Home Office

NON-TECHNICAL SUMMARY

Generation and screening of companion animal therapeutics.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Companion animal, monoclonal antibody, Veterinary medicine, Cancer, Atopic dermatitis

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Rats	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To complete genetically engineered mouse platforms containing a collection of genes (immunoglobulin loci) from companion animals that are responsible for the generation of species-specific antibodies that would normally recognise and eliminate harmful substances like bacteria, viruses or toxins. The mice will be used to generate antibody sequences against a range of companion animal diseases. Once antibody sequences with desirable characteristics have been identified the antibodies will be produced using stable cell line culture techniques and molecules will be assessed in a range of studies using rodent models to test how well they remain detectable and/or functional over a defined period of time. The information gathered here will inform us which drug is best suited to take forward to test in the target animal species.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This programme of work is expected to discover new drugs which can be used to treat a range of diseases that companion animals suffer from. Companion animals are medically underserved compared to humans with only a handful of antibody drugs available compared to more than 120 human FDA approved antibody (mAb) drugs available.

Cell based efficacy tests provide valuable information, however it is necessary to confirm their anticipated characteristics in an animal by using mouse or rat models before progressing to the target animal species.

This project aims to generate drugs which address a range of diseases such as cancer, skin conditions, disease induced weight loss and pain.

What outputs do you think you will see at the end of this project?

Genes which are used in the creation of antibodies are well understood. By introducing these genes from a companion animal species into a mouse model, we expect to complete a genetically altered mouse which contains the vast majority of either cat (felinised), dog (caninised) or horse (equinised)

immunoglobulin heavy and light chain genes. We will introduce enough genes so that each species specific platform can reflect the naturally occurring diverse antibody repertoire.

The companion animal platforms will then be presented with different antigens to create a range of therapeutic drugs to address prevalent and regularly occurring diseases where there is a clinical veterinary need, such as cancer, skin conditions such as atopic dermatitis, pain, and weight loss associated with long term disease.

Once therapeutic candidate drugs have been generated they will be tested extensively in vitro (in cells) and the best and most likely to succeed candidates will be administered into rodent models to understand how they behave in a whole animal, we can also gather information on how safe these candidates are too prior to administration in their target species. For some indications such as cancer or anaemia we can use rodent models that reflect critical elements of the target disease to determine how likely our therapeutics are to succeed before testing in companion animal species.

Summary;

- New veterinary drugs addressing 5-10 disease targets such as cancer, atopic dermatitis, pain, anaemia and cachexia.
- Genetically engineered mouse models which produce species specific antibodies for companion animals that can be used to address additional therapeutic targets.
- Publications in the peer reviewed scientific literature
- Presentation of scientific data at meetings
- We will file patents, thereby placing in the public domain detailed knowledge of the discoveries we have made.

Who or what will benefit from these outputs, and how?

Antibody therapeutics being identified in this license will be used to validate and eventually treat companion animal disease indications. Candidate therapeutics that prove to be safe and effective may progress from this license into a preclinical testing phase in their target species. Following a demonstration of efficacy and safety, candidate therapeutics discovered under this license will progress into field studies and clinical trials in compliance with market authorisation standards detailed by the United States Department of Agriculturem, the European Market Authorisation and the Veterinary Medicines Directorate. Once market approval is obtained, therapeutics discovered under this license will progress towards use in general clinical veterinary practices.

Short term:

Monoclonal antibody therapeutics being discovered in this license that prove to be safe and effective may progress through to preclinical and clinical trials within the field of clinical veterinary medicine. Therapeutics discovered under this license will also progress towards obtaining USDA (United States Department of Agriculture) approval so that they can be used in the United States and EMA approval (European Market Authorisation) to enable their use within Europe. Following Brexit additional market

authorisation is expected to be required after 2025 through the Vetereinary Medicine Directorate in order for therapeutics to enter veterinary practices within the United Kingdom.

Medium term:

Therapeutics discovered under this license (or in future using the genetically altered mouse models) are expected to become available for use in clinical veterinary medicine to improve the health and welfare of our companion animals, treating a range of potentially serious and debilitating illnesses, such as cancer and atopic dermatitis. Monoclonal antibodies are expected to become a new standard of care in veterinary medicine.

