essential to note that 'relevant' signifies not only that the monoclonal antibody binds appropriately in a tissue-specific manner but also that it generates the expected pharmacology (i.e. epitope and signaling/functional comparability). The language in the ICH S6 guidance allows an exploration of nontraditional animal models when no pharmacologically relevant traditional model can be identified [40]. Mouse transgenic models can be informative tools in screening for relevant pharmacology and contribute to the totality of the prediction of safety outcomes in humans. With an increasing number of biopharmaceuticals under development, results generated from studies in transgenic animals will become important for demonstrating their utility as alternative animal models in assessing antibody safety profiles.

Several types of transgenic animals, such as knockout (KO) and humanized knockin (KI) models, have been used to assess drug specificity, investigate mechanisms of toxicity and screen for mutagenic and carcinogenic activities of therapeutic candidates. The effect of total target blockade by novel therapeutic candidates can be approximated in KO mice (e.g. generation of viable and fertile animals with null mutations for a potential target protein might suggest that pharmacological inhibition of the molecule *in vivo* is unlikely to elicit major adverse effects on normal physiological functions) [41]. By the same token, care must be taken concerning the value and interpretation of these studies because KO mice might have uncharacterized compensatory mechanisms or redundant pathways to replace the function of the absent protein(s) or target antigen that are not readily apparent [42–44].

'Humanized' KI animals, in which the human gene is inserted into the mouse genome, are becoming especially useful in evaluating the efficacy and toxicity of human biopharmaceuticals that are not pharmacologically active in traditional toxicology species [45,46]. Using mouse molecular genetics approaches, for example, a humanized form of the antigen can be used to replace the endogenous mouse gene via homologue recombination. The goal of this approach is to create a hybrid mouse/human gene within the context of the native genomic loci, whereby natural control of transcription and cell-type-specific expression would be retained, producing a human gene product in replacement of the mouse protein. In doing so, the resulting animal retains the normal physiological function of the target yet is amenable to change in function based on binding of the introduced therapeutic antibody candidate.

An example of the utility of this approach was recently described by Kostenuik *et al.* [47], who created a human RANKL KI mouse line to assess the bioactivity of the RANKL-neutralizing antibody Denosumab. Denosumab is a fully human IgG2 kappa antibody that does not cross-react with rodent forms of the antigen [48]. Human forms of the RANKL protein, however, have been shown previously to interact with murine forms of the receptor, to stimulate osteoclastogenesis from mouse hematopoietic cells and to induce bone resorption when systemically introduced into mice [49]. Furthermore, the human RANKL protein was shown to bind the murine forms of osteoprotegerin, suggesting that the human KI gene product would be subject to endogenous regulatory controls *in vivo* and during development of the organism. The viable humanized RANKL mice exclusively expressed the chimeric (human/murine) RANKL product and were found to develop as normal animals with physiologic remodeling of the skeleton. Injection of Denosumab into adult humanized RANKL mice reduced bone resorption, increased both cortical and cancellous bone mass, and improved trabecular microarchitecture in a dose-dependent manner, as would be predicted from previous analysis of RANKL inhibitors in a mouse system [47].

Although specific approaches similar to those described above should allow administration of the clinical candidate to a mouse, there is a risk to this approach; despite the presence of a human gene, the mouse may probably develop antibodies against the clinical candidate. This could make long-term dosing for safety assessment impossible. Further considerations must be given to comparable tissue target expression, receptor density and the physiological effects of human–mouse protein interactions that might differ slightly or substantially from that of the normal human–human association (e.g. Toll-like receptors) [50,51]. In addition, despite the challenges in using novel tools to assess safety of monoclonal antibodies, this approach has been used successfully in development of antibodies [52].

Mouse strain

Selection of an appropriate mouse strain is an important consideration for profiling antibody-mediated immune responses.

