EARA/EFPIA response to EURL ECVAM Recommendation on Non-Animal-Derived antibodies

European Animal Research Association

efpia
European Federation of Pharmaceutical Industries and Associations

animalhealth europe

Contents

About

European Animal Research Association (EARA)

EARA represents 100 public and private institutions in the biomedical research and pharmaceutical sectors in 20 countries across Europe. The Association informs people about the continued need for, and benefits of, the humane use of animals in scientific research, and provides a unified voice for the biomedical research community on matters in support of the essential use of laboratory animals for scientific purposes.

European Federation of Pharmaceutical Industries and Associations (EFPIA)

The EFPIA represents the biopharmaceutical industry operating in Europe. Through its direct membership of 36 national associations, 39 leading pharmaceutical companies and a growing number of small and medium-sized enterprises (SMEs), EFPIA's mission is to create a collaborative environment that enables our members to innovate, discover, develop and deliver new therapies and vaccines for people across Europe, as well as contribute to the European economy.

AnimalhealthEurope

AnimalhealthEurope is the association representing manufacturers of animal medicines, vaccines and other animal health products in Europe. It is a not-for-profit body representing both corporate members and national animal health associations in Europe. It represents both innovators and generics alike, as well as large, medium-sized and small companies. AnimalhealthEurope's membership covers 90% of the European market for animal health products.

Section 1 – Executive Summary

The JRC recommends that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications and that EU countries should no longer authorise the development and production of antibodies through animal immunisation, where robust, legitimate scientific justification is lacking for animal use in a call for adherence to the legal obligations under Directive 2010/63/EU.

European Animal Research Association (EARA) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) contend that such recommendation is premature, in particular in the field of vaccines and therapeutics which were not part of the ESAC opinion, even if the scientific community acknowledges the forward-thinking approach that the EURL ECVAM have adopted in exploring the potential for increasing the scientific impact from the generation of high-quality antibodies and technology development in non-animal-derived antibody libraries and display technologies.

Indeed, there are technological, scientific and regulatory questions that need to be addressed to fully deploy the methods in all areas where antibodies are used, meet the needs of patients and increasing human and animal health research demand. The existence of a method and its scientific validity in the context of research, does not necessarily and automatically translate into industrial processes in a regulated environment. This step requires demonstration of feasibility in specific contexts and the ability to scale up the production, adaptation and validation of manufacturing processes.

The current healthcare crisis and strategic technological resilience ambition of the EU (as expressed in the industrial strategy and pharmaceutical strategy roadmap) underpins the need to discuss what constitutes sufficient justification, including technical parameters leading to a scaling-up, for large-scale use in R&D and regulated industrialised contexts, of development and production of medicines.

The research community also calls for support in diversifying the production capacity, investing in cutting down the cost of novel production methods, and offering appropriate support to the evaluation of projects/applications in a fast-evolving area of science that still has a relatively small subject matter expert community. It is an observation that, when in doubt, competent authorities tend to delay or halt decisions.

EARA/EFPIA therefore recommend the following:

- It has been identified that presently it is premature to consider the EURL ECVAM Recommendation for applications involving non-animal-derived antibody production and use for research, diagnostic, therapeutic and regulatory application. EARA/EFPIA would particularly welcome a thorough evaluation on the feasibility of therapeutic applications in this context.

- There is a need to discuss what constitutes a robust, legitimate scientific justification in practice, including the feasibility of technical parameters leading to a scaling-up for use in the R&D and regulated industrialised contexts. EARA/EFPIA recommends that EURL ECVAM develop a Q&A, along with experts, to help with nuancing. This will provide support in the short-term, to Member State authorities for them to be better informed, about the considerations to be taken into account.

- The research community calls for regulatory support and legal certainty, in line with the EU industrial and pharmaceutical strategies, to diversify the production capacity, investing in cutting down the cost of novel production methods and offering appropriate support to the evaluation of projects/applications.

