

# Challenges and opportunities for monoclonal antibody therapy in veterinary oncology



**Review**

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**Abstract**

Monoclonal antibodies (mAbs) have come to dominate the biologics market in human cancer therapy. Nevertheless, in veterinary medicine very few clinical trials have been initiated using this form of therapy. Some of the advantages of monoclonal antibody therapeutics over conventional drugs are high specificity, precise mode of action and long half-life which favours infrequent dosing of the antibody. Further advancement in the field of biomedical sciences has led to the production of different forms of the antibody such as single chain antibody fragment (scFv), Fab, bi-specific and drug conjugates for use in diagnostic and therapeutic purposes. In this review we describe the potential for monoclonal antibodies in veterinary oncology patients in supporting both diagnosis and therapy of cancer. We explore the technical and financial hurdles to facilitate clinical acceptance of mAbs and offer insights into novel technologies and targets that could support more rapid clinical development.

*Keywords*: Monoclonal antibodies; Veterinary oncology; Therapy; Idiotype; Biologicals

## Introduction

The history of monoclonal antibody (mAb) therapy can be traced back to the start of the hybridoma technology, in 1975 (Köhler and Milstein, 1975). Fig. 1 describes the chronology of mAb development.

The greatest advances in monoclonal antibody therapeutics began when recombinant DNA technology could be applied to antibody design. This facilitated the reduction in their immunogenicity and optimisation of antibody half-life and effector functions. Despite that, mAbs have taken a long time since the early days of discovery until the current "golden phase" of cancer therapies using biologicals (Waldmann, 1991). Since then, anti-cancer mAbs – and also mAbs for other therapeutic purposes – have become some of the highest selling medicines ever (King, 2013).

The importance of monoclonal antibodies as therapeutics is increasing, and currently there is an average of four new molecules reaching the human therapeutics market every year. This class of treatment is seen as critical for the future of the pharmaceutical industry, especially in a time when the development of new drugs and the discovery of new targets is declining (Munro et al., 2011; Arrowsmith, 2012; Ecker et al., 2014).

## Mechanisms of action

Several mechanisms of action of the monoclonal antibodies have been identified. They are summarised in Table 1 per the molecular mechanism to which they are relevant (Glennie and Johnson, 2000; Adams and Weiner, 2005).

Many antibody functions shown in Table 1 are dependent on the Fc region of the mAb. The interaction between the Fc region of the mAb and Fc receptors (FcR) is crucial for the development of immune-mediated functions of the antibody, such as antibody-dependent cell-mediated cytotoxicity (ADCC). Complement-dependent cytotoxicity (CDC) can also be activated by interaction with the Fc fraction of the mAb. This interaction is specific, such that not all antibody isotypes interact with the same intensity with the FcR, even within the IgG subclass in a single species, for instance (Bergeron et al., 2014; Strietzel et al., 2014). Therefore, the choice of Fc region during the chimerisation process (described below) affects the effector functions of the therapeutic speciated mAb. In dogs, IgGB and IgGC are capable of driving ADCC and CDC, while IgGD is only capable of activating the complement cascade (for CDC), but not of binding to FcγRs (for ADCC or phagocytosis). The other IgG isotypes of dogs, "A" and "D" are not drivers of antibody-mediated immune responses. Human IgG1 and mouse IgG2a also bind to canine FcγRI, and are potentially useful as therapeutics in dogs (Helfand et al., 1994; Bergeron et al., 2014). Indeed, mAb 231, a murine antibody previously used against canine lymphoma (described below in detail), is reported to show antibody-mediated cytotoxicity and complement activation in dogs (Rosales et al., 1988). In cats, feline IgG1a and IgG1b bind to Fc receptors and also seem capable of mediating CDC, while IgG2 does not (Strietzel et al., 2014).

IgE-Fc receptor binding is also being explored as a potential mechanism to treat cancer. There are two views on the use of IgE antibodies against cancer: 1) IgE seems to have a role in preventing cancer; 2) IgE can be used to target FcεR-expressing mast cell tumours. As for the first approach on the use of IgE, this isotype is more effective in inducing ADCC against cancer cells, as well as inducing stronger responses (Singer and Jensen-Jarolim, 2014). The second approach takes advantage of the high expression of IgE-binding Fc

receptors (FcεR) on mast cell tumours; thus, IgE can be used as a platform for the delivery of therapies to these cells (Elders et al., 2014).

## Monoclonal antibody therapy strategies for cancer in veterinary medicine

### *The past of mAb therapy in veterinary medicine: mAb 231*

One of the first clinical monoclonal antibody licenced for cancer treatment, denominated mAb231, was for use in canine lymphoma, although it was later discontinued. (Jeglum, 2009; USDA, 2016). Three years before the licencing of the first cancer-targeted monoclonal antibody in Germany (Panorex), and five years before the first monoclonal antibody was licenced by the FDA for the treatment of cancer in humans (Rituximab), a monoclonal antibody received approval in the US to treat lymphoma in dogs, called mAb 231. The selection of the monoclonal antibody was carried out against tumour cells and the molecular target of this antibody was never unveiled. Immunoprecipitation using the antibody did not display any protein. The antibody was nevertheless able to bind to 73% of the formalin-fixed and paraffin-embedded lymphomas tested (Steplewski et al., 1987; Jeglum, 2009). The antibody is of the IgG2aκ subclass, with a detailed study of its structure having already been performed (Harris et al., 1997). Its mechanisms of action were reported to be due to antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) (Rosales et al., 1988) and inhibition of tumour growth (Steplewski et al., 1987).

The antibody was to be administered to dogs in remission after two four weeks' cycles of vincristine, cyclophosphamide, L-asparaginase and doxorubicin (modified VCAA) and a three-week rest. The total dose was 100mg/m$^2$, to be divided into 5 sequential days and to be administered over two hours. If lymphoma recurred after the administration of the Mab

231, a new 4-weeks cycle of the modified VCAA was to be administered, followed by a 3 weeks' rest and a new round of antibodies (Jeglum, 1996, 2009).

The complete remission of the 215 dogs tested was 80.9%, with a mean survival time of 624.28 days for the responders (in complete remission) against 153.67 days for the non-responders. Non-responders tended to be from the group of animals to which mAb231 does not bind on immunohistochemistry (Jeglum, 1996). Anecdotally, the administration of mAb 231 showed a disproportional rate of achieving indefinite remission when compared to chemotherapeutics alone (Crow, 2008).

The drug was discontinued in 1996, apparently due to lack of demand (Crow, 2008; Kaplan, 2008). This mAb is no longer licenced for use by the USDA (USDA, 2016). Yet, it must be brought to attention that the drug was marketed in a time when antibody therapy to cancer was non-existent in human medicine, nor had the subject the same attention it receives currently (Reichert, 2012).

*The present of mAb therapy in veterinary medicine: antibodies reaching the market*

The veterinary market is currently going through its first intense wave of interest in monoclonal antibodies (Harvey, 2015; Ledford, 2016). Two product launches by Aratana Therapeutics, against B (product AT-004, anti-CD20) and T cell lymphomas (AT-005, anti-CD52) were widely regarded in the market as great promises for veterinary oncology. AT-004 was shown to increase median progression-free survival of dogs with B cell lymphoma (Anderson and Modiano, 2015). However, recent post-USDA licencing trials have not shown encouraging results; in randomized clinical trials combining AT-005 to two different chemotherapy protocols, mAb therapy did not seem to improve clinical outcomes compared

to placebo. Aratana indicated that both AT-004 and AT-005 showed poor specificity to their respective targets. Despite this, both antibodies are currently still commercially available[1].

While other commercially available options of mAbs for the treatment of cancer in animals are not yet available, there are candidates approaching or at market launch for other clinical conditions. Zoetis has recently been granted a conditional licence by the USDA for lokivetmab, to be used for treatment of canine atopic dermatitis. This mAb blocks IL-31, which is important for the development of pruritus (Michels et al., 2016), with the intent of interrupting the cycle of itching and inflammation typical of atopic dermatitis (Zoetis, 2015)[2]. Nexvet Biopharma is investigating a target for chronic pain, with an anti-nerve growth factor mAb. Interestingly, they have already demonstrated the efficacy of this mAb both in caninised and in felinised formats (Gearing et al., 2013, 2016)[3].

*The future of mAb therapy in veterinary medicine I: possible targets*

There is a limited number of publications evaluating the effects of monoclonal antibodies or related molecules in veterinary species. Shown below in Table 2 are antibodies that have been trialled or used in domestic animals, as well as future potential targets for these patients.

*The future of mAb therapy in veterinary medicine II: 'speciation'*

Veterinary medicine has always borrowed tools from human practice. However, contrarily to chemical drugs, biologicals such as mAbs are in most cases species-specific.

[1] See: http://aratana.investorroom.com/2015-09-24-Aratana-Therapeutics-Provides-Product-Updates (accessed 15 June 2016).
[2] See: http://news.zoetis.com/press-release/companion-animals/zoetis-receives-conditional-license-usda-canine-atopic-dermatitis-im (acessed 16 June 2016)
[3] See: http://ir.nexvet.com/phoenix.zhtml?c=253841&p=irol-newsArticle&ID=2176442 (accessed 16 June 2016).

Although the process of antibody caninisation is now becoming more widely used, it is still a time-consuming method (Gearing et al., 2013). Also, producing mAbs exclusively for one animal species may not be always commercially viable, and may impede further developments of new candidates. This is clearly illustrated by the fact that while there are now tens of monoclonal antibodies approved worldwide for cancer therapy in humans (Modjtahedi et al., 2012; Reichert, 2012; Ecker et al., 2015), in veterinary oncology, however, therapeutic antibodies are only just reaching the market (USDA, 2016).

The use of antibodies that have been developed for human therapy in animals is an attractive approach. Some groups have explored the potential use of human mAbs in dogs. Stein and colleagues, for instance, successfully tested an anti-human leukocyte antigen DR in dogs. While it was then concluded that dogs were validated as good models for the trial of an antibody, the authors also note how the study and the patients would have benefited from a species-specific antibody (Impellizeri et al., 2006; Stein et al., 2011; Biller et al., 2014).

Monoclonal antibody 231, the best example of mAb therapies in veterinary medicine, may have had reduced efficacy due to its xenogeneic origin. The canine anti-mouse antibody (CAMA) levels were raised after therapy with this antibody, and, as expected, further applications resulted in quicker and higher production of CAMA. No clinical disease seemed to arise from the use of this murine antibody in dogs. The investigators believed that length of remissions and survival times were not affected by the murine nature of the antibodies, but it was never directly compared to a 'caninised' version of the antibody (Jeglum, 1996, 2009). Possible side effects were occasionally observed, including facial oedema, erythema, fever, pruritus, vomiting and/or diarrhoea, joint pain and myalgia. Although the antibody seemed to

provide clinical benefits, it is possible that its murine origin was part of the reasons why it was later discontinued (Jeglum, 1996, 2009).