Long term:

We are in a unique position to be able create therapeutic antibodies to address new targets more quickly than in the human pharmaceutical industry. It is anticipiated that the success or failure of novel companion animal therapeutic areas may infuence target selection and prioritisation within human medicine in cases where the biological mechanisms are similar.

How will you look to maximise the outputs of this work?

Drugs discovered under or due to this license will likely become available for use in clinical veterinary medicine. Appropriate business development will result in these drugs being administered internationally.

Data obtained under this license will be published in the form of patents and it is expected to be published in scientific journals so that some of the knowledge gained under this license can be utilised by other research groups.

Where possible technical or ethical refinements may be published in open access journals.

Species and numbers of animals expected to be used

- Mice: 42450
- Rats: 2400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Well established and sophisticated technologies are available to create genetically altered mouse models, where their genetic composition has been manipulated. Technology capable of introducing large amounts of genetic material (BACs) will be used to substitute part of the immunoglobulin loci in the mouse genome, which is responsible for generating antibodies. Companion animal immunoglobulin

genes will be introduced so that they can be used to generate species specific antibodies. These methods are very efficient and have been refined over many years so that less animals are needed compared to achieving the same goals in other species. When challenged with a particular foreign molecule, these mouse models will produce companion animal antibodies against it. These antibodies will then be identified and screened for desirable characteristics.

The canine transgenic platform that we are creating is the most complete to date, and displays similar immunological characteristics to dogs (Beagles) with very similar V(D)J recombination. This process shuffles and combines Variable (V), Diversity (D) and Joining (J) gene segments to create trillions of possible antibody receptor sequences which could recognise new disease target molecules. The canine mouse platform reproduces gene fragment combination and usage that is very similar to the target species, this provides excellent validation that our approach with a transgenic platform will be successful.

In some cases, the disease target molecule (antigen) will be similar in to one which occurs naturally in the mouse, in order to avoid inducing an immune response against self, some mice will have the mouse version 'knocked-out' or deleted so that it is recognised as foreign. This will only be completed if no negative side effects are expected form 'deleting' the specific gene.

All life stages of mice will be used to generate the genetically altered companion animal mouse models.

Rodent models represent the least sentient species which still demonstrate the physiological and genetic similarities to the companion animal species that we are generating drugs for. There are currently no better animal model steps that could validate our therapeutic drugs before progressing into target species preclinical testing.

After screening of new candidate drugs in cell lines (non licensed), they will be injected into mice or rats to gather information on how well they remain functional and available in the blood or organs. Also, what impact they have on the injected animal. Blood will be collected at established time points to obtain this data.

New drugs will also be tested in adult mice or rat disease models where the animals will recapitulate a particular target disease.

Examples of disease models include using mice in tumour engraftment studies. There are already well established protocols available to grow tumours under the skin (subcutaneous) on the flanks of mice, which are easy to monitor and measure, and which cause very little discomfort to the animal. The tumours generated from specific cell lines will be engineered to present companion animal versions of the disease target antigens so that new companion animal drugs can be assessed first in mice before progressing to target animal studies. Rodent models will also be used to assess how well new therapeutic antibodies perform which target energy intake disorders.

Typically, what will be done to an animal used in your project?

Superovulation (stimulate the release of additional oocytes)- female mice over three weeks will normally receive two substance administrations over a period of three days. Substances will be

injected into the abdomen (intraperitoneal) or under the skin (subcutaneous). To conduct this procedure mice will be scruffed and will remain conscious.

Vasectomy (to generate sterile mice) - male mice over the age of four weeks will be anaesthetised using gaseous anaesthesia and small 3mm incision will be made to the scrotum. The duct through which mature sperm pass, will be identified and then severed to remove a 4mm section. The small incision in the skin will be closed with one or two sutures (stitches). This process takes roughly 5 minutes. Pain relief is given before the procedure so the male mice will not suffer as a result.

Embryo transfer - Female mice which have been time mated to vasectomised males will be anaesthetised using gaseous anaesthesia. A small 5mm incision will be made along the midline of their back to allow access to either side of the reproductive tract. On each side of the mouse an incision will be made through the body wall to expose relevant reproductive organs (ovary, oviduct and uterus). Early stage preimplantation embryos will then be transferred to the section of reproductive tract which best matches their stage of development. Following embryo transfer the exposed tissues will be replaced and the incisions closed either by surgical glue, or using wound clips that will be removed 7-10 days later. This process takes up to 7 minutes. Pain relief is given before the procedure so the recipient mice will not suffer as a result.