- EFPIA/EARA recommend to use existing (EPAA) - potentially with a broader stakeholder participation - or new platforms for a wider exchange amongst researchers, industry, regulators and civil society on the production and use of non-animal alternatives to animal-derived antibodies and how they evolve over time and are fit-for-purpose, taking into account the scientific, technical and ethical issues.

- Building on the recommendation above, EARA/EFPIA would welcome the establishment of a permanent stakeholder platform which includes balanced representation of all relevant stakeholders where the objective would be to identify areas of alignment in progressing the animal research and welfare agenda together.

- The scientific community will continue to invest in the research and development of robust technologies and high quality science which will replace, reduce and refine the use of animals. Public private partnerships, like IMI and the future health Partnership, are excellent opportunities to progress science in this area. EARA/EFPIA therefore recommend that future projects are considered which are in line with EU policies, to progress in this field. The annex offers case studies illustrating the points above.

EARA/EFPIA look forward to a continued constructive dialogue on these matters with the JRC and with competent authorities to avoid unintended consequences while supporting scientific progress.

Summary of key points:

- Animal-derived antibodies have unique properties, being based on the immune systems of humans and animals, that make them critical tools throughout the biomedical sector from basic research to the development of life-saving drugs.
- Although non-animal-derived antibody technologies have developed considerably, they still cannot recapitulate many of the properties which make animal-derived antibodies so useful, and there are numerous situations in which non-animal-derived antibodies simply cannot replace their animal-derived counterparts.
- Within the field of therapeutics and drug development, non-animal-derived antibodies cannot currently compete and the vast majority of approved therapeutic antibodies are from animal-derived origin.
- In COVID-19 research, we have seen the benefits of animal-derived antibodies which have played a key role in potential therapeutic development and will be essential in the large-scale production of approved therapeutics which counter coronavirus infections.
- Animal health and veterinary medicine requires development of antibodies suitable for a vast range of animal species, which currently is not possible with non-animal-derived methods.
- Restricting the use of animal-derived antibodies would have implications for the competitive nature of EU research, and access of European patients to the best medicines.

Section 2 - Setting the Scene

Antibodies are complex proteins that form a critical part of our immune response to disease and are a vital source of ***therapeutics*** for the treatment of a wide spectrum of life-threatening disorders as well as providing ***research tools*** to advance scientific understanding across a vast range of scientific disciplines. Animal-derived, or *in vivo* antibodies are produced by injecting an animal with a small amount of the target protein, generating an immune response and resulting in antibodies that are specific to the target in question. For decades, the discovery and development of many important drugs for cancer, autoimmune, inflammatory and infectious disease patients have utilised the unique ability of an intact animal immune system to deliver high quality antibodies and this approach remains as relevant today as ever as evidenced by the global scientific response to the SARS-CoV-2 pandemic.

A survey[1] conducted in 2020, highlights that the scientific community acknowledges the forward-thinking approach that EURL ECVAM has adopted in exploring the potential for increasing the scientific impact from the generation of high-quality antibodies – a position EFPIA and EARA support. We acknowledge the continuous technology development in non-animal-derived antibody libraries and display technologies and many of the responder groups are themselves actively involved in the development and implementation of non-animal alternatives for antibody generation.

Key findings from the survey include that 90% of researchers questioned utilised animal-derived antibodies for exploratory and pre-clinical research and in the discovery of therapeutics, whereas less than half of the recipients use non-animal-derived antibodies (primarily within certain aspects of drug discovery). Therefore, it is the opinion of the EFPIA and EARA that non-animal methods cannot fully replace the animal-derived methods, since non-animal-derived antibodies are not suitable for all uses and applications that are vital for scientific research and drug discovery for unmet patient and animal needs.