Much more likely to be successful is the interchange of therapeutic antibodies between dogs and cats, for instance. Fc receptors, which are crucial for antibody half-life and effector functions, are very similar between these two domestic species (tested using Ensembl Genome Browser and ClustalOmega[4]) (Lobo et al., 2004; Strietzel et al., 2014). This could prove to be an important feature for veterinary practitioners when new mAbs become available for either species, since development of mAbs for these patients is slow and receives relatively little attention, especially for cats, for whom no mAb is available currently in the market.

Even though the majority of the literature indicates that anti-globulin responses limit the function of mAbs, as discussed above, there are still licensed antibodies for use in humans that are not humanised. Understanding how they are effective may set new parameters that may be useful for the production of innovative antibodies for domestic animals.

At least two mouse antibodies are still currently in use for the treatment of malignancies in humans. Both of these target CD20, and both are conjugated to radionuclides. The use of murine antibodies in humans is permitted in these cases because the mAbs are clinically useful with single dose schemes. Rituximab, a chimeric mAb that also targets CD20 in humans, is used in a course of several doses, in comparison (Witzig et al., 2002; Burdick and Macklis, 2009). Further possible explanations for the low seroconversion from the use of these antibodies include the occurrence of immune suppression. It is expected

[4] See: http://www.ensembl.org/index.html and http://www.ebi.ac.uk/Tools/msa/clustalo/ (accessed 6 March 2015).

214 that both normal and malignant B cells are depleted by the treatment, therefore limiting the
215 capacity of the host to react to the mAbs (Mirick et al., 2004). Therefore, these results leave
216 open the possibility of using non-speciated mAbs in animals if these are administered in
217 single-dose regimens; mAbs coupled to radionuclides are potential candidates.
218
219 **The process of speciation**
220 As should be expected, xenogeneic antibodies are more immunogenic than syngeneic
221 ones. Therefore, speciation was probably the single most important step towards the success
222 of mAbs in clinical practice, since anti-mAb responses are responsible for rapid clearance of
223 circulating mAbs as well as for several side effects (Glennie and Johnson, 2000).
224 Chimerisation was the first stage towards antibody speciation (Hwang and Foote, 2005). In
225 this technique, after the antibody is raised and selected from mouse hybridomas, the murine
226 variable regions are cloned with human (or from any other species of interest) constant
227 regions by fusing the immunoglobulin genes in an expression vector (Fig. 2). This is then
228 expressed in a host system, such as Chinese hamster ovary (CHO) cells (Köhler and Milstein,
229 1975; Persic et al., 1997).
230
231 Humanisation is a further step in the engineering of the antibodies. This technology
232 starts from the chimerised version of the mAb, and attempts to further reduce the
233 immunogenicity of the antibody. In speciation, the framework regions of the variable fraction
234 (the parts of the variable fraction which do not contact the antigen) of murine antibodies are
235 substituted with human germline sequences. In this approach of speciation, CDRs are
236 retained from the original murine mAb sequence.
237

To completely avoid murine sequences, additional strategies have been developed to generate fully speciated monoclonal antibodies through display technologies (such as phage display) and transgenic mice (Deckert, 2009; Bradbury et al., 2011). In fully human antibodies, all of the mAb molecule, including the CDRs, which bind to the antigen, are from the species of interest (Thie et al., 2008). Phage display relies on libraries of antibody fragments or whole IgGs (reviewed by Miersch & Sidhu, 2012) (Fig. 3). These can be constructed from samples of the species of interest; thus, a display library can contain the virtually the entirety of the antibodies being expressed in a blood sample, or a spleen, for instance. The ultimate aim of the display technology is the selection of the recombinant antibody that can bind to the antigen with highest affinity from the immense number of non-specific antibodies in the library. After the construction of a display library putatively containing all the possible antibodies from one animal – or from several animals, if desired – this library can be screened against different therapeutic targets by the process of biopanning (Barbas et al., 1991). Since the library can be constructed from the target species, instead of mice, the antibodies it generates will be fully human (or canine, for example). Mice modified to contain a 'human' immune system are also used as an alternative for the production of fully human mAbs, but such a technology is not available for production of antibodies for domestic animals (Shultz et al., 2012). The aforementioned technologies associated with antibody production are illustrated in Fig. 4.

Although full speciation of antibodies has become the industry standard (Modjtahedi et al., 2012), more recently opinions have emerged questioning the real benefits of engineering antibodies beyond chimerisation. As stated previously, chimerisation is responsible for the main reduction of immunogenicity of the mAbs. Indeed, in many cases chimerisation is enough to prevent anti-mAb formation. Full speciation also has the

disadvantages that it is a lengthy and labour-intensive laboratory process, delaying product launch, and in many cases leads to lower antibody affinity, since it interferes with the antibody variable regions (Kettleborough et al., 1991). New speciation techniques have been developed to try and circumvent these problems; one such example is the "PETization platform", developed by a veterinary company (Gearing et al., 2013). However, full speciation does not guarantee that a mAb will not raise a host response, as has been seen with some fully humanised mAbs (Clark, 2000; Singh et al., 2012; van Meer et al., 2013; Waldmann, 2014).

In veterinary medicine, the first speciated mAbs are now attaining the necessary legal approvals and are entering the market (Acharya et al., 2015), and it will be interesting to analyse the responses of large cohorts of animals to such caninised antibodies, in special with regards to side effects.

**Potential side effects of mAb therapy**

The side-effects of mAb therapy can arise as a consequence of a host reaction against the antibody or of the direct activity of the mAb. The host reactions against mAbs can be either acute (infusion reactions or anaphilactoid) or dependent on the formation of anti-mAbs. Acute reactions include the cytokine release syndrome (CRS) and systemic inflammatory response syndrome (SIRS). A number of counter-measures are recommended, such as hydration, premedication and gradual increase in the rate of infusion. Anaphylactic and 'anaphylactoid' reactions occur more frequently with some antibodies than with others, probably due to the generation of IgE by the host. Adverse reactions can also be derived from the formation of anti-mAb antibodies after administration. These tend to appear and increase with subsequent doses, when the immune response has matured to secondary and adaptive

reactions. In these cases, high titres of anti-mAb IgGs can be found in the circulation of the patient. Monitoring anti-mAb responses relies on specialized techniques, not commonly available to the veterinary clinician; it depends on the concomitant development of the mAb with its coupled monitoring tests. Anti-mAb responses can be monitored, among other possibilities, by specific enzyme-linked immune sorbent assays (ELISAs), such as anti-idiotype ELISA (for an explanation on anti-idiotypes, see Fig. 5 below and associated discussion) or antigen capture methods (Shankar et al., 2007; Renaudineau et al., 2009).

Anti-mAb reactions may cause CRS and influenza-like symptoms in humans (McLaughlin et al., 1998; Hansel et al., 2010). In dogs, the murine mAb 231 caused clinical signs such as oedema and fever, but the occurrence of these signs did not impede subsequent administration of the treatment; however, the dogs were preventively treated with antihistamine, corticosteroids and the rate of infusion was reduced (Jeglum, 1996). It must be noted that side effect management and mAb treatment withdrawal should be considered on a case-by-case basis (Melosky et al., 2009). These host reactions tend to be more frequent when non-speciated (humanised or caninised) formulations are used, demonstrating the relevance of speciation (Adams and Weiner, 2005). The speciation process of antibodies, from chimerisation to full speciation, followed by fully human (or canine, feline) mAbs, reduces but does not exclude the probability of formation of strong host adverse reactions against the antibody. Moderate and mild host reactions may continue to occur, nonetheless (Hwang and Foote, 2005).

Reactions against fully speciated mAbs (canine against canine antibodies, for instance) occur due to the inherent variability of antibodies. The antigen-binding region (named the complementarity-determining region, or CDR) is by definition always structurally

313 novel to the immune system itself – that means that antibodies are always immunogenic,
314 regardless of how speciated the constant region of the mAb is. Although it is possible to
315 change a few (1-2) amino acids in the CDR of a mAb to reduce its immunogenicity, these
316 alterations always risk reducing the functionality of the antibody, since its very binding
317 region is being altered (Harding et al., 2010). The immunogenic property of the CDRs create
318 the phenomenon of the idiotype cascade presented in Fig. 5 (Herlyn et al., 1986). The main
319 importance of full speciation seems to be to reduce "marked" anti-antibody responses. When
320 reactions do occur against speciated mAbs, these tend to be "tolerable" or "negligible"
321 (Hwang and Foote, 2005).
322
323 The most common signs that follow the rapid infusion of rituximab, a human chimeric
324 antibody, are vomiting and nausea, rash and hypotension. The prophylactic use of
325 antipyretics and antihistamines is widespread to prevent these effects (Lang et al., 2011).
326 However, major acute reactions such as CRS can occur. Similar effects are also known to
327 occur after the administration of other chimeric mAbs such as Cetuximab (an anti-EGFR
328 mAb). These strong adverse reactions are less common for fully human mAbs, such as
329 Panitumumab (also anti-EGFR), but signs, such as skin rashes, still occur widely with the use
330 of this antibody (Hansel et al., 2010). Nevertheless, it has been highlighted that the most
331 common biological consequence of anti-mAb responses tends to be loss of efficacy, which is
332 deleterious but not a safety concern (Shankar et al., 2007).
333
334 The second type of possible reactions is dependent on antibody activity. One possible
335 effect of mAbs is the formation of Tumour Lysis Syndrome (TLS), where the release of
336 intracellular contents from the tumour after being targeted by treatment leads to metabolic
337 disorders, such as hyperkalaemia, hyperphosphatemia and hypocalcaemia (Jeha, 2001;

Gilbert and Wright, 2015). Undesired antibody activity has led to life threatening situations in the famous case of the CD28 superagonist antibody TGN1412, used with the goal of stimulating immune responses. This mAb had been trialled in human cells *in vitro*, in mice and in primates before reaching a human phase I clinical trial. However, in humans the mAb induced a severe cytokine storm (CRS): within minutes of administration patients had headaches, nausea, vomiting, pyrexia, a systemic inflammatory response with hypotension. This was followed by severe respiratory deterioration and several other signs of metabolic failure. It was later found that these effects were not predicted by pre-clinical tests due to immune differences between primates and mice compared humans – CD4 from macaques lose CD28 expression at the effector memory level, while mice induce strong regulatory T lymphocyte responses when administered with the superagonist. Additionally, the mere detail of cell density in cell culture assays seems to have affected the results so that the cytokine storm was also not predicted from these tests (Suntharalingam et al., 2006; Hünig, 2012).

Other side-effects related to antibody function may derive from off-targets. None of the currently approved anti-cancer mAbs target a tumour-specific molecule. The targets are also expressed in normal tissues, albeit often at different concentrations. Adverse reactions therefore may originate from the off-targeting of the mAbs to molecules associated with physiological functions, but these events are rarely life threatening or debilitating (Modjtahedi et al., 2012).

When an antibody is used to block the function of leukocytic cancers, the possibility of side effects related to suppression of normal immune cells exists. Alemtuzumab, used for lymphoid malignancies, targets CD52, which also exists on the surface of normal circulating lymphocytes, leading to lymphocytopoenia for up to one year after treatment is discontinued.