Breeding and maintenance - Genetically engineered mice will be mated together in either pairs or in trio/harem breeding systems and the resulting offspring may carry genetically engineered genes.

Blood withdrawal - Mice which have a suitable combination of engineered genes may receive several blood withdrawals all from a superficial blood vessel by puncture with a needle whist they are conscious. Veins such as the saphenous (leg) or lateral tail vein will be used. Guidelines for blood withdrawal will be followed - no more than 10% in 24 hours or 15% in 28 days.

Immunisation - Mice may receive up to 5 administrations of an antigen over the space of 4 months. Injections may contain mild adjuvants (substances) that will induce a stronger immune response. Substances will normally be injected into the abdominal cavity (intraperitoneal), under the skin (subcutaneous), into a blood vessel (intravenous), rarely substances will be injected into muscle (intramuscular). Occasionally hydrodynamic tail vein injections will be used as a route of immunisation, where a large volume (8 to 10% of the body weight as a volume) will be injected into a mouse tail vein over a period of 8 to12 seconds. This solution will contain DNA which encodes for the target antigen, that will integrate primarily into the liver after organ swells and it will begin to express the target antigen. This procedure is conducted under anaesthesia and pain relief is provided. Mice will remain anaesthetised for 4 minutes after the injection so that the most significant side effects wear off. Mice should be fully recovered within 2 to 4 hours.

Tumour studies - Some mice will have tumour cells injected under the skin (subcutaneous) on their flank and be left to form tumours potentially until they reach a mean diameter of 15mm. Normally this will take 4 to 6 weeks after administration however the experiment may persist longer if they are given a drug which decreases the size of the tumour or delays the growth.

Pharmacokinetic, pharmacodynamic and safety studies - Novel drugs will be administered under the skin (subcutaneously) or more commonly into a vein (intravenous) of mice or rats. By performing routine scheduled blood withdrawals we are able to monitor how well the drug remains available in the

blood and in certain organs of the animals over time. Antibodies typically have a long half life so the animals may be monitored for several months.

Metabolism targets - Mice or rats will receive a substance either in their food or drinking water, or given through oral dosing (oral gavage) or as an injection into the peritoneal cavity (Intraperitoneal) that will begin to replicate a weight loss disease up to a maximum of 10% weight loss. Some animals will be given a drug as an injection under the skin (subcutaneous) or into a blood vessel (intravenous) that aims to increase weight. In some cases a high fat diet may be given to the animals so we can assess a difference between drugs over a shorter time period. Experiments are not expected to last more than 6 weeks.

Pain studies - Mice or rats will receive an injection into one knee joint that will simulate the onset and progression of arthritis. Candidate drugs will then be administered with the aim of alleviating pain (measured by limb weight bearing ratio and joint swelling) and slowing or preventing disease progression. Experiments will last up to 28 days.

Terminal blood withdrawal - In some cases where a terminal blood sample is required, animals will be placed under terminal anaesthesia and blood will be withdrawn using a needle and syringe either directly from the heart or from an internal easily identifiable blood vessel (caudal vena cava). Animals will then be killed by an approved method without regaining consciousness.

Animals will not be reused during this project.

What are the expected impacts and/or adverse effects for the animals during your project?

Some female mice will receive a couple of injections to stimulate egg release for time point mating/harvest. This is not expected to exceed the minimum level of suffering associated with the insertion of a hypodermic needle following good practice and will have no lasting harm.

Most genetically altered mice used under this license will be used to breed for 3 to 6 months and are not expected to experience any adverse effects associated with any genetic modification.

Some of the animals will experience transient pain from the minor surgery required for embryo transfer and vasectomy. These procedures will take a roughly 5 minutes and will always be conducted under anaesthesia with a suitable pain management program in place to support the recovery over a few days.