The need to continue to utilise *in vivo* systems to generate suitable research tools and therapeutics must of course be balanced with moral and societal commitments to 3R practices (***R***educing the number of animals used, ***R***efining experiments to minimise the impact on animals, and ***R***eplacing animal experiments wherever possible with alternatives), and in strict compliance with Directive/2010/63/EU on the protection of animals used for scientific purposes. Here, implementation of new methods and technologies such as single B cell cloning which has significantly increased the number of antibodies that can be identified per animal, the refinement of immunisation processes and the non-terminal immunisation techniques used for larger animals have further increased the quality of antibodies that can be generated whilst reducing the number of animals euthanised. The following pages will explain how the use of complementary antibody discovery platforms, a focus on high-quality antibodies and modern technologies, justify the continued use of a very limited amount of animals for development of therapeutic antibodies in the future, to be able to address both today's and future drug targets for unmet disease.

[1] in July/August 2020, EARA and EFPIA surveyed the scientific community to and 133 responses were received from the private (25%), public (68 %) and other respondents (6%), representing 9 Member States (Germany, France, Belgium, Spain, Portugal, Denmark, Hungary, Italy, and the Netherlands), the UK and Switzerland.

Summary of animal-derived and *in vitro* synthetic antibodies in therapeutics, research and diagnostics

There are two key methodologies used for the generation of antibodies, *in vitro* display technologies and *in vivo* immunisation based platforms (Table 1). Increasingly, these two technologies are combined with modern molecular biology techniques (not described in the EURL ECVAM Recommendation) to harness the power of *in vitro* display, with the unique ability of an animal immune system to generate exquisitely selective antibodies with higher potency, via the natural mammalian process of *in vitro* maturation and selection. It is exactly this process that is exploited by the human body to fight disease, thus *in vivo* generated antibodies (from human or animal species) are an important high quality starting point for therapeutic antibodies. Recent advances in molecular biology i.e. single cell sequencing have been embraced enthusiastically by the antibody discovery community and have accelerated and improved discovery workflows. This has resulted in significant reductions in the number of animals utilised and, simultaneously, has improved antibody quality via early integration of recombinant molecules in discovery pipelines. This and other technology enhancements (for example mass spectrometry-based methods for direct antibody De Novo sequencing) have generally superseded the need to use hybridoma-based technologies for modern drug discovery.

	in vivo animal-derived	*in vitro* synthetic
Advantages	• Large numbers of top quality antibodies can be identified • High specificity and affinity • High solubility and stability • Integrates mammalian *in vivo* post-translational modifications • Highly available in the antibody market • Technically easy to generate • Technology well-established in academic and applied research • Enables generation of antibodies against cDNA encoded targets or in forms not suitable for *in vitro* technologies • Can be combined with *in vitro* technologies to maximise success of antibody recovery	• Fast process (once libraries have been established) • Recombinant, sequence-known • Possible to directly screen human libraries • Possible to screen non-immunogenic antigens • Possible to screen toxic antigens • No animal use (for naïve libraries)
Drawbacks	• 6-8 months generation time (although rapid immunisation protocols can be 3-4 months) • May require humanisation, or use of proprietary transgenic animal strains for therapeutic antibodies • Limitations with non-immunogenic antigens • Limitations with toxic antigens • Requires immunisation of animals	• Small numbers of top-quality binders can be identified, dependent on size of phage library • Some challenging target classes, e.g. small molecules or post-translational modifications • Binders often have lower affinity, specificity or immunogenicity issues, requiring additional affinity maturation processes (still underdeveloped, extra time and cost) • Binders often have low solubility and stability • Lack mammalian post-translational modifications • Low availability in the antibody market • Technically more difficult to generate • Difficult and expensive to implement in academia, start-ups and small-medium sized companies • 6-7 months generation time for one-off development of a library. Currently a lack of libraries for species other than humans, rabbits & camelids (issue for the veterinary research field) • Requires animal usage for immune libraries.

Table 1: Current pros and cons of in vivo and in vitro technology for antibodies production for research.

The immediate transition to only non-animal-derived antibodies would have serious negative implications and impacts on research, innovation and discovery of new life-saving drugs. Currently, no single technology is able to supply suitable antibodies for all applications, and a complementary approach is necessary to avoid impacting both basic and pre-clinical research, and ultimately the development of innovative treatments.