One of the possible consequences is the appearance of opportunistic infections. Rituximab targets CD20 on the surface of B cells, but secondary infection is normally only a problem in patients that were already immunosuppressed or taking immunosuppressive drugs concomitantly. Nevertheless, transient decline in leukocytes can occur after use of Rituximab and caution is recommended when administering multiple courses of the antibody. Additionally, reactivation of latent hepatitis B infections has been recorded in patients receiving Rituximab (Sharma et al., 2007; Kelesidis et al., 2011; Uettwiller et al., 2011).

*Benefits of immunosuppression*

Several factors influence the formation of anti-antibody responses by the patient receiving a mAb, such as the number of injections of the antibody, its immunogenicity and the immunocompetence of the recipient (Kuus-Reichel et al., 1994). Reducing the immune response against the mAb is therefore an intuitive procedure that could make possible the use of extraneous antibodies or reduce the responses against antibodies. Indeed, it is believed that the lack of a negative reaction to some antibodies is due to the immunosuppressed status of the patients, either by the use of chemotherapy or because of the cancer activity itself (Glennie and Johnson, 2000). Suppression of human anti-mouse (HAMA) responses has been attempted with the administration of cyclosporine and other compounds. The use of cyclosporine was able to reduce the formation of anti-mAb responses and increase the amount of useful doses of mAbs that could be administered, but could not completely suppress HAMA in all the individuals tested or for an indefinite amount of administered doses (Ledermann et al., 1988; Weiden et al., 1994; Dhingra et al., 1995). CTLA-4 inhibits T lymphocyte activation by affecting the CD28-B7 co-stimulatory signal. Healthy beagle dogs simultaneously receiving an antibody fraction and the recombinant human CTLA-4 had better antibody responses. While dogs treated with the CTLA-4 had no side-effects after 5

doses of human antibody fractions, dogs that did not receive CTLA-4 simultaneously with the antibody showed hypersensitivity responses after 4 doses (pale mucous membranes, tremors, nasal discharge, and other signs). Also, the treatment seemed to reduce anti-antibody formation, allowing antibody plasma levels to be higher for longer periods (Siegall et al., 1997). However, another study could not reproduce these results. The addition of CTLA-4 did not inhibit the formation of anti- antibodies in this second study (Henry et al., 2005).

**Hurdles and opportunities**

The hurdles and opportunities in the study and production of new antibodies for veterinary use are discussed below and are summarised in Table 3.

The immunogenicity of therapeutic molecules, including monoclonal antibodies, affects the efficacy and safety of the product (Chirino et al., 2004). For the veterinary field this still represents the greatest barrier for mAb usage in the clinical setting, although much still needs to be studied about the possible use of non-speciated mAbs. Clinical use of antibodies will benefit from the production of species-specific molecules (Gearing et al., 2013).

Cost represents an important concern for monoclonal therapies in humans. In veterinary medicine, this issue is even more relevant. The need for high cumulative doses and the fact that cancer is a chronic disease add to the fact that current methods for antibody production in mammalian cells are intrinsically expensive. Novel technologies such as transgenic animals and use of yeast, bacteria and insect culture systems are probable paths to be followed in the future for the production of antibodies (Daniell et al., 2001; Farid, 2007). This highlights the importance of better understanding the possibilities of using single-dose

non-speciated mAbs, which are substantially cheaper to produce, since the price of production will likely hinder the market accessibility of the veterinary biologics industry.

Another limiting factor is the ability of the antibody to penetrate into the tumour. This accounts for one of the reasons why many mAbs that are promising during the developmental stage are not so successful clinically. Clearance from circulation, blood vessel density, vascular permeability and binding site barrier are some of the factors that influence mAb delivery (Lobo et al., 2004; Niu et al., 2009). Solutions include the use of antibody fragments such as scFv or Fab which exhibit better pharmacokinetics for penetration into the cancerous tissue and also possess high binding specificity (Batra et al., 2002). Direct intratumoural antibody administration and the administration of microencapsulated whole antibody-producing cells or other *in situ* antibody producing technologies are possible means to circumvent these difficulties (Pelegrin et al., 1998; Azemar et al., 2003; Orive et al., 2003). Imaging techniques such as positron emission tomography (PET) offer high sensitivity which is required to monitor distribution of the drug throughout the body, to study pharmacokinetics and pharmacodynamics and also provide the information on optimal timing and antibody dosage (Rudin et al., 2005). These techniques will be central in the establishment of better mAb tumour penetration.

Reduced efficiency of monoclonal antibodies during human clinical trials when compared to mouse trials is of major concern (Stein, 1997), and this might also be an issue when mAbs are to be used in veterinary medicine. Dogs represent a good model to study cancer on in respect to possible future applications in human patients. The genetic diversity of dogs is comparable to that of humans, and is larger than that of most rodent models; their cancer-associated genes show great similarities; cancers are naturally occurring, and appear

in animals with intact immune systems; similar factors influence the appearance of cancer in dogs and humans, such as age and environmental exposure to carcinogens. Indeed, clinical studies in dogs have shown that they serve as good predictors of both clinical toxicity and response to therapy (Paoloni and Khanna, 2008; London et al., 2009; Richards and Suter, 2015). It would be in the interests of both veterinary medicine and of human medicine that dogs became an established intermediate in the studies of monoclonal antibodies. Experiments using human or murine mAbs in animals need to consider the potential benefits of the idiotype responses and the possibility of using mAbs in short dosing protocols. Quick chimerisation of antibodies and the study of cheaper expression systems would facilitate this process. It is very likely that once an antibody is fully arranged to be tested in dogs, the relevance of the results for human clinical trials would be improved when compared to mouse studies.

## Conclusions

The current trend of biological therapeutics is now reaching veterinary medicine. While there are still many challenges to overcome in order to achieve commercial viability and wide accessibility of monoclonal antibodies for animal use, there are many opportunities in this field, as can be seen by the renewed interest of many research laboratories and private companies. Testing and using monoclonals clinically in veterinary medicine will be of benefit for both the animal patients and the development and improvement of human therapeutics.

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464

465 **Conflict of Interest**

466 The authors declare no conflicts of interest.

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468 **References**

469 Acharya, N., Gohel, D., Kuberkar, V., Natesan, S., 2015. Recent advances in immunotherapy
470 for the treatment of cancer. Adv. Anim. Vet. Sci 3, 23–29.
471 Adams, G.P., Weiner, L.M., 2005. Monoclonal antibody therapy of cancer. Nature
472 biotechnology 23, 1147–57.
473 Anderson, K.L., Lins, D., Volkmer, J.-P., Shimizu, Y., Mescher, I.L.W.M., Modiano, J.,
474 2016. Melanoma cell resistance to phagocytosis is unrelated to expression of
475 conventional "eat-me/don't eat-me" signals, in: Inaugural International Cancer
476 Immunotherapy Conference. p. A153.
477 Anderson, K.L., Modiano, J.F., 2015. Progress in Adaptive Immunotherapy for Cancer in
478 Companion Animals: Success on the Path to a Cure. Veterinary sciences 2, 363–387.
479 Anidjar, M., Villette, J.-M., Devauchelle, P., Delisle, F., Cotard, J.P., Billotey, C., Cochand-
480 Priollet, B., Copin, H., Barnoux, M., Triballeau, S., et al., 2001. In vivo model
481 mimicking natural history of dog prostate cancer using DPC-1, a new canine prostate
482 carcinoma cell line. The Prostate 46, 2–10.
483 Aratana Therapeutics Inc, 2015. Aratana Therapeutics Provides Product Updates. Kansas
484 Citu.
485 Arrowsmith, J., 2012. A decade of change. Nat Rev Drug Discov 11, 17–18.
486 Azemar, M., Djahansouzi, S., Jäger, E., Solbach, C., Schmidt, M., Maurer, A.B., Mross, K.,
487 Unger, C., von Minckwitz, G., Dall, P., 2003. Regression of cutaneous tumor lesions in
488 patients intratumorally injected with a recombinant single-chain antibody-toxin targeted
489 to ErbB2/HER2. Breast cancer research and treatment 82, 155–164.
490 Barbas, C.F., Kang, A.S., Lerner, R.A., Benkovic, S.J., 1991. Assembly of combinatorial
491 antibody libraries on phage surfaces: the gene III site. Proceedings of the National
492 Academy of Sciences 88, 7978–7982.
493 Batra, S.K., Jain, M., Wittel, U.A., Chauhan, S.C., Colcher, D., 2002. Pharmacokinetics and
494 biodistribution of genetically engineered antibodies. Current opinion in biotechnology
495 13, 603–608.
496 Bergeron, L.M., McCandless, E.E., Dunham, S., Dunkle, B., Zhu, Y., Shelly, J., Lightle, S.,
497 Gonzales, A., Bainbridge, G., 2014. Comparative functional characterization of canine
498 IgG subclasses. Veterinary Immunology and Immunopathology 157, 31–41.
499 Biller, B.J., Page, R., Sharkey, R., Goldenberg, D.M., 2014. Abstract A07: Phase I evaluation
500 of anti-human leukocyte antigen-DR monoclonal antibody therapy in spontaneous
501 canine lymphoma. Molecular Cancer Research 12 , A07–A07.
502 Binder, M., Otto, F., Mertelsmann, R., Veelken, H., Trepel, M., 2006. The epitope recognized
503 by rituximab. Blood 108, 1975–1978.
504 Bradbury, A.R.M., Sidhu, S., Dübel, S., McCafferty, J., 2011. Beyond natural antibodies: the