The majority of mice which are immunised under this license will experience transient discomfort from a series of injections which are likely to occur every 2 to 4 weeks for up to 5 occasions. Adjuvants may also be administered alongside a target antigen to stimulate an immune response. Mild, non-ulcerating adjuvants will be used but mild injection site reactions may occur, such as the formation of small granulomas, localised inflammation. Rarely animals may exhibit reduced movement, hunched posture, piloerection (erect hairs over their body) for a period up to 3 to 4 hours following immunisation. Blood will be collected following a needle stick to a superficial vessel at predetermined points. This is expected to cause mild discomfort that will resolve within minutes.

A small proportion of immunisations will be conducted under anaesthesia to inject DNA directly into a vein. Appropriate pain relief will be provided and mice will be recovered from anaesthesia when the

majority of adverse effects have resolved after approximately 4 minutes. Mice will be subdued and lethargic after injection but will recover fully within 2 to 4 hours following injection.

The procedures used for tumour cell implantation under the skin will involve needle pricks. They will be conducted under anaesthesia to ensure refined placement and the mice will be expected to be subdued and lethargic for 30 minutes after the injection whilst they recover from anaesthesia.

The tumours will be allowed to grow to a size where they may cause some discomfort and may cause other clinical signs. We will carefully monitor mice with tumours to make sure that the tumour is not having a major impact on the health of the mice. We monitor weight, mobility and general condition of the mice. We also routinely measure tumour size, which may involve feeling it through the skin or using imaging techniques.

Tumour growth will occur from roughly two weeks after injection however it is unpredictable and they make take up to two months to grow. Monitoring will be regular but will increased during critical periods of growth. Tumour bearing mice will be humanely killed at the point that tumours reach a predetermined size in line with current UK guidance.

Some genetically altered animals which are being produced may have an altered immune system that may make them more susceptible to infection. To keep mice with immune deficiencies healthy, they are maintained in a very clean facility where they are not exposed to harmful viruses or bacteria.

Mice and rats used in dose ranging studies will experience several blood withdrawals, they are expected to cause mild discomfort that will subside very quickly after the procedure and have no lasting harm. Due to how well antibodies persist within an animal it is not expected that we will require blood withdrawals frequent enough to warrant the use of catheters.

For some disease model studies we need to induce a metabolic intake pathway disease. Whilst the administration regime is not expected to cause any adverse effects it is likely that a small proportion of control animals will lose up to 10% of their body weight, and may experience discomfort as a result. Clinical signs may include becoming less active and adopting a hunched posture and their coat quality deteriorating. Animals on this protocol will be weighed up to 3 times a week to monitor weight loss. The pathway induction step will commonly coincide with the provision of a high fat diet - this will ameliorate weight loss but may also cause animals to gain weight and become obese. The experiment duration is not long enough to expect significant adverse effects as a result of the weight gain however measures will be in place to regularly change food to lower excess levels in the home cage environment. Animals on high fat diets will often develop greasy unkempt coats partly due to residual levels of diet around the cage. In rare cases this may cause irritation or skin reactions.

Mice and rats used in osteoarthritis like pain studies will experience localised pain and inflammation to a single knee joint. The vast majority of animals will receive a type of drug to alleviate this pain however some animals will not receive any treatment so we can understand how much of an effect each drug has. These animals are expected to show weight bearing preference to the unaffected limb and may move less. If ant any point mice or rats exceed the end points of extreme limb preference or significant weight loss then will be killed.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice

Mild: 90%

Moderate: 10%

Rats

Mild: 75%

Moderate: 25%

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Within each animal trillions of different antibodies are presented on the cell surface of B cells, which circulate in the blood. Naturally they recognise and kill bacteria and virus infected cells by binding to a foreign substance known as an antigen. Because each antibody is different, once it binds an antigen it is already quite specific, but other antibodies will evolve from this sequence so that they become more and more specific and bind more tightly. This specificity, affinity and ability to recruit an immune response to destroy the target antigen presenting cell makes them useful as a therapeutic drug. Depending on how antibodies recruit a killing response from the immune system, they may also act to bind and block a target antigen so to prevent further activity in a particular biological pathway.

The immune response is highly complex and the adaptive immune response we are interested in matures over several months. It involves the interaction of many different cell types and migration of these cells to a variety of sites in the body. The intricate interaction between the different cell types simply can't be reproduced in a laboratory. Antibodies which have matured through this process are more likely to have better characteristics which are key to creating viable therapeutics such as thermal stability, solubility and specificity. By introducing companion animal immunoglobulin genes into a mouse platforms we are able to fully utilise this biological mechanism to make species specific therapeutic antibodies.