Section 3 – Research Tool Antibodies

Basic research provides the foundation for our understanding of biological processes. Antibodies are a key tool used by the research community, and for that reason it is of utmost importance to utilise a wide range of antibodies to identify the highest-quality molecule (in terms of affinity, solubility, stability, low immunogenicity, epitope targeting) for a particular application. The challenge of creating appropriate antibodies for research directly impacts the progression of the science and the timeliness in which scientific breakthroughs can be made.

The technology to retrieve potentially useful antibodies from synthetic libraries has been significantly advanced over the last three decades, and is still improving. However, for a variety of applications this approach cannot fully compete with the antibodies derived from immunised animals, which are typically used for the recovery of antibodies as either polyclonal or monoclonal reagents. This was highlighted in the EFPIA/EARA survey, where respondents indicated that a transition to full replacement of the animal-derived antibodies is viewed as not possible by over 50 % of the respondents, with only 15 % feeling it is achievable in the near future. Whilst the EFPIA and EARA strongly support the move to evaluate alternative methods of antibody generation, any move to no longer authorise the use and development of animal-derived antibodies will in the medium term greatly impact scientific research leading to new discoveries.

One example worthy of specific attention is the use of antibodies in immunohistochemistry (IHC) which is an essential tool in diagnosis, targeted therapeutic patient selection, and research in cancer and other diseases. Such approaches are vital to ensure not only accurate diagnosis of disease, but also play an increasingly important role in precision medicine, ensuring that the correct drug is selected that is anticipated to provide the most benefit to patients. Synthetic antibodies have not historically worked well for IHC methods, and to date there are only a limited number of non-animal-derived antibodies that adequately work in IHC[1]. This situation is particularly difficult when developing drug target immunohistochemistry assays for novel targets. Additionally, synthetic human antibodies are not suitable for chronic use in animal models critical to the increased understanding of the target pharmacology and safety of antibodies for human use, due to the potential for risks of immune responses. While efforts screening non-animal-derived antibodies continue, it is not a replacement for animal-based antibodies. This process is empirical in that one usually does not have advanced knowledge of the protein to allow antibody design, and the best antibody that performs for IHC will need to be selected through large-scale screens. Difficulties remain in identifying suitable antibody reagents for challenging antigens/proteins in this situation. Limiting approaches to developing antibodies would also greatly slow drug and diagnostic development. In these instances, it is necessary to resort to animal-derived antibodies to allow research to continue.

Another application in which animal-derived antibodies are often required is in the generation of antibodies specific to proteins bearing post-translational modifications (PTM) with small molecules, such as phosphorylated forms. These have proven to be challenging to make without the use of specific hosts, such as rabbits. The application of *in vitro* display in this area is complicated by the cost and time of producing reagents of suitably high affinity and specificity.

The catalogue of research tool antibodies available worldwide is largely made up of animal-derived antibodies, and their elimination would mean a lack of available research tools in Europe and worldwide. While currently a very minor percentage of non-animal-derived antibodies (compared to the total number of antibodies available) exist for scientific use in Europe, EFPIA/EARA do support investment in increasing the supply of such tools, including for example, recombinant production of antibodies currently available from hybridoma. However, a science-based transition period is necessary to move towards reliance on the non-animal-derived antibodies in the future; this currently has an uncertain time frame (>10 years). Thus, it would be counterproductive to have strict restrictions in Europe on the use of animals for immunisation for antibody generation, and this could lead to companies moving offshore and a reduction of the attractiveness of Europe to perform basic scientific research.