For example, C57BL/6 mice predominantly produce Th1 helper cells in response to antigenic stimulation and thus exhibit a Th1 cytokine bias characterized by IFN-γ production. However, BALB/c mice display Th2-dominated immune responses characterized by IL-4 production [53,54]. It is important to note that target antigens might exhibit different pharmacological roles in animal species comparison to its function in human; for example, TGN1412, an anti-CD28 superagonist antibody, induced a life-threatening cytokine storm in human patients in a recent clinical trial [55,56]. Interestingly, this toxicity was not predictable from preclinical animal models. In rodents, CD28 superagonists induced the preferential expansion of regulatory T cells [57], whereas in humans, TGN1412 induced a sustained calcium response in naïve and memory $CD4^+$ T cells, leading to pronounced cytokine release [57]. In contrast to humans, cynomolgus macaques did not exhibit any adverse reaction at the clinical dose (0.1 mg/kg) [56,57]. The loss of CD33-related Siglec expression in man is one potential mechanism that might explain the diversity observed in CD28 pharmacology across species [58]. Siglecs are sialic-acid-recognizing Ig-superfamily lectins prominently expressed in immune cells. CD33-related Siglecs are inhibitory receptors, and their expression seems to have been lost during evolution from monkey to human [58]. The absence of CD33rSiglec expression on human T cells might have contributed to the marked stimulation of these cells in human subjects.

Translational considerations

As highlighted, effective translational strategies should deliver superior and safe therapeutic candidates for clinical development in the treatment of the intended patient population. An understanding of the relationship between the 'unit dose' and 'unit effect' with respect to both beneficial and deleterious effects is essential in accomplishing this objective [1,3,4,59]. Because there are currently no established criteria or regulatory guidance regarding the application of surrogate approaches, a science-based approach for transition of preclinical information is vital for effective antibody development [1–3,59]. Successful strategies for the development of the lead candidate, therefore, will require comparative investigations and integration of knowledge with respect to target antigen properties (expression and density), pharmacology, antibody design criteria (such as affinity, isotype selection and Fc domain interaction), as well as evaluation of the factors that impact antibody exposure–response relationships across species [1,3,59].

Pharmacodynamic system efficiency

The stimulus–response mechanisms that convert receptor occupancy into pharmacological response are usually nonlinear and, in some instances, disproportional [4]. Diversity in the relationships between receptor occupancy and response intensity could range from highly efficient (Figure 1a) to non-efficient responses (Figure 1b). For highly efficient responses, the binding curves denoting receptor occupancy lie to the right of the concentration–response curves ($K_D > EC_{50}$) owing to large amplification factors in signaling cascades. By contrast, for the non-efficient responses, loss of signal in the cascade might contribute to the underlying system inefficiency and, hence, receptor occupancy curves might lie to the left of the concentration–response curves ($K_D < EC_{50}$) [60]. Highly efficient pharmacological systems have been described previously for production of glucose via glucagon or β-adrenoceptor pathways [60,61]. For antibodies in which effector functions (ADCC and CDC) contribute significantly to the underlying mechanism of action, such as depleting antibodies with enhanced effector function properties [23,62–65], highly efficient pharmacological responses are anticipated (Figure 1a). Hence, it is evident that application of surrogate approaches for this class of antibodies will require accurate attention to comparative investigations for Fc receptor expression, effector function pharmacology and target distribution