There are already more than 100 antibody therapeutic drugs approved by the FDA/USDA/EMA for the treatment of human diseases which target specific antigens present on disease-associated cells, such as cancer.

Regardless of their quality in cell based tests, it would be unethical to use experimental therapeutics on target animal species or patient owned animals without first performing some safety studies in a controlled laboratory setting, in this case using mice and rats. Before any candidate therapeutic is administered to an animal under this license a comprehensive selection and screening cascade is completed using at least two cell based tests.

Which non-animal alternatives did you consider for use in this project?

Display technologies

An alternative, artificial antibody discovery process known as 'phage display' uses bacteria. As part of the phage display process, viruses which naturally infect bacteria are modified in such a way that they carry antibody fragments. A 'library' of different sequences is then generated which may contain millions of combinations. It is then possible that some will bind the particular drug target molecule (antigen). Yeast display libraries can also be used in a similar way.

When monoclonal antibody combinations generated in this way bind to an antigen of interest, there are associated issues which are problematic when creating therapeutic antibodies. The binding is normally not very strong, because the pairing is immature and they also often lack specificity which could result in off target binding, which can cause serious side effects.

When antibody sequences are obtained from our transgenic mouse platform they are considered to be mature. They will have gone through rounds of 'optimisation' against the target antigen by repeat antigen administrations (immunisations). Biological processes responsible for this adaptive response have been highly conserved across species over millions of years indicating how fundamental this process is. Mature antibodies typically make much better therapeutics, they will bind to a target antigen up to a thousand times more tightly than those generated through display technologies and they will have much better specificity so that they can be developed as drugs without further modification. Modifying antibody sequences is more likely to result in side-effects when injected into their target species.

Artificial intelligence & machine-learning

In recent years, artificial intelligence and machine-learning (AI/ML) for both the prediction of unknown protein structures and also the design of protein-protein interactions has received a high profile in mainstream media and in specialist areas of research. Many factors contribute to this, including a number of academic breakthroughs, advances in computing power and, arguably the most important contribution is due to the increased availability of large datasets for AI training.

In order to predict protein structures and design new proteins, large language models (LLMs) are used. These are based on neural networks which are a series of pattern-recognising algorithms. These algorithms are loosely modelled on the current understanding of how neurons function in the brain.

Generative artificial intelligence for the de novo or novel generation and design of proteins (including monoclonal antibodies) has developed significantly over the past 5 years. The ability to rationally design binding properties in silico for a protein of choice has immediate therapeutic applications, this has been led by small molecule drugs for over two decades. However, large biological molecules such as an antibody are significantly larger and more complex than small molecules, they are therefore more difficult to model and design in a rational way. For an antibody to be a therapeutic candidate, it must satisfy a set of stringent criteria or “drug-like properties”. For example, it must usually bind the target in a precise place (epitope) which is determined by the biology of that target, it must bind to the target with an affinity that permits standard medical doses (usually <10 nM), it must be amenable to manufacturing at scale (“developable”), sometimes cross-reactivity with different protein families is necessary or conversely be highly selective of a single protein and it must fail to be recognised as foreign in the body.

To achieve these properties, selecting a therapeutic antibody from a discovery campaign has meant screening hundreds or thousands of candidate antibodies in order to find one which displays the complete set of characteristics. AI/ML promises to design-in many of these features in silico instead of relying on discovering them “by chance” during antibody discovery.

Why were they not suitable?

Display technologies

Artificial phage display technologies are a viable option for some fields such as toxicology and anti-venom research and in some cases, they can be used in experimental applications such as tool antibodies used for cell based tests such as generation anti-idiotypes, biomarker detection, sandwich ELISAS or FC/IgG isotype detection. However, when trying to develop therapeutic drugs unfortunately, this technology has significant limitations which restricts its usefulness.

The EURL ECVAM publication which provided the controversial recommendation that "animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications. In the EU, the provisions of Directive 2010/63/EU should be respected and EU countries should no longer authorise the development and production of antibodies through animal immunisation, where robust, legitimate scientific justification is lacking". The report acknowledges it did not consider the field of therapeutic applications despite including the field in their recommendation, particularly the use of transgenic mouse platforms. This is surprising and concerning given the lack of successful market authorisations for monoclonal antibodies generated by display technologies.