505 power of in vitro display technologies. Nature biotechnology 29, 245–254.
506 Burdick, M., Macklis, R., 2009. Update on the rational use of tositumomab and iodine-131
507 tositumomab radioimmunotherapy for the treatment of non-Hodgkin's lymphoma.
508 OncoTargets and therapy 229–242.
509 Campos, L.C., Lavalle, G.E., Estrela-Lima, A., Melgaço de Faria, J.C., Guimarães, J.E.,
510 Dutra, Á.P., Ferreira, E., de Sousa, L.P., Rabelo, É., Vieira da Costa, A.F.D., 2012.
511 CA15. 3, CEA and LDH in Dogs with Malignant Mammary Tumors. Journal of
512 Veterinary Internal Medicine 26, 1383–1388.
513 Cannon, C.M., 2015. Cats, cancer and comparative oncology. Veterinary Sciences 2, 111–
514 126.
515 Chirino, A.J., Ary, M.L., Marshall, S.A., 2004. Minimizing the immunogenicity of protein
516 therapeutics. Drug Discovery Today 9, 82–90.
517 Clark, M., 2000. Antibody humanization: a case of the "Emperor"s new clothes'?
518 Immunology Today 21, 397–402.
519 Coomer, A.R., 2008. A Murine Xenograft Model of Canine Osteosarcoma: Anti-Tumor
520 Effects of an Angiogenesis Inhibitor and Combinations of Radiation, Chemotherapy,
521 and a Vascular Targeting Agent. University of Florida.
522 Crow, S.E., 2008. Chemoimmunotherapy for canine lymphoma: tumor vaccines and
523 monoclonal antibodies. Cancer Therapy 6, 181–186.
524 Daniell, H., Streatfield, S.J., Wycoff, K., 2001. Medical molecular farming: production of
525 antibodies, biopharmaceuticals and edible vaccines in plants. Trends in Plant Science 6,
526 219–226.
527 Deckert, P.M., 2009. Current constructs and targets in clinical development for antibody-
528 based cancer therapy. Current drug targets 10, 158–175.
529 Demaria, S., Bhardwaj, N., McBride, W.H., Formenti, S.C., 2005. Combining radiotherapy
530 and immunotherapy: a revived partnership. International Journal of Radiation
531 Oncology* Biology* Physics 63, 655–666.
532 Dhingra, K., Fritsche, H., Murray, J.L., LoBuglio, A.F., Khazaeli, M.B., Kelley, S., Tepper,
533 M.A., Grasela, D., Buzdar, A., Valero, V., et al., 1995. Phase I Clinical and
534 Pharmacological Study of Suppression of Human Antimouse Antibody Response to
535 Monoclonal Antibody L6 by Deoxyspergualin. Cancer Research 55 , 3060–3067.
536 Ecker, D.M., Jones, S.D., Levine, H.L., 2015. The therapeutic monoclonal antibody market,
537 in: MAbs. Taylor & Francis, pp. 9–14.
538 Ecker, D.M., Jones, S.D., Levine, H.L., 2014. The therapeutic monoclonal antibody market.
539 mAbs 7, 9–14.
540 Eissler, N., Ruf, P., Mysliwietz, J., Lindhofer, H., Mocikat, R., 2012. Trifunctional Bispecific
541 Antibodies Induce Tumor-Specific T Cells and Elicit a Vaccination Effect. Cancer
542 Research 72 , 3958–3966.
543 Elders, R.C., Holder, A., Smith, K.C., Baines, S.J., Catchpole, B., 2014. Recombinant canine
544 IgE Fc and an IgE Fc-TRAIL fusion protein bind to neoplastic canine mast cells.
545 Veterinary Immunology and Immunopathology 159, 29–40.
546 Farid, S.S., 2007. Process economics of industrial monoclonal antibody manufacture. Journal
547 of Chromatography B 848, 8–18.
548 Friedman, P.N., Chace, D.F., Trail, P.A., Siegall, C.B., 1993. Antitumor activity of the
549 single-chain immunotoxin BR96 sFv-PE40 against established breast and lung tumor
550 xenografts. The Journal of Immunology 150 , 3054–3061.
551 Gama, A., Alves, A., Schmitt, F., 2008. Identification of molecular phenotypes in canine
552 mammary carcinomas with clinical implications: application of the human classification.
553 Virchows Archiv 453, 123–132.
554 Gama, A., Gärtner, F., Alves, A., Schmitt, F., 2009. Immunohistochemical expression of

555 Epidermal Growth Factor Receptor (EGFR) in canine mammary tissues. Research in
556 veterinary science 87, 432–437.
557 Gearing, D.P., Huebner, M., Virtue, E.R., Knight, K., Hansen, P., Lascelles, B.D.X., Gearing,
558 R.P., Drew, A.C., 2016. In Vitro and In Vivo Characterization of a Fully Felinized
559 Therapeutic Anti-Nerve Growth Factor Monoclonal Antibody for the Treatment of Pain
560 in Cats. Journal of Veterinary Internal Medicine n/a–n/a.
561 Gearing, D.P., Virtue, E.R., Gearing, R.P., Drew, A.C., 2013. A fully caninised anti-NGF
562 monoclonal antibody for pain relief in dogs. BMC veterinary research 9, 226.
563 Gianni, L., Eiermann, W., Semiglazov, V., Manikhas, A., Lluch, A., Tjulandin, S., Zambetti,
564 M., Vazquez, F., Byakhow, M., Lichinitser, M., 2010. Neoadjuvant chemotherapy with
565 trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone,
566 in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a
567 randomised controlled superiority trial with a parallel HER. The Lancet 375, 377–384.
568 Gilbert, S.J., Wright, S., 2015. Tumor Lysis Syndrome, in: Onconephrology. Springer, pp.
569 163–181.
570 Glennie, M.J., Johnson, P.W.M., 2000. Clinical trials of antibody therapy. Immunology today
571 21, 403–410.
572 Golay, J., Introna, M., 2012. Mechanism of action of therapeutic monoclonal antibodies:
573 Promises and pitfalls of in vitro and in vivo assays. Archives of biochemistry and
574 biophysics 526, 146–153.
575 Grunnet, M., Sorensen, J.B., 2012. Carcinoembryonic antigen (CEA) as tumor marker in lung
576 cancer. Lung Cancer 76, 138–143.
577 Hansel, T.T., Kropshofer, H., Singer, T., Mitchell, J.A., George, A.J.T., 2010. The safety and
578 side effects of monoclonal antibodies. Nat Rev Drug Discov 9, 325–338.
579 Harding, F.A., Stickler, M.M., Razo, J., DuBridge, R.B., 2010. The immunogenicity of
580 humanized and fully human antibodies: Residual immunogenicity resides in the CDR
581 regions. mAbs 2, 256–265.
582 Harris, L.J., Larson, S.B., Hasel, K.W., McPherson, A., 1997. Refined structure of an intact
583 IgG2a monoclonal antibody. Biochemistry 36, 1581–1597.
584 Harvey, J., 2015. Pet cancer treatment: R&D dead end or the next blockbuster goldmine?
585 VetDC.
586 Hay, R. V, Cao, B., Skinner, R.S., Su, Y., Zhao, P., Gustafson, M.F., Qian, C.-N., Teh, B.T.,
587 Knudsen, B.S., Resau, J.H., et al., 2005. Nuclear Imaging of Met-Expressing Human
588 and Canine Cancer Xenografts with Radiolabeled Monoclonal Antibodies
589 (MetSeekTM). Clinical Cancer Research 11 , 7064s–7069s.
590 Helfand, S.C., Soergel, S.A., Donner, R.L., Gan, J., Hank, J.A., Lindstrom, M.J., Sondel,
591 P.M., 1994. Potential to Involve Multiple Effector Cells with Human Recombinant
592 Interleukin-2 and Antiganglioside Monoclonal Antibodies in a Canine Malignant
593 Melanoma Immunotherapy Model. Journal of Immunotherapy 16.
594 Henry, C.J., Buss, M.S., Hellström, I., Hellström, K.E., Brewer, W.G., Bryan, J.N., Siegall,
595 C.B., 2005. Clinical evaluation of BR96 sFv-PE40 immunotoxin therapy in canine
596 models of spontaneously occurring invasive carcinoma. Clinical cancer research : an
597 official journal of the American Association for Cancer Research 11, 751–755.
598 Herlyn, D., Ross, A.H., Koprowski, H., 1986. Anti-idiotypic antibodies bear the internal
599 image of a human tumor antigen. Science (New York, N.Y.) 232, 100–102.
600 Hünig, T., 2012. The storm has cleared: lessons from the CD28 superagonist TGN1412 trial.
601 Nat Rev Immunol 12, 317–318.
602 Hwang, W.Y.K., Foote, J., 2005. Immunogenicity of engineered antibodies. Methods 36, 3–
603 10.
604 Iannello, A., Ahmad, A., 2005. Role of antibody-dependent cell-mediated cytotoxicity in the

efficacy of therapeutic anti-cancer monoclonal antibodies. Cancer metastasis reviews 24, 487–99.
Impellizeri, J.A., Howell, K., McKeever, K.P., Crow, S.E., 2006. The role of rituximab in the treatment of canine lymphoma: an ex vivo evaluation. The Veterinary Journal 171, 556–558.
Ito, D., Brewer, S., Modiano, J.F., Beall, M.J., 2015. Development of a novel anti-canine CD20 monoclonal antibody with diagnostic and therapeutic potential. Leukemia & Lymphoma 56, 219–225.
Jakobovits, A., Amado, R.G., Yang, X., Roskos, L., Schwab, G., 2007. From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice. Nature biotechnology 25, 1134–1143.
Jeglum, K.A., 2009. The history and future of canine lymphoma monoclonal antibody 231 Review Article. Cancer Therapy 7, 59–61.
Jeglum, K.A., 1996. Chemoimmunotherapy of canine lymphoma with adjuvant canine monoclonal antibody 231. Veterinary Clinics of North America: Small Animal Practice 26, 73–85.
Jeha, S., 2001. Tumor lysis syndrome. Seminars in Hematology 38, Supple, 4–8.
Jubala, C.M., Wojcieszyn, J.W., Valli, V.E.O., Getzy, D.M., Fosmire, S.P., Coffey, D., Bellgrau, D., Modiano, J.F., 2005. CD20 expression in normal canine B cells and in canine non-Hodgkin lymphoma. Veterinary Pathology Online 42, 468–476.
Kaplan, L., 2008. Help Your Dog Fight Cancer: What Every Caretaker Should Know About Canine Cancer, Featuring Bullet's Survival Story. JanGen Press.
Kelesidis, T., Daikos, G., Boumpas, D., Tsiodras, S., 2011. Does rituximab increase the incidence of infectious complications? A narrative review. International Journal of Infectious Diseases 15, e2–e16.
Kettleborough, C.A., Saldanha, J., Heath, V.J., Morrison, C.J., Bendig, M.M., 1991. Humanization of a mouse monoclonal antibody by CDR–grafting: the importance of framework residues on loop conformation. Protein Engineering 4, 773–783.
King, S., 2013. The Best Selling Drugs of All Time; Humira Joins The Elite. Forbes.
Köhler, G., Milstein, C., 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256, 495–497.
Kubota, T., Niwa, R., Satoh, M., Akinaga, S., Shitara, K., Hanai, N., 2009. Engineered therapeutic antibodies with improved effector functions. Cancer science 100, 1566–1572.
Kuus-Reichel, K., Grauer, L.S., Karavodin, L.M., Knott, C., Krusemeier, M., Kay, N.E., 1994. Will immunogenicity limit the use, efficacy, and future development of therapeutic monoclonal antibodies? Clinical and Diagnostic Laboratory Immunology 1 , 365–372.
Lai, C.-L., van den Ham, R., van Leenders, G., van der Lugt, J., Mol, J.A., Teske, E., 2008. Histopathological and immunohistochemical characterization of canine prostate cancer. The Prostate 68, 477–488.
Lang, D.S.P., Hagger, C., Pearson, A., 2011. Safety of rapid rituximab infusion in adult cancer patients: A systematic review. International Journal of Nursing Practice 17, 357–369.
Ledermann, J.A., Begent, R.H., Bagshawe, K.D., Riggs, S.J., Searle, F., Glaser, M.G., Green, A.J., Dale, R.G., 1988. Repeated antitumour antibody therapy in man with suppression of the host response by cyclosporin A. British journal of cancer 58, 654–657.
Ledford, H., 2016. Stem cells for Snoopy: pet medicines spark a biotech boom. Nature 303–304.
Lewis, M.R., Bryan, J.N., Pardo, I.D.R., Jia, F., Kunz, D.A., Besch-Williford, C.L.,