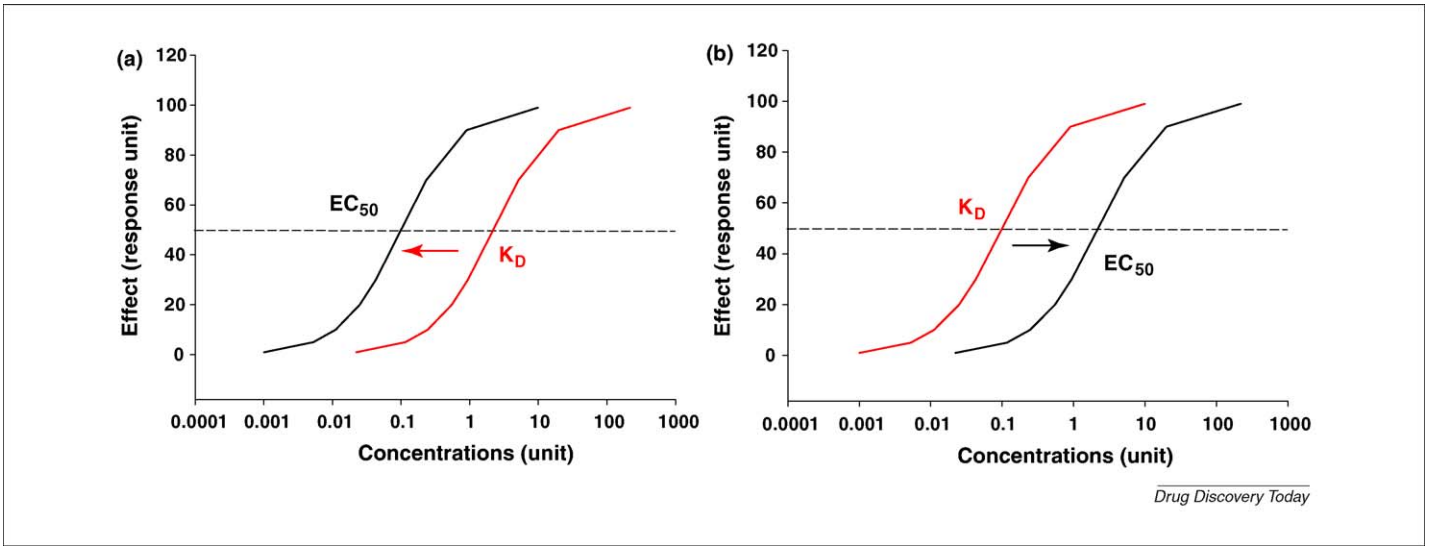

FIGURE 1

Relationships between receptor occupancy (K_D) and response intensity (EC_{50}) for (a) highly efficient and (b) non-efficient responses. For the former, the binding curves denoting receptor occupancy lie to the right of the dose (or concentration)-response curves owing to large amplification factor in signaling cascades, whereas for the latter, the loss of signaling or abbreviation of signal in the cascade might contribute to the underlying system inefficiency.