The "reproducibility crisis" will not be solved by using only non-animal-derived antibodies

The EURL ECVAM Recommendation and associated texts state that animal-derived antibodies are the cause of the "reproducibility crisis", concluding that they are an unreliable tool for research[17]. The quality of many research tool antibodies in the market are of poor quality, particularly in terms of specificity, but this does not apply to therapeutic antibodies, which are rigorously characterised to be devoid of such polyreactivity. The solution to this issue relies on the use of high-quality reagents, proper validation of those antibodies, and in the training of how to select and use antibodies in research. A lack of proper validation may indeed result in the improper use of a high number of animals needed to confirm antibody specificity and efficiency. All antibodies have to be validated and tested in all applications, regardless of whether they are animal- or non-animal-derived (as already mentioned in the EURL ECVAM Recommendation). These core issues will remain, regardless of the source of antibody generation. Therefore, the notion that the reproducibility crisis is due to the use of animal-derived antibodies and may be fixed by using non-animal-derived antibodies, is a significant oversimplification. A lack of proper validation may indeed result in the improper use of a high number of animals needed to confirm antibody specificity and efficiency.

EFPIA and EARA are in complete agreement with the EURL ECVAM Recommendation that highlights the pitfalls of poorly generated and characterised antibody reagents, that often result in misleading findings and poor quality research and support any efforts that raise the standards for biomedical science to this end. However, the actual antibody generation procedure per se is not at fault, more the use of poor quality reagents and poor scientific practice, which is also highly relevant for *in vitro* methods as well.

Section 4 - Therapeutic antibodies

Overview

Therapeutic antibodies and other antibody-derived biologic drugs are some of the most versatile modalities available to treat disease today. Through their unique selectivity they offer relief and hope to tens of thousands of patients daily, including those suffering from some of the most burdensome and difficult to treat diseases faced by man and animal. Antibodies and antibody reagents are used throughout the drug discovery process, to develop and test hypotheses regarding the efficacy of an approach, and to study the way the body responds to a specific biologic drug (Figure 1).

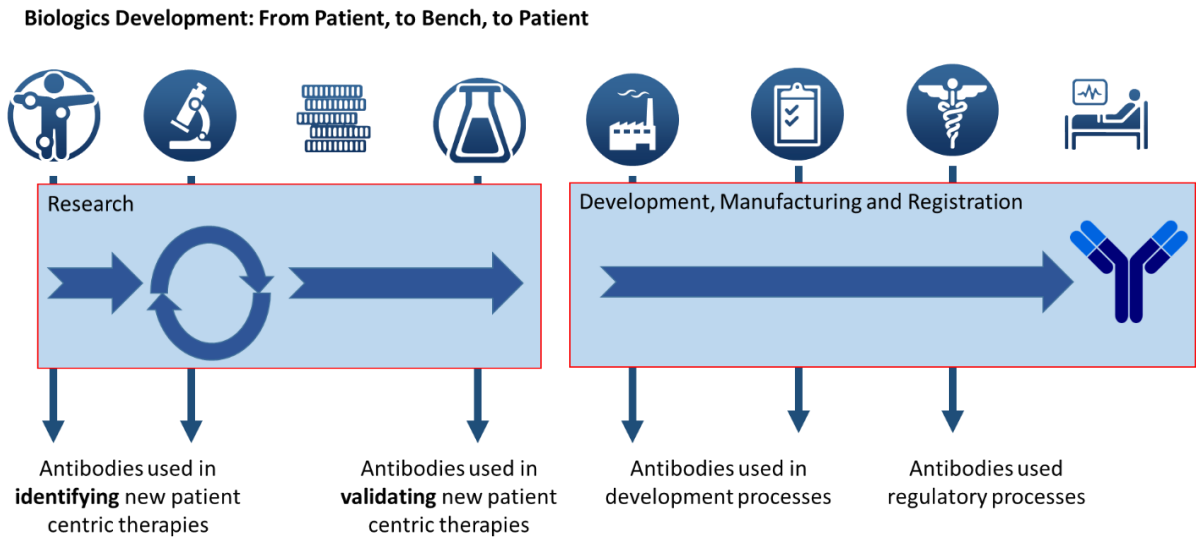


Figure 1: The key role that antibodies play in biologics drug research and development

To deliver the full therapeutic promise of biologics drugs to those patients that need them, a diversity of antibody discovery approaches is required to increase the chance of a successful hit. This is especially true in addressing unmet medical needs, which often require intervention in more complex biological settings, with increasingly difficult targets to modulate in order to achieve efficacy. Consequently, a diverse range of antibody discovery approaches is essential. Each of these methods has unique and occasionally shared advantages and disadvantages; however, when used in combination they permit the prosecution of almost any drug target accessible to the systemic circulation, encompassing a range of diseases from COVID-19 to cancer.