Theodore, L.J., Axworthy, D.B., 2005. Annual VCS Conference Proceedings - Antibody pretargeting for molecular imaging of canine metastatic prostate cancer. Veterinary and Comparative Oncology 4, 241–304.
Lobo, E.D., Hansen, R.J., Balthasar, J.P., 2004. Antibody pharmacokinetics and pharmacodynamics. Journal of pharmaceutical sciences 93, 2645–68.
London, C.A., Malpas, P.B., Wood-Follis, S.L., Boucher, J.F., Rusk, A.W., Rosenberg, M.P., Henry, C.J., Mitchener, K.L., Klein, M.K., Hintermeister, J.G., et al., 2009. Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. Clinical cancer research : an official journal of the American Association for Cancer Research 15, 3856–3865.
Maekawa, N., Konnai, S., Ikebuchi, R., Okagawa, T., Adachi, M., Takagi, S., Kagawa, Y., Nakajima, C., Suzuki, Y., Murata, S., 2014. Expression of PD-L1 on canine tumor cells and enhancement of IFN-γ production from tumor-infiltrating cells by PD-L1 blockade. PloS one 9, e98415.
Maekawa, N., Konnai, S., Okagawa, T., Nishimori, A., Ikebuchi, R., Izumi, Y., Takagi, S., Kagawa, Y., Nakajima, C., Suzuki, Y., et al., 2016. Immunohistochemical Analysis of PD-L1 Expression in Canine Malignant Cancers and PD-1 Expression on Lymphocytes in Canine Oral Melanoma. PLoS ONE 11, e0157176.
McLaughlin, P., Grillo-López, A.J., Link, B.K., Levy, R., Czuczman, M.S., Williams, M.E., Heyman, M.R., Bence-Bruckler, I., White, C.A., Cabanillas, F., et al., 1998. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. Journal of Clinical Oncology 16 , 2825–2833.
Melosky, B., Burkes, R., Rayson, D., Alcindor, T., Shear, N., Lacouture, M., 2009. Management of skin rash during EGFR-targeted monoclonal antibody treatment for gastro intestinal malignancies: Canadian recommendations. Current Oncology 16, 14–24.
Michels, G.M., Ramsay, D., Walsh, K., Martinon, O., Mahabir, S., Hoevers, J., Walters, R., Boucher, J., Álvarez-García, G., 2016. A blinded, randomized, placebo-controlled trial investigating three dose levels of lokivetmab (ZTS-00103289), a caninized anti-canine-IL-31 monoclonal antibody (mAb), for the reduction of pruritus and associated skin lesions in dogs with atopic dermatitis, in: 8th World Congress of Veterinary Dermatology. p. WCVD8/FC – 1106.
Michishita, M., Uto, T., Nakazawa, R., Yoshimura, H., Ogihara, K., Naya, Y., Tajima, T., Azakami, D., Kishikawa, S., Arai, T., 2013. Antitumor effect of bevacizumab in a xenograft model of canine hemangiopericytoma. Journal of pharmacological sciences 121, 339–342.
Miersch, S., Sidhu, S.S., 2012. Synthetic antibodies: concepts, potential and practical considerations. Methods 57, 486–498.
Millanta, F., Lazzeri, G., Vannozzi, I., Viacava, P., Poli, A., 2002. Correlation of vascular endothelial growth factor expression to overall survival in feline invasive mammary carcinomas. Veterinary pathology 39, 690–696.
Millanta, F., Silvestri, G., Vaselli, C., Citi, S., Pisani, G., Lorenzi, D., Poli, A., 2006. The role of vascular endothelial growth factor and its receptor Flk-1/KDR in promoting tumour angiogenesis in feline and canine mammary carcinomas: A preliminary study of autocrine and paracrine loops. Research in Veterinary Science 81, 350–357.
Mirick, G.R., Bradt, B.M., Denardo, S.J., Denardo, G.L., 2004. A review of human anti-globulin antibody (HAGA, HAMA, HACA, HAHA) responses to monoclonal

antibodies. Not four letter words. The quarterly journal of nuclear medicine and molecular imaging : official publication of the Italian Association of Nuclear Medicine (AIMN) [and] the International Association of Radiopharmacology (IAR), [and] Section of the Society of.. 48, 251–257.
Modjtahedi, H., Ali, S., Essapen, S., 2012. Therapeutic application of monoclonal antibodies in cancer: advances and challenges. British medical bulletin 104, 41–59.
Mottolese, M., Morelli, L., Agrimi, U., Benevolo, M., Sciarretta, F., Antonucci, G., Natali, P.G., 1994. Spontaneous canine mammary tumors. A model for monoclonal antibody diagnosis and treatment of human breast cancer. Laboratory investigation; a journal of technical methods and pathology 71, 182–187.
Munro, T.P., Mahler, S.M., Huang, E.P., Chin, D.Y., Gray, P.P., 2011. Bridging the gap: Facilities and technologies for development of early stage therapeutic mAb candidates. mAbs 3, 440–452.
Nexvet, 2016. Nexvet and Zenoaq announce PD-1 mAb candidate for canine cancer.
Niu, G., Li, Z., Xie, J., Le, Q.-T., Chen, X., 2009. PET of EGFR Antibody Distribution in Head and Neck Squamous Cell Carcinoma Models. Journal of Nuclear Medicine 50, 1116–1123.
Orive, G., Hernandez, R.M., Gascón, A.R., Domínguez-Gil, A., Pedraz, J.L., 2003. Drug delivery in biotechnology: present and future. Current opinion in biotechnology 14, 659–664.
Paoloni, M., Khanna, C., 2008. Translation of new cancer treatments from pet dogs to humans. Nature reviews. Cancer 8, 147–156.
Pedersen, I.M., Buhl, A.M., Klausen, P., Geisler, C.H., Jurlander, J., 2002. The chimeric anti-CD20 antibody rituximab induces apoptosis in B-cell chronic lymphocytic leukemia cells through a p38 mitogen activated protein-kinase-dependent mechanism. Blood 99, 1314–1319.
Pelegrin, M., Marin, M., Noel, D., Del Rio, M., Saller, R., Stange, J., Mitzner, S., Günzburg, W.H., Piechaczyk, M., 1998. Systemic long-term delivery of antibodies in immunocompetent animals using cellulose sulphate capsules containing antibody-producing cells. Gene therapy 5, 828–834.
Persic, L., Roberts, a, Wilton, J., Cattaneo, a, Bradbury, a, Hoogenboom, H.R., 1997. An integrated vector system for the eukaryotic expression of antibodies or their fragments after selection from phage display libraries. Gene 187, 9–18.
Queiroga, F.L., Perez-Alenza, M.D., González-Gil, A., Silván, G., Peña, L., Illera, J.C., 2015. Quantification of epidermal growth factor receptor (EGFR) in canine mammary tumours by ELISA assay: clinical and prognostic implications. Veterinary and Comparative Oncology n/a–n/a.
Reichert, J., 2012. Marketed therapeutic antibodies compendium. MAbs 3, 413–415.
Renaudineau, Y., Devauchelle-Pensec, V., Hanrotel, C., Pers, J.-O., Saraux, A., Youinou, P., 2009. Monoclonal anti-CD20 antibodies: Mechanisms of action and monitoring of biological effects. Joint Bone Spine 76, 458–463.
Restucci, B., Papparella, S., Maiolino, P., De Vico, G., 2002. Expression of vascular endothelial growth factor in canine mammary tumors. Veterinary pathology 39, 488–493.
Richards, K.L., Suter, S.E., 2015. Man's best friend: what can pet dogs teach us about non-Hodgkin's lymphoma? Immunological Reviews 263, 173–191.
Rosales, C., Jeglum, K.A., Obrocka, M., Steplewski, Z., 1988. Cytolytic activity of murine anti-dog lymphoma monoclonal antibodies with canine effector cells and complement. Cellular immunology 115, 420–428.
Ross, J.S., Sheehan, C.E., Fisher, H.A.G., Kaufman, R.P., Kaur, P., Gray, K., Webb, I., Gray,

G.S., Mosher, R., Kallakury, B.V.S., 2003. Correlation of Primary Tumor Prostate-Specific Membrane Antigen Expression with Disease Recurrence in Prostate Cancer. Clinical Cancer Research 9 , 6357–6362.
Rudin, M., Rausch, M., Stoeckli, M., 2005. Molecular imaging in drug discovery and development: potential and limitations of nonnuclear methods. Molecular Imaging and Biology 7, 5–13.
Rue, S.M., Eckelman, B.P., Efe, J.A., Bloink, K., Deveraux, Q.L., Lowery, D., Nasoff, M., 2015. Identification of a candidate therapeutic antibody for treatment of canine B-cell lymphoma. Veterinary Immunology and Immunopathology 164, 148–159.
Scharf, V.F., Farese, J.P., Coomer, A.R., Milner, R.J., Taylor, D.P., Salute, M.E., Chang, M.N., Neal, D., Siemann, D.W., 2013. Effect of bevacizumab on angiogenesis and growth of canine osteosarcoma cells xenografted in athymic mice. American journal of veterinary research 74, 771–778.
Scheidegger, P., Weiglhofer, W., Suarez, S., Kaser-Hotz, B., Steiner, R., Ballmer-Hofer, K., Jaussi, R., 1999. Vascular endothelial growth factor (VEGF) and its receptors in tumor-bearing dogs. Biological chemistry 380, 1449–54.
Seimetz, D., Lindhofer, H., Bokemeyer, C., 2010. Development and approval of the trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3) as a targeted cancer immunotherapy. Cancer treatment reviews 36, 458–467.
Selvarajah, G.T., Verheije, M.H., Kik, M., Slob, A., Rottier, P.J.M., Mol, J.A., Kirpensteijn, J., 2012. Expression of epidermal growth factor receptor in canine osteosarcoma: Association with clinicopathological parameters and prognosis. The Veterinary Journal 193, 412–419.
Shankar, G., Pendley, C., Stein, K.E., 2007. A risk-based bioanalytical strategy for the assessment of antibody immune responses against biological drugs. Nat Biotech 25, 555–561.
Sharma, R., Koller, L., Barclay, P., Liddle, C., 2007. Evaluation of the off-label usage of rituximab in a large teaching hospital in New South Wales. Internal Medicine Journal 37, 569–571.
Shiomitsu, K., Johnson, C.L., Malarkey, D.E., Pruitt, A.F., Thrall, D.E., 2009. Expression of epidermal growth factor receptor and vascular endothelial growth factor in malignant canine epithelial nasal tumours*. Veterinary and comparative oncology.
Shultz, L.D., Brehm, M.A., Garcia, J.V., Greiner, D.L., 2012. Humanized mice for immune system investigation: progress, promise and challenges. Nature reviews. Immunology 12, 786–798.
Siegall, C.B., Haggerty, H.G., Warner, G.L., Chace, D., Mixan, B., Linsley, P.S., Davidson, T., 1997. Prevention of immunotoxin-induced immunogenicity by coadministration with CTLA4Ig enhances antitumor efficacy. The Journal of Immunology 159 , 5168–5173.
Singer, J., Fazekas, J., Wang, W., Weichselbaumer, M., Matz, M., Mader, A., Steinfellner, W., Meitz, S., Mechtcheriakova, D., Sobanov, Y., et al., 2014. Generation of a Canine Anti-EGFR (ErbB-1) Antibody for Passive Immunotherapy in Dog Cancer Patients. American Association for Cancer Research 13, 1777–1790.
Singer, J., Jensen-Jarolim, E., 2014. IgE-based Immunotherapy of Cancer -A Comparative Oncology Approach. Journal of carcinogenesis & mutagenesis 5, 1000176.
Singer, J., Weichselbaumer, M., Stockner, T., Mechtcheriakova, D., Sobanov, Y., Bajna, E., Wrba, F., Horvat, R., Thalhammer, J.G., Willmann, M., 2012. Comparative oncology: ErbB-1 and ErbB-2 homologues in canine cancer are susceptible to cetuximab and trastuzumab targeting. Molecular immunology 50, 200–209.
Singh, S.K., Cousens, L.P., Alvarez, D., Mahajan, P.B., 2012. Determinants of immunogenic response to protein therapeutics. Biologicals 40, 364–368.