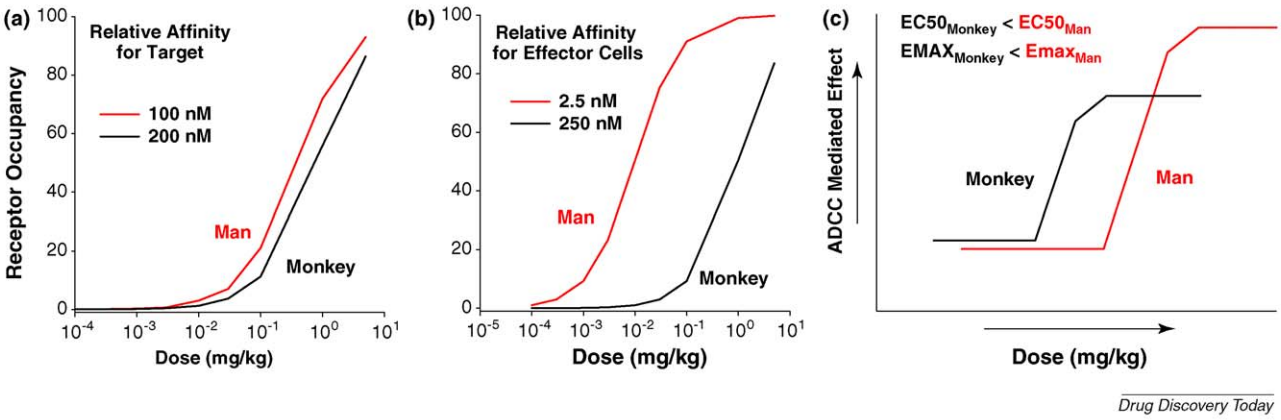

FIGURE 2

Theoretical predicted differences in receptor occupancy for the (a) target and (b) effector cells for the surrogate (cross-reactive to monkey) and lead antibody at a comparable doses. (c) The differences in the efficiency (E_{MAX}) and potency (E_{C50}) of the effector systems for surrogate and the lead antibody across species.

across species. The importance of these considerations is highlighted in the example below.

A case study

Biophysical measurements are one of the crucial components necessary for developing effective translational strategies when surrogate approaches are employed. The relevant experimental knowledge obtained from biophysical studies will enable adjustment of affinity differences between the lead and surrogate(s) across species for appropriate dose selection [1]. Such an approach was implemented recently for the design of preclinical safety studies in monkeys during development of a bispecific antibody. The lead bispecific antibody was designed to recruit effector functions for depletion of the cancer cells that expressed the target of interest. Because the lead antibody did not recognize the effector cells in monkey or rodents, a surrogate molecule was developed. Like the lead antibody, the surrogate recognized the target antigen in monkey tissues with a comparable affinity (lead vs surrogate: 100 nM vs 200 nM; Figure 2a). A 100-fold affinity difference was however, observed after evaluation of the lead affinity for human effector cells as compared to the surrogate affinity for the monkey effector cells (lead vs surrogate: 2.5 vs 250 nM; Figure 2b). As shown in Figure 2, theoretical evaluation of binding curves predicted crucial differences in receptor occupancy for the effector cells for the surrogate at a comparable dose to the lead. Additionally, *in vitro* results indicated significant differences with respect to potency (EC_{50}) and efficiency (E_{max}) for the effector functions across species (Figure 2c). Hence, considerations with respect to affinity differences and the diversity in the efficiency and potency for the effector systems across species were of crucial significance for the design of effective preclinical safety studies and dose selection across species.

Emerging antibody-based modalities

With the advancement in technologies employed in generation of antibodies, more advanced antibody-based modalities are emerging [63–69]. Currently, many bispecific or trispecific antibodies are in development, and many of these constructs rely on recruiting endogenous immune effector cells to exert their pharmacological effects. For example, the bispecific T cell engagers, or BiTE®, carry dual specificity in their binding arms and rely on recruiting cytotoxic T lymphocytes of the host for killing cancerous cells [62,67–69]. Although current clinical results underline the potential significance for these bispecific constructs as therapeutics, their development path to the clinic has been complex because of limited cross-reactivity across species. In general, the development path into the clinic for these molecules has required application of specific surrogate approaches [69]. It is evident that as the complexities in antibody-based therapeutics increase, it is progressively more challenging to achieve a desirable cross-reactivity profile for the lead molecule across species; hence, the reliance on surrogate approaches will become a necessity. As highlighted, many factors will need to be evaluated for designing the most effective translational strategies necessary for the successful development of antibody-based therapeutics.

Concluding remarks

A science-based decision-making approach for translation of preclinical information is vital for effective development of antibody-based therapeutics. When limited cross-reactivity of the lead candidate necessitates the use of alternative approaches for preclinical safety and pharmacology assessment, many crucial factors must be considered to assure the integrity of the results in identifying potential risks to humans and in establishing the safe starting dose in the clinic. Particular attention must be given to an understanding of relevant species selection, target pharmacology, effector function efficiency, Fc receptor expression and distribution, as well as comparable antibody isotype selection across species.

Conflict of interest statement

At the time of submission of this article, M.T. and W.J.B. were employed by AnaptysBio, Inc. and G.G.B., S.K. and L.A. were employed by AstraZeneca, Takeda and Genzyme Corporation, respectively.

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