Monoclonal antibodies generated by transgenic platforms achieve twice the revenue of monoclonals generated by display technologies and have four times as many blockbuster drugs (>1 billion USD in sales annually). It is worth noting that one of the earliest display technology generated monoclonal antibodies accounts for over 80% of revenue for all display generated monoclonal antibodies. Despite the investment into display technologies over a comparable time period, market authorisations have been lacking. It is noteworthy that this first blockbuster display generated monoclonal antibody was not isolated de novo from a proprietary company library, but instead it was isolated using a “guided selection” approach in which a human antibody was derived by switching the original murine antibody binder chain for a human equivalent.

In vivo antibody maturation in an immunocompetent mouse allows antibodies to develop and mature in their natural biological context, which provides the necessary selection pressure and interactions with immune cells to refine the antibody's binding properties. In vivo maturation involves a process called affinity maturation, where B cells that produce antibodies with higher affinity for the target antigen are preferentially selected and undergo further rounds of mutation and selection. This process of continual improvement gradually improves the antibody's binding affinity after repeat exposure to an antigen. Immunisation also takes advantage of the interaction between B cells and T cells within the complete immune system. T cells provide signals which help B cells optimise their antibody production, leading to the creation of antibodies with improved specificity and functionality. In vivo maturation generates antibodies with natural modifications, such as somatic hypermutations, glycosylation patterns, and alternative splicing. These in vivo modifications impact an antibody's efficacy, stability, and interaction with other immune components which are fundamental for a therapeutic antibody.

The generation of phage display antibodies only uses in vitro selection, which lacks the complexity and intricacies of the complete mammalian immune system, in some cases the display prokaryotes will not be able to correctly fold an antibody protein in the same way as a Eukaryote cell (used for therapeutic antibody production). These processes cannot fully replicate the complex affinity maturation process occurring in a whole organism nor the complex interactions between B cells and T cells. For example it is difficult to generate antibodies with long HCDR3 regions (which are more appropriate for certain antigens and certain species) and it is common that phage display libraries are unable to express certain immunoglobulin gene families which limits their ability to capture natural diversity and pairing, something that is crucial when generating therapeutic antibodies.

In summary, when used to address native targets (such as a virus) their lack of initial specificity (binding more than one target), have weaker binding and associated developability issues which can make it difficult to formulate into a drug that can be given (in our case) to a companion animal. To fix these issues requires a huge laboratory resource and has a low success rate. Rounds of additional biopanning can be used to affinity mature phage candidates however artificially modifying antibody sequences is also more likely to result in serious side effects associated with immunogenicity, the likes of which in some cases have led to fatalities within human clinical trials.

It is also worth considering that phage display libraries are not truly animal free as they are often attained from a target species after they have been immunologically challenged.

Artificial intelligence (AI) & machine-learning (ML)

A US-based drug-discovery company at the forefront of antibody generation de novo (from nothing) using AI/ML techniques and their recent publication provides a good summary of current capabilities in this space. Authors used AI/ML to design new antibodies which bind the cancer target antigen HER2 at a site which neutralises its function with therapeutically-relevant characteristics. 3 antibodies which have a slightly higher affinity than an existing therapeutic 'blockbuster drug. This is a significant achievement. These antibodies had been designed to contain high likeness to human antibodies (“humanness”) so that the likelihood of them being immunogenic after administration is lowered. A closer inspection of the paper reveals that for the design of these AI-generated antibodies they first required structural knowledge of both the target antigen HER2 and the ideal place for the antibody to bind (epitope). This is identical to the existing therapeutic. Unfortunately for most novel antibody discovery projects this information is simply not known or available when generating therapeutics and importantly it is not a requirement to discovery antibodies by other means such as through animal

immunisation. The AI-designed antibodies were only fully novel in one small region of the antibody variable region (heavy chain CDRs which account for about 10% of the antibody variable sequence) and the rest of the antibody sequence including the whole light chain was taken from the pre-existing therapeutic antibody. In typical novel discovery campaigns, a template sequence is not usually available. Furthermore, there was no indication of the developability properties of these AI-designed antibodies.