Stein, K.E., 1997. Overcoming obstacles to monoclonal antibody product development and approval. Trends in biotechnology 15, 88–89.
Stein, R., Balkman, C., Chen, S., Rassnick, K., Mcentee, M., Page, R., Goldenberg, D.M., 2011. Evaluation of anti-human leukocyte antigen-DR monoclonal antibody therapy in spontaneous canine lymphoma. Leukemia & lymphoma 52, 273–284.
Steplewski, Z., Jeglum, K.A., Rosales, C., Weintraub, N., 1987. Canine lymphoma-associated antigens defined by murine monoclonal antibodies. Cancer immunology, immunotherapy 24, 197–201.
Strietzel, C.J., Bergeron, L.M., Oliphant, T., Mutchler, V.T., Choromanski, L.J., Bainbridge, G., 2014. In Vitro functional characterization of feline IgGs. Veterinary Immunology and Immunopathology 158, 214–223.
Sturgeon, C.M., Duffy, M.J., Stenman, U.-H., Lilja, H., Brünner, N., Chan, D.W., Babaian, R., Bast, R.C., Dowell, B., Esteva, F.J., 2008. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Clinical chemistry 54, e11–e79.
Suntharalingam, G., Perry, M.R., Ward, S., Brett, S.J., Castello-Cortes, A., Brunner, M.D., Panoskaltsis, N., 2006. Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412. New England Journal of Medicine 355, 1018–1028.
Terragni, R., Gardini, A.C., Sabattini, S., Bettini, G., Amadori, D., Talamonti, C., Vignoli, M., Capelli, L., Saunders, J.H., Ricci, M., 2014. EGFR, HER-2 and KRAS in canine gastric epithelial tumors: a potential human model? PloS one 9.
Thie, H., Meyer, T., Schirrmann, T., Hust, M., Dubel, S., 2008. Phage display derived therapeutic antibodies. Current Pharmaceutical Biotechnology 9, 439–446.
Uettwiller, F., Rigal, E., Hoarau, C., 2011. Infections associated with monoclonal antibody and fusion protein therapy in humans, in: MAbs. Taylor & Francis, pp. 461–466.
USDA, 2016. Veterinary biological products, in: Licensees and Permittees. p. 100.
Valabrega, G., Montemurro, F., Aglietta, M., 2007. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. Annals of Oncology 18 , 977–984.
van Meer, P.J.K., Kooijman, M., Brinks, V., Gispen-de Wied, C.C., Silva-Lima, B., Moors, E.H.M., Schellekens, H., 2013. Immunogenicity of mAbs in non-human primates during nonclinical safety assessment. mAbs 5, 810–816.
Waldmann, H., 2014. Human monoclonal antibodies: the residual challenge of antibody immunogenicity, in: Human Monoclonal Antibodies. Springer, pp. 1–8.
Waldmann, T.A., 1991. Monoclonal antibodies in diagnosis and therapy. Science 252 , 1657–1662.
Watine, J., Miédougé, M., Friedberg, B., 2001. Carcinoembryonic antigen as an independent prognostic factor of recurrence and survival in patients resected for colorectal liver metastases. Diseases of the colon & rectum 44, 1791–1799.
Weichselbaumer, M., Willmann, M., Reifinger, M., Singer, J., Bajna, E., Sobanov, Y., Mechtcherikova, D., Selzer, E., Thalhammer, J.G., Kammerer, R., 2011. Phylogenetic discordance of human and canine carcinoembryonic antigen (CEA, CEACAM) families, but striking identity of the CEA receptors will impact comparative oncology studies. PLoS currents 3.
Weiden, P.L., Wolf, S.B., Breitz, H.B., Appelbaum, J.W., Seiler, C.A., Mallett, R., Bjorn, M.J., Su, F.M., Fer, M.F., Salk, D., 1994. Human anti-mouse antibody suppression with cyclosporin A. Cancer 73, 1093–1097.
Witzig, T.E., Gordon, L.I., Cabanillas, F., Czuczman, M.S., Emmanouilides, C., Joyce, R., Pohlman, B.L., Bartlett, N.L., Wiseman, G.A., Padre, N., et al., 2002. Randomized

Controlled Trial of Yttrium-90–Labeled Ibritumomab Tiuxetan Radioimmunotherapy Versus Rituximab Immunotherapy for Patients With Relapsed or Refractory Low-Grade, Follicular, or Transformed B-Cell Non-Hodgkin's Lymphoma. Journal of Clinical Oncology 20 , 2453–2463.

Zoetis, 2015. Zoetis Receives a Conditional License from USDA for Canine Atopic Dermatitis Immunotherapeutic.

Zwingenberger, A.L., Kent, M.S., Liu, R., Kukis, D.L., Wisner, E.R., DeNardo, S.J., Taylor, S.L., Chen, X., Lam, K.S., 2012. In-vivo biodistribution and safety of 99mTc-LLP2A-HYNIC in canine non-Hodgkin lymphoma. PloS one 7, e34404.

Zwingenberger, A.L., Kent, M.S., Shi, C., Taylor, S.L., Chen, X., Lam, K.S., 2012. Affinity of the alpha4–beta1 integrin-targeting peptide LLP2A to canine lymphoma. Veterinary Immunology and Immunopathology 145, 298–304.

887 **Table 1**

888 Molecular mechanisms of action of monoclonal antibodies in cancer.

889

| Classes | Mechanisms | Mediators | mAbs | Effects |
|---|---|---|---|---|
| **Direct effects** | | | | |
| **Blockade of signalling** | Neutralisation of mediators, cytokines, blockade of receptors, induction of apoptosis | Apoptosis mediators | Daclizumab<br>Trastuzumab (other mechanisms also involved)<br>Cetuximab<br>Panitumumab<br>Nivolumab<br>Ipilimumab<br>[nearly all commercially available antibodies for cancer affect signalling to some extent] | Inhibition of proliferation and apoptosis |
| **Induction of signalling** | Protein tyrosine phosphorylation, upregulation of Myc | p38 Map kinase, protein kinase C, Myc | Rituximab | Regulation of cell growth |
| **Conjugated antibodies** | Induction of cell death and inhibition of proliferation | Drugs, toxin fragments, radioisotopes | Tositumomab<br>Ibritumomab tiuxetan | Direct cell death |
| **Immune Mediated Effects** | | | | |
| **ADCC**[a] | Interaction of mAb with FcγR bearing cells (NK, macrophages, neutrophils) with mAbs of IgG2a and IgG3 (in dogs receiving mouse mAb) or IgG1 and IgG3 (in humans)* | [NK cells] IFNγ, cytotoxic granules (perforin, granulysin, granzymes), [Macrophages] ROS, proteases | Rituximab<br>Trastuzumab (other mechanisms also involved)<br>Alemtuzumab | Inhibition of cell proliferation, anti-angiogenesis, cell lysis. The antigen presentation following cell death may induce a host |

| | | | | |
|---|---|---|---|---|
| | | | | response. |
| **CDC** | Classical activation of complement system | IgG1 and IgG3 Fc region (in humans)[b]; IgG2 region (in mice) and complement cascade (initiating with C1q) | Alemtuzumab<br>Rituximab | Lysis of cells by the complement through the creation of pore on cell membrane. Enhancement of ADCC. |
| **Immunomodulation**<br>**Checkpoint inhibitors** | Blockade of immune inhibitory receptors; approximation of immune cells to its target (trifunctional antibodies); activation of cells and induction of immune memory | CTLA-4, PD-1, CD40, CD3, Fcγ receptors (mouse IgG2a and rat IgG2b in combination, for humans)[c] | Catumaxomab; Ipilimumab | Induction of T mediated lysis, ADCC, phagocytosis; reduction of tumour-induced immune anergy |

[a]ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; IFN, interferon; FcγR, Fc gamma receptor; ROS, reactive oxygen species; NK, natural killer cells.

[b]Shuffled (engineered) isotypes of IgG1 and IgG3 in humans show a strong enhancement in the ADCC and CDC capacity (Kubota et al., 2009)

[c]Shuffled isotypes of mouse IgG2a and rat IgG2b show increased activation of human FcγR.

References: (Rosales et al., 1988; Helfand et al., 1994; Pedersen et al., 2002; Iannello and Ahmad, 2005; Seimetz et al., 2010; Eissler et al., 2012; Golay and Introna, 2012; Anderson and Modiano, 2015) .

908

909 **Table 2**

910 Potential targets in domestic animal species for monoclonal antibodies in oncology.

911

| Target (name of mAb) | Condition targeted | Potential to be used in animals | Outcome in dogs (cats) | Hurdles | References |
|---|---|---|---|---|---|
| **CD20** | B-cell lymphoma | Dog PBMC stained with Rituximab. CD20 occurs in canine B-cell lymphomas. New antibodies developed for dogs (AT-004, 6C8, 1E4)<br><br>mAb 6C8 had specific binding to canine B-cells<br><br>mAb 1E4 (Elanco) has 'rituximab-like' properties and depletes B-cells; chimeric version maintains function | Rituximab does not bind peripheral blood dog cells. mAbs AT-004 reached the market [Aratana/Novartis], with poor outcomes. | Difference in aa[a] where Rituximab binds.<br><br>Low affinity of AT-004 for its target | (Jubala et al., 2005; Binder et al., 2006; Impellizeri et al., 2006; Acharya et al., 2015; Aratana Therapeutics Inc, 2015; Ito et al., 2015; Rue et al., 2015) |
| **CD52** | T-cell lymphoma | mAb received USDA approval for therapeutic use in dogs (AT-005) | Canine mAbs reached the market, but with poor responses [Aratana] | Low affinity of the antibody for its target | (Acharya et al., 2015; Aratana Therapeutics Inc, 2015) |
| **CD47** | B-cell lymphoma | Innate anti-tumor immune response such as phagocytosis increased against canine lymphoma by anti-CD47 mAb | mAb treatment increased canine lymphoma phagocytosis *in vitro* | None described | (Anderson and Modiano, 2015)(Anderson et al., 2016) |
| **α4β1 receptor (LLP2A)** | NHL | Biodistribution of LLP2A studied in dog as model for human | Tumour affected tissues strongly targeted (preferentially B cells) | None described | (A L Zwingenberger et al., 2012; Allison L Zwingenberger et al., 2012) |
| **Lymphoma [target not defined] (mAb 231)** | Lymphoma | FDA approved for dog lymphoma. Binds 73% of tested lymphomas | Increased complete remission in dogs combined with VCAA* | Discontinued due to lack of demand. No longer USDA licensed | (Steplewski et al., 1987; Jeglum, 1996, 2009; USDA, 2016) |
| **VEGF (Bevacizumab)** | Various cancers | VEGF correlation with outcome ambiguous in dogs and cats. Molecular structure of VEGF/R similar between dog and man.<br><br>Bavacizumab inhibited canine osteosarcoma and | Delayed tumour growth and blood vessel formation in mice grafted with canine osteosarcoma | Humanized mAb and cost of therapy | (Scheidegger et al., 1999; Millanta et al., 2002, 2006; Restucci et al., 2002; Coomer, 2008; Michishita et al., 2013; Scharf et |