Focusing exclusively on one or another antibody generation process alone will endanger the flow of medicines from research and preclinical development to the clinic, and will rob a generation of EU-based scientists (and the patients they serve) of the tools they require to develop the science that underpins the therapeutic efficacy of these molecules.

The large majority of therapeutic antibodies used in clinics today are animal-derived; only 10 of more than 100 FDA-licenced antibodies used were discovered using phage display technology and in the last five years 90% of approved therapeutic antibodies are derived from animals [2,3,4]. There is also a small, but significant number of lifesaving polyclonal therapies developed from animal serum, for example, Sanofi's Thymoglobulin (polyclonal from rabbit immunisation) or anti-venom (polyclonal from horse immunisation). In these cases, the polyclonal approaches have been developed as life saving drugs and no monoclonal or synthetic alternatives are comparable. Any major process change would stop the marketing authorisation and these drugs would not then be available for patients.

Therapeutic antibody discovery

In order for an antibody to successfully qualify as a therapeutic antibody it has to simultaneously satisfy a composite set of criteria including, but not limited to:

- Specific binding to the target of interest
- Cross-reactivity to relevant animal targets for non-human safety studies
- Optimal functional activity
- Optimal absorption, action and breakdown of the drug
- Low immunogenicity
- No off-target effects to ensure patient safety

- Manufacturing characteristics permitting the market production of the molecule
- Biophysical properties ensuring suitable shelf-life, device compatibility (i.e. infusion bag) and safe in-patient use

Identifying antibodies that fulfil all these criteria is exceptionally challenging and it is rare that any antibody is ready for clinical development without further extensive optimisation, regardless of their origin and of these, even fewer still will reach patients. Organisations developing therapeutic antibodies need to access large libraries of antibodies, with as high a diversity as possible, to find a successful candidate.

Antibody discovery technologies based on animal immunisations take advantage of the ability of the intact immune system to very efficiently generate a dynamic and highly diverse antibody response. The natural immune system adopts multiple diversification features, like somatic hyper-mutation, or gene conversion, during the antigen-specific B cell maturation, favouring a highly diverse repertoire**.** In parallel, a natural selection/deselection process takes place to simultaneously enrich antibodies with enhanced binding affinity, specificity and protein stability. This unique process is not yet recapitulated to its full extent by current synthetic antibody discovery platforms,[5,6] resulting in synthetic display platforms which are less diverse than the total repertoire that can be generated via a complete functional immune system.

Due to the sophisticated selection and maturation process that occurs *in vivo*, an intact immune system generates high-quality antibodies that by their very nature of having arisen from living cells are preselected for certain characteristics attractive for drug discovery. Perhaps most importantly, antibodies from immunised animals are in general higher in affinity (meaning that they are more efficient at modulating a given target) than those derived from *in vitro* methods. Often *in vitro* derived antibodies require subsequent molecule engineering that can be exceptionally challenging and increases not only the time required to bring the molecule to the clinic, but also the risk associated with a given therapeutic target, and potentially increases the need for greater animal use during the drug development pathway.