In summary AI/ML algorithms are currently capable of producing which given the complexities of designing an entirely new protein capable of binding another, is remarkable. However, this relies on a wealth of pre-existing information that just isn't available in novel drug discovery campaigns. There has so far been no demonstration that in silico approaches can generate a fully-novel, fully drug-like monoclonal antibody.

Summary

The ethical and cost arguments against animal-derived antibodies are strong, however this must be balanced against bringing the best antibodies possible to a clinical setting. At the moment, the best and most likely avenue for generating therapeutic grade antibodies combining the required developability profile is clearly from in vivo-derived immunisations. However given the speed of development in the area of artificial intelligence and machine learning, its limitations and weaknesses should be thoroughly re-investigated in line with the anticipated end of this project in 5 years time.

Until replacement technologies are truly equal in their capability to generate therapeutic antibodies we must focus on reduction and refinement by selecting therapeutic targets with as much scientific diligence as possible and continue to optimise immunisation protocols, by ensuring the best and most appropriate immunogens are used and lastly by fully extracting, analysing and exploiting all data generated.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The majority of the companion animal discovery platform colonies have already been completed so we are able to estimate the number of mice required quite confidently based on existing experience. However generating mice from highly engineered embryonic stem cells can be challenging and somewhat variable.

The complex breeding regimes to create the multi-allele discovery platforms has been calculated using in-house developed algorithms which are used routinely to predict which mice and how many of them

to use in breeding. These calculations have also been applied when considering the generation of genetically altered disease models.

The number of animals required for screening studies has been calculated on the number of anticipated new drugs expected to be tested under this license.

Following harm/benefit analysis, re-using animals is not considered.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Control groups provide an essential contrast to the drug treatment groups. They will provide critical baseline data that drugs can be assessed against. Animals will be allocated randomly to experimental groups by using a randomisation tool however social housing will be taken into consideration where possible.

Technicians and veterinary staff will also be blinded to the candidate therapeutics being administered as part of the experimental design strategy.

Quality control will be performed on all materials used with in vivo studies, such as cell lines and proteins, to make sure that when they are administered into mice or rats there is the highest likelihood of producing good data.

Standard operating procedures will be used to ensure that we get the maximum amount of useful biological information from each animal.

For every study plan type the NC3R's EDA tool will be used to help validate and provide clarity on the structure of each study.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In silico and in vitro screening programs will be completed first for any therapeutic being administered under this license. Those with obvious liabilities will be excluded at the earliest time point possible so that only the most likely to succeed will progress into animal studies.

Colony management software will be used to coordinate study plans and to monitor rodent colonies. This database will link with in-house developed algorithms to predict the most beneficial mating so that the fewest animals are used to achieve a desired genotype or colony size. The software records a comprehensive amount of data including pedigree, regulated procedures and an electronic health record for individual animals from birth until death.

Mouse colonies will be cryopreserved and removed as a live resource at the earliest opportunity when they are no longer required.

Pilot studies will be conducted using a small number of animals to confirm the expected performance of particular antigen materials or disease models before initiating experiments with larger numbers of animals (or not if they are unsuccessful).

Experimental noise will be limited by controlling as many variables as possible, including genetic background, age, sex, the environment. Microbiome screening will also be utilised to this end.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Both Mice and rats will be used in this project

Genome Engineering

Most of the genome engineering steps required in this project will be for the replacement of mouse antibody producing genes with corresponding companion animal genes. These replacement genes will work as the mouse genes do so that the mice will maintain a fully functional immune system.

Where mice are used to model disease they may be genetically engineered so they express a companion animal version of a protein that will interact with a therapeutic drug so we can simulate interactions which may occur in the target species.

Some gene knock out models will be created if the drug target antigen is highly conserved between species. By deleting a conserved gene, the mouse will then recognise the drug target antigen as 'foreign' and mount an immune response against it. Literature and databases will always be consulted to confirm whether the deletion is viable and that it wont have an associated harmful phenotype.

Immunisations

The immunisation methods in this license are refined and widely used and a standardised approach to each discovery target is adopted. State-of-the-art technologies such as B cell isolation and sequencing are well established to insure that we gather as much information as possible from every immunised mouse.