| | | | | | |
|---|---|---|---|---|---|
| | | hemangiopericytoma xenografts in mice. | | | al., 2013) |
| **Lewis$^{y}$ (BR96)** | Breast and lung carcinomas | 33% of dog tumour samples tested express Le$^{y}$ (several carcinomas) | Partial response in 25% of dogs tested. Stable disease in 33%. Dogs negative for Le$^{y}$ had progressive disease. | 75% cases had anti-mAb reactions not preventable by immunosupression. Anti-mAb reactions curtailed clinical benefits | (Friedman et al., 1993; Henry et al., 2005) |
| **HER2/neu (trastuzumab)** | Breast cancer overexpressing HER2/neu | Dog tumours expressing HER2/neu have been identified, but prognostic value is uncertain. Canine and human receptors are similar; dogs have a similar antigenic phenotype to humans. Prognostic value and tumour behaviour are more similar to humans in cats | Human monoclonal antibodies are able to bind dog antigens. | In cats no adaptation is possible from trastuzumab, since human and feline receptors differ at the EC* region | (Mottolese et al., 1994; Demaria et al., 2005; Valabrega et al., 2007; Gama et al., 2008; Gianni et al., 2010; Singer et al., 2012)(Cannon, 2015) |
| **CEA*** | Tumours of colon; other epitelial tumours | CEA ligand and receptor aa* are 99% conserved in dogs compared to humans. | CEA receptor is expressed in canine mammary carcinoma cell lines | The CEA ligand is not differentially expressed in cancer in dogs | (Watine et al., 2001; Sturgeon et al., 2008; Weichselbaumer et al., 2011; Campos et al., 2012; Grunnet and Sorensen, 2012) |
| **EGFR (cetuximab)** | Colorectal; head and neck cancer | In canine mammary tumours, EGFR has been associated with malignancy and poor prognosis. Human and canine receptors are similar in the epitopes for cetuximab. A caninised version of Cetuximab was developed | Cetuximab is tumoristatic to canine mammary carcinoma cell lines.<br>Caninised cetuximab (can225IgG) maintained function | Cetuximab is a human chimeric antibody. Caninised version is in trial phase. | (Gama et al., 2009; Shiomitsu et al., 2009; Selvarajah et al., 2012; Singer et al., 2012, 2014; Terragni et al., 2014; Acharya et al., 2015; Queiroga et al., 2015) |
| **Human leucocyte antigen-DR [MHC II] (Murine version: L243; humanised: IMMU-114)** | Lymphoma | L243 and IMMU-114 bind human lymphocytes and malignant cells in nodal tissue | Transient disease stabilisation.<br>Murine led to fever, vomiting and short mAb half-life. Humanised had lymphocytopenia and half-life of 2h | Author recommended the canine chimerisation of mAb | (Stein et al., 2011) |
| **Prostatic tumour antigens** | Prostate cancer and human colon | Met5 binds to the EC region of human and canine Met; dog prostate express PSMA mRNA | Met5 binds to canine prostate cancer metastatic cell line | None described | (Anidjar et al., 2001; Ross et al., 2003; Hay et al., |

| | | | | | |
|---|---|---|---|---|---|
| **(Met5; PSM-P12; CC49)** | cancer | and 50% of cancers have the antigen (but not benign tissue) | GN4; PSM-P12 binds to a prostate cell line (DPC-1); CC49 was successful in imaging canine metastases | | 2005; Lewis et al., 2005; Lai et al., 2008) |
| **PD-1** | Several tumour types | Several canine tumours express PD-1, and some also express its ligand. Intra-tumoural lymphocytes in canine melanoma also express PD-1. Anti-PD-1 increases IFNγ production | PD-1 being researched by Nexvet/Zenoaq | None described | (Maekawa et al., 2014, 2016; Nexvet, 2016) |

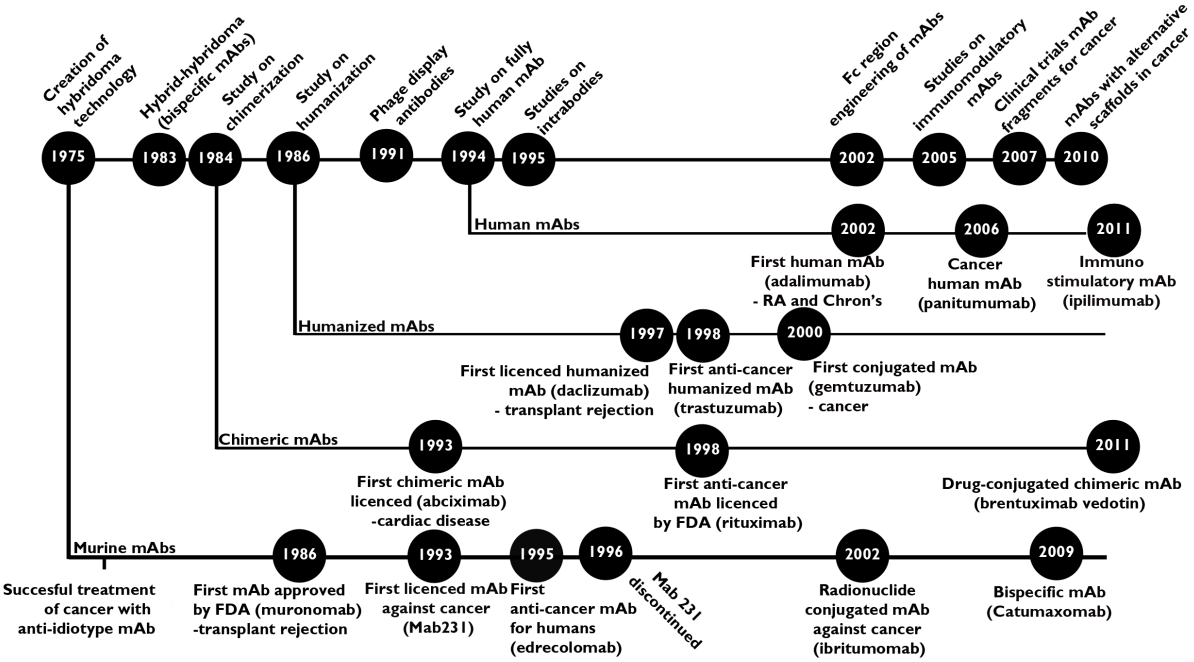
[a]aa = aminoacids; CEA = carcinoembryonic antigen; EC = extracellular; EGFR = epidermal growth factor receptor; PSMA = prostate specific membrane antigen; VCAA = vincristine, cyclophosphamide, L-asparaginase and doxorubicin; VEGF/R = Vascular endothelial growth factor/receptor;

## Table 3

Hurdles and opportunities in monoclonal antibody therapies in veterinary medicine.

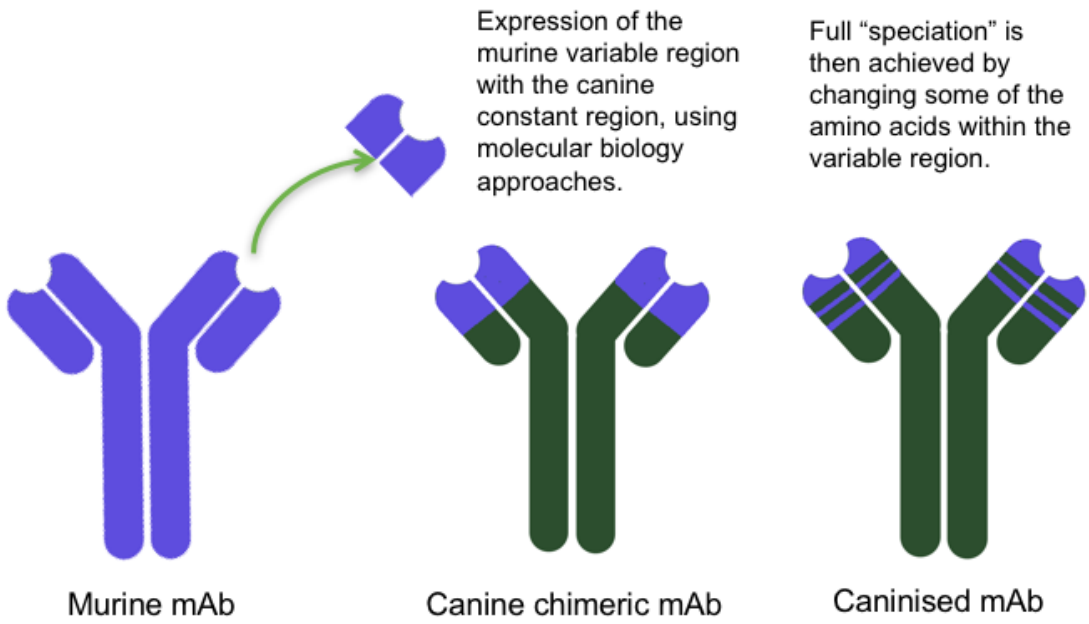
| Hurdles | Opportunities |
|---|---|
| **Immunogenicity of human and mouse mAbs in dogs** | Caninised and chimeric mAbs |
| **Cost of production of conventional mAbs, with cumulative doses** | Alternative mAb production systems and the use of non-speciated single-dose mAbs. Possible expression systems:<br>Insect and yeast cells, transgenic plants and animals. Bacterial production of scFv |
| **Difficult tumour penetration** | *In vivo* production of the antibody by the host, through viral transduction or direct DNA injection; use of scFv antibody fragments |
| **Weak results *in vivo* following promising results in mice** | Use of naturally occurring cancers in animals as models to study the therapies |