Advances in modern, high throughput, molecular biological techniques mean that a therapeutic antibody library is expressed via recombinant means and thoroughly characterised, regardless of whether it is derived from animal or non-animal technologies. This occurs early on in the discovery process, ensuring a high-quality and robust molecule set for drug discovery. Additionally, by immunising animals, large repertoires of antibodies are generated against the antigen of interest and by using Next Generation Sequencing (NGS) and single B Cell sequencing approaches, researchers are able to focus on a deeper mining of the immune-response, and also recovering vast numbers of diverse antibodies as starting points for therapeutics. Thus, more antibodies can be generated per animal, reducing animal usage from what has been traditionally needed from hybridoma approaches, as well as providing a library of molecules with higher molecular diversity (determined from the DNA sequence). By characterising the molecular diversity, large numbers of antibodies are generated from each animal with a higher success of finding antibodies with the required features.

The discovery of new targets is essential to ensure researchers can address unmet and emerging medical needs. Synthetic libraries often rely on having prior knowledge of the target, including its sequence and structure, in order to generate sufficient protein for new antibody production. For complex cellular targets this is not always possible, as the target is difficult to obtain or loses some of its essential characteristics when isolated from a cell, and therefore is not yet proved reliable enough in real-world drug discovery settings to reliably enable therapeutic antibody generation. This also excludes the possibility to discover new targets. In such cases, an immunisation-based approach is necessary, as the target can be provided in the format it would appear in the body.

This feature is especially important for hard-to-isolate protein targets, such as G-protein coupled receptors (GPCRs). These are large proteins which play a crucial role in how the cell interacts with its environment and is implicated in a wide range of diseases. As an example, nanobodies against these GPCRs were isolated from llamas which had been immunised with the protein, allowing researchers to work out the structure of these proteins and how they function, while also providing the potential for a huge range of therapeutic antibodies to be developed. So far, antibodies against these targets, from animal-derived sources, have consistently been shown to be better at acting upon the target than non-animal-derived antibodies[7,8,9].

As described above, the complex relationship between an antibody sequence, its ability to modulate a target and its intrinsic pharmaceutical properties are poorly understood. This creates challenges with the current inability to predict toxicity, or even efficacy of the antibody. Thus, regardless of the source of a therapeutic antibody, during its progress from research idea through to a medicine fit to treat patients, testing of this molecule in either preclinical efficacy or *in vivo* toxicology models cannot be avoided. Selecting the best possible antibody, based on a multiparameter analysis during the research phase, reduces the need for repeated preclinical testing and reduces the risk of program failure in toxicological testing. Thus, early investment in diverse, high quality antibodies during the drug discovery phase may ultimately help reduce the total number of animals spent overall, especially when it is considered the number of animal studies performed to satisfy efficacy and regulatory requirements will almost exclusively far exceed the number of animals used for *in vivo* antibody discovery.

Given their versatility and widespread use in scientific research, it is very important that high quality antibodies, and antibody fragments, remain available to the research community at low cost and with short delivery times. Currently, the very best synthetic *in vitro* display platforms are in the hands of a small number of companies and institutions. A full switch to library-derived antibodies would deprive the scientific community of a very important antibody source. This type of switch can only be justified if the alternative is able to provide high quality antibodies that are fit-for-purpose. This starts with access to libraries that contain the largest possible antibody diversity. If the diversity is not high enough, the number of hits will be very limited, and the quality of the hits will be relatively low.

Regulatory Requirements: Vaccines, Biologics and Animal Health

Animal-derived antibodies may be used for potency assays used for drug analysis and release, and as capture reagents, detection reagents and positive controls used in pharmacokinetic (PK), pharmacodynamic (PD) and anti-drug antibody assays (ADA) methods. These methods are validated, regulated and used over the long-term (years) to support drug development. Replacing validated reagents with in vitro-derived monoclonal antibodies (mAbs) can result in delays and challenges when comparing results across studies. For example, animal-derived polyclonal antibodies are necessary in order to generate drug-specific assay positive controls, which are critical for all ADA. ADA assays are used in non-clinical studies to interpret toxicokinetic data and to interpret immune-related reactions. In clinical studies, immunogenicity analysis is an integral part of the safety assessment of every biotherapeutic mAb. *In vitro* antibody specific approaches for this do not represent the full immune response to therapeutic drugs, and broad comprehensive testing of *in vitro* antibodies for this purpose is needed in order to capture the potential risks of generating ADAs in patients - an important safety consideration.