A significant amount of work is performed in the laboratory to maximise the chance that an antigen has the correct composition to bring about a good and meaningful immune response. This includes ensuring that the antigen is pure, free of toxins and has not been damaged during its preparation and that it has not been degraded or aggregated together.

Thorough preparation of the antigen will also ensure that each mouse experiences the fewest and mildest adverse effects necessary whilst still producing the desired immune response.

Dose ranging and safety studies

Following the administration of a therapeutic drug into mice or rats, blood withdrawals are crucial and will be taken at set time points to understand how long our drugs remain active in an animal. This procedure is simple and well established for both species that will be used under this protocol.

Disease models

When addressing cancer targets, genetically engineered or wildtype mice may be used to conduct experiments where cancer cell lines presenting the disease target are injected into mice. In some cases the mice may have immune defects so that we can manipulate their immune response. Typically the tumours will be injected under the skin which allows us to monitor the size of the tumour to limit suffering. We can then confirm how well our drugs modulate the disease progression.

Wildtype rats will also be used alongside or instead of mice in some disease models where their larger size offers a scientific or ethical advantage, for example if they can reduce the variability and number of animals required in a study.

Well established protocols exist in both mice and rat species which use chemicals to create localised pain and inflammation like that experienced from arthritis. Rats will be used when there is a need to perform more tests on the fluid from within the knee joint because it is not possible to recover a large enough volume from mice. Using a chemical to create this type of pain and inflammation is more consistent and refined method when compared to surgical methods which physically damage or cut ligaments or membranes in the knee joint. After administering the particular chemicals into the knee joint, the speed of cell death is well established and we plan to use the lowest possible dose to simulate arthritis. Also, lower concentration injections of chemicals may better reflect real companion animal arthritis pain and inflammation.

Why can’t you use animals that are less sentient?

The immune system requires several months to develop a robust immune response against a particular target antigen. This process only happens in mature adult animals so it is not possible to use younger animals or those which have been terminally anaesthetised.

Organisms deemed to be less sentient are not suitable either due to the lack of genome editing technology available and because they do not possess the same immune system mechanisms.

When assessing how well a drug persists within an animal, or testing the effects upon the animal it is necessary to use a species which has a physiologically similar immune system to our target animal species.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The production of genetically altered mice is very refined and streamlined. One recent refinement that I will also implement on this project is the co-housing of excess female mice with stud males which have been vasectomised who would otherwise would spend ~70% of their time singly housed.

When starting immunisation campaigns the mildest adjuvants and antigen presentation methods are used, only if these are not successful will stronger adjuvants and methods be used. Where mice already produce a protein which is very similar (highly conserved) this escalation of adjuvants can in some cases be circumvented by producing mice which have a genetic 'knock out' of the target so that the mouse will recognise the antigen as 'foreign' and mount an immune response against it. Resources such as the international mouse phenotyping consortium and the international mouse strain resource will be used to identify any viability issues of 'knock out' models, also whether they already exist to prevent duplicating mutations.

Wherever regulated procedures are completed the use of pain relief will be carefully considered and an appropriate management regime implemented under the direction of the Named Veterinary Surgeon. In addition to comprehensive pain management, animals recovering from surgical procedures or those on disease model studies will be provided with floor food and soft diet if necessary. A programme of more frequent monitoring will be implemented for all animals on experimental procedures so that any adverse effects are quickly identified.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Where appropriate we will act in best accordance with the ARRIVE (2.0) and PREPARE guidelines from norecopa/RSPCA particularly with regard to the equivalent methods sections.

The Experimental design assistance (EDA) developed by the NC3Rs will also be utilised to help validate study design and also to enhance understanding of overall study design by providing useful visualisations of experimental groups and commissions.

The NC3Rs good practice guidance documents will also be utilised when performing administrations and blood withdrawals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Subscription to regular newsletters from groups like the NC3Rs, LASA and the RSPCA as well as attending relevant courses and conferences will help to stay informed on best practice. Close liaison with the Named Information Officer at the establishment will also facilitate efficient passage of information.

I am a member of several transgenic technologies groups where advances in genome engineering techniques and best practice are frequently discussed.

The NC3Rs 3Rs resource library will also be referred to during the project to help maintain best practice.