924 **Figure legends**



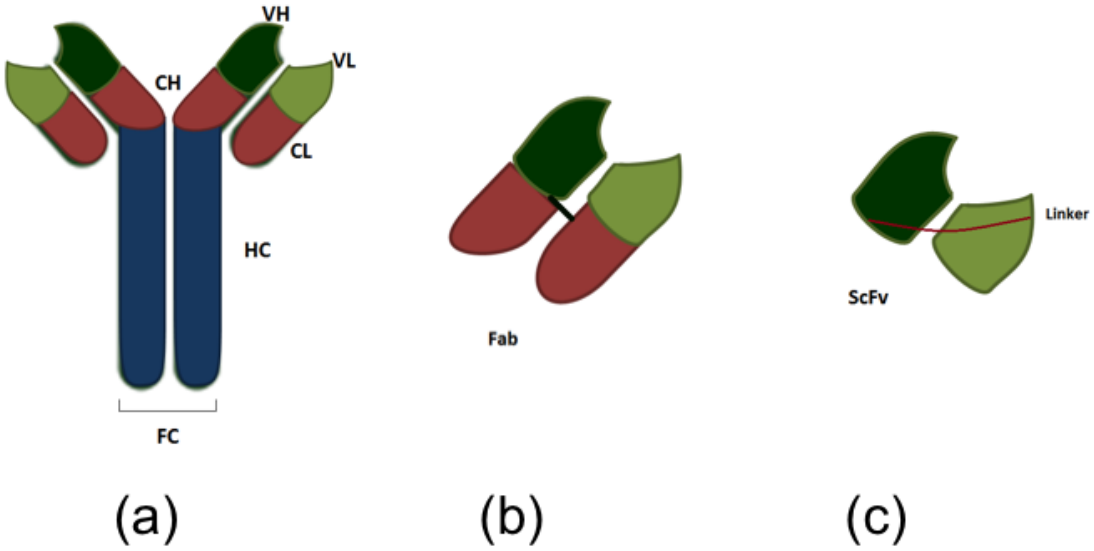

925

926 Fig. 1. History of monoclonal antibodies and its clinical uses. The top horizontal line presents
927 the main technological breakthroughs and the year in which they occurred (or the year around
928 which significant research was done on that subject). The parallel lower lines represent the
929 mAbs used clinically categorised by their structural classes with regard to humanisation. The
930 mAbs cited represent a milestone in the use of the technology; other dates of interest are also
931 shown. RA = rheumatoid arthritis; References: Carter et al., 2001; Lobato and Rabbitts, 2001;
932 Fong and Small, 2008; Reichert, 2012; Kellerman and Green, 2002; Enever et al., 2009.



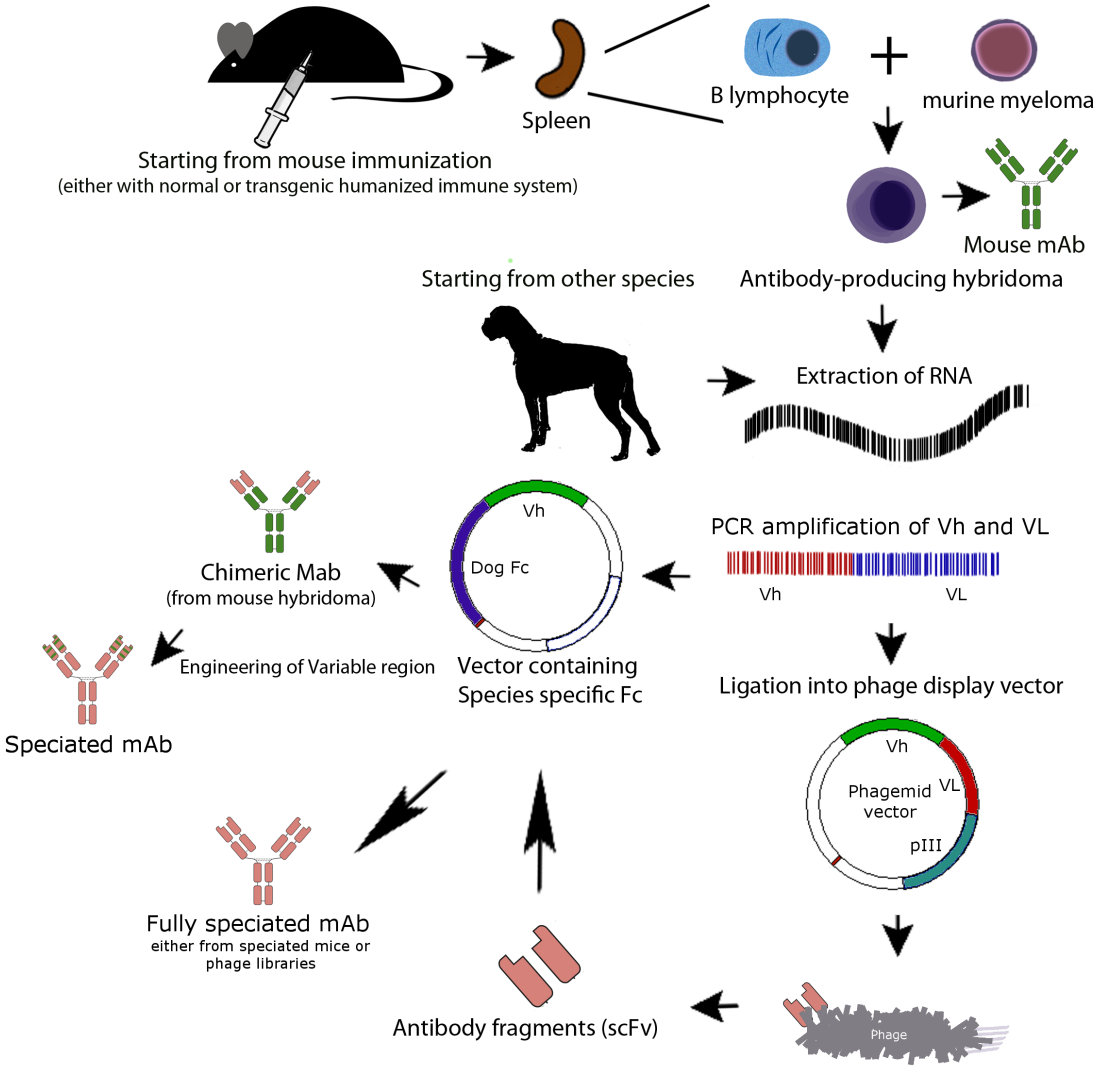

933

934 Fig. 2. The most common methods of antibody production and the resulting classes of
935 antibodies (Jakobovits et al., 2007).




936

937 Fig. 3. Representation of different formats of the antibody molecule. (a) Full length
938 monoclonal antibody showing heavy as well as light chain comprised of variable and
939 constant regions, also the hinge region and the Fc portion which interacts with cell surface
940 receptors; (b) Fab molecule which contains no Fc portion of the antibody, thus containing
941 variable as well as constant regions from heavy and light chains; and (c) scFv which contains
942 only variable domains of the heavy and light chains for antigen binding.

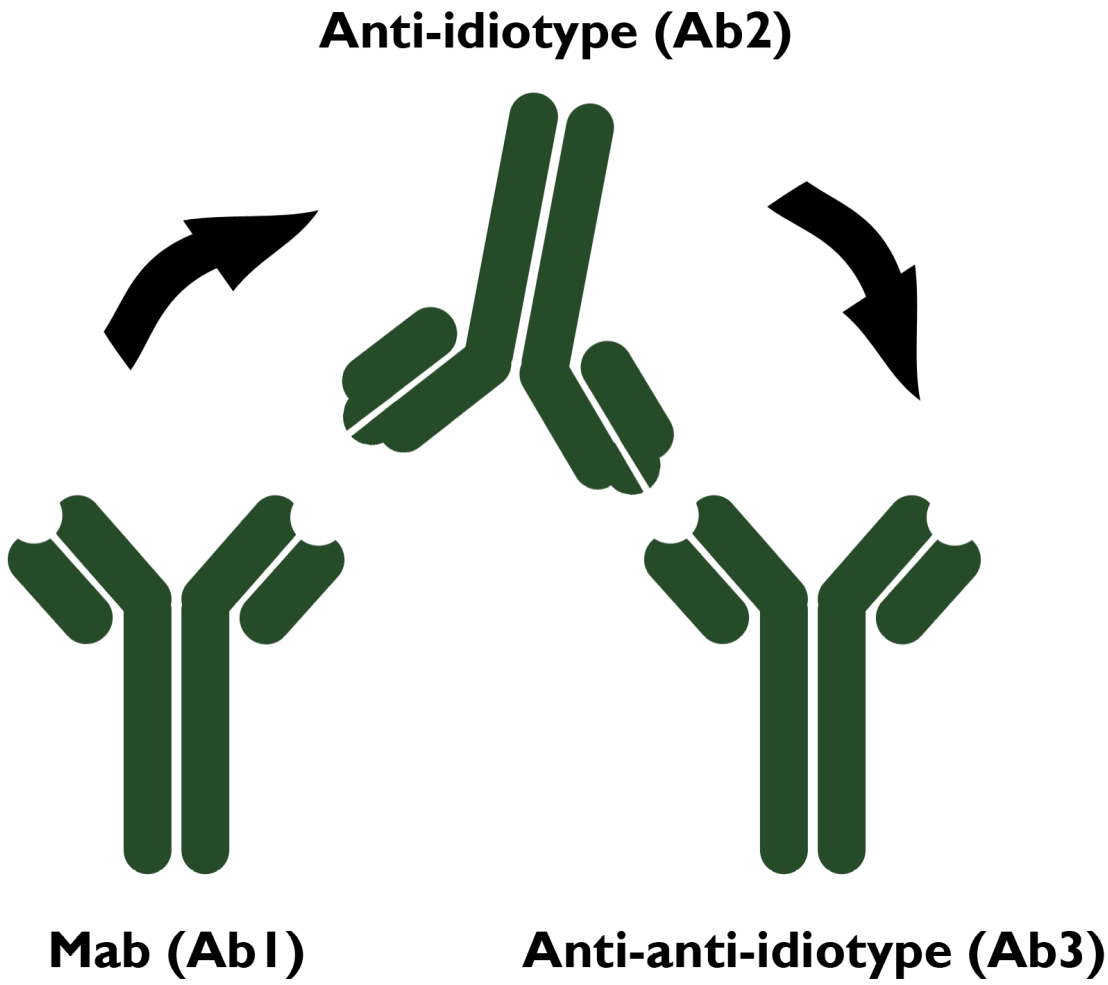



943

944 Fig. 4. Techniques for producing antibodies of different speciation levels. Antibodies can be

945 derived either from: 1) mice or other laboratory animals immunized with the antigen of

946 interest; unless the mouse was altered genetically to express the immune system from another
947 species (such as the XenoMouse), antibodies derived from laboratory species will be at most
948 'speciated' (humanized, for instance); 2) directly from the species of interest, in which case
949 phage display can be used to select the antibody of interest from the vast repertoire available
950 in any animal. In this case, the antibodies that are derived are 'humanized', or 'caninised',
951 etc.




952

953 Fig. 5. The idiotype cascade. The mAb itself is an antigen, and the patient receiving it will
954 raise an immune response against all the foreign parts of the mAb. Ab2 are created by the
955 host as a response against the antigen-binding region of the mAb (Ab1). Ab3 is then raised
956 recognizing the antigen-binding region of Ab2. Therefore, the antigen-binding region of Ab3
957 is identical to that of the therapeutic mAb (Ab1).

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