Additionally, there is the need for reagents in the assessment of immune responses in efficacy studies in humans and across a wide range of species. In veterinary medicine, the challenge is unique in that suitable reagents are often not available for multiple animal species. Therefore, it is necessary to generate antibody reagents internally, and these are mainly derived from animal sources via hybridoma technology or polyclonals from sera. Where possible, non-animal-derived antibodies are

used and the need for animals is kept to a minimum. However, limiting options for this will cause concern for reagent production as, at present, the spectrum of antibodies obtained from *in vitro* methods is narrower than when animals are used.

To replace *in vivo* methods with valid *in vitro* potency assays for vaccines, antibodies need to be biologically relevant. Biologically relevant assays for complex vaccines are best developed with reagents from immunised animals. Without animal use, it will be difficult to demonstrate biological relevance for antibodies developed via purely *in vitro* methodologies.

Collectively, these restrictions, if applied, could realistically delay, or even preclude, the discovery, development, authorisation and availability of new medicinal products (mAbs, other therapeutic proteins, vaccines and diagnostic products) in the EU and jeopardise the batch release of currently authorised veterinary medicinal products, with associated concerns in the field.

Novel therapeutic antibody formats

The natural world offers unconventional formats and/or unique properties of antibodies, and new potential thanks to characteristics that are not possible to obtain through classic antibody *in vitro* platforms. This will ultimately broaden the scientists' toolbox, increasing the probability of success when identifying new therapeutics and addressing unmet patient needs. Denying or strongly limiting the scientific community access to *in vivo* antibody discovery would therefore have immediate detrimental consequences for existing programs relying on well-established technologies and formats. It would also jeopardise the discovery of alternative antibody formats that could in the future be important for treating serious diseases.

Examples of such antibodies include:

- Nanobodies derived from camelids, where their small size and adaptability have paved the way for a new class of therapeutics that have entered clinical development. Llama-derived nanobodies have been of particular interest given their potential in the treatment of COVID-19.
- Antibodies derived from chickens and rabbits have diverse potential thanks to unique features in the adaptive immune response to a given target in these animals. These unique features are not found in classic *in vivo* approaches, and are not captured or simulated in library-based platforms. Chicken-derived antibodies, like camelid-derived nanobodies, are currently in clinical development.
- In veterinary medicine, monoclonal antibodies derived from veterinary species are being used to treat a wide range of important diseases and conditions, and it would be very difficult to derive these therapeutics from synthetic approaches at this time.

COVID-19

At a time when the world is depending on biomedical researchers to find a vaccine to combat the COVID-19 pandemic, the benefits of animal-derived antibodies have been of critical importance in the struggle to better understand and rapidly advance therapeutics to combat COVID-19. Many SARS-CoV-2 neutralising therapeutic antibody programs have entered clinical development at an unprecedented speed. Currently, there are 14 active clinical programs trialling antibodies, all of which use cells isolated from patients or immunised animals. Here are four examples that have come from research involving animal-derived antibodies:

- Regeneron isolated an antibody cocktail consisting of two neutralising antibodies from immunised transgenic mice, or infected human donors. The cocktail binds non-overlapping epitopes on RBD and prevents viral escape. The cocktail has advanced into phase 3 clinical trials at an unprecedented speed[10,11].
- VIB, in Belgium, and the German Primate Research Centre, were able to generate specific antibodies[12] against the virus using camelids, and demonstrated that they were able to neutralise the coronavirus.
- The Karolinska Institute[13], Sweden, found that alpacas can generate a smaller-sized form of antibody against the virus, and that these are remarkably effective at preventing the virus from entering cells and thus causing infection.
- In a similar manner, a collaboration between the University of Texas at Austin, USA and Ghent University, Belgium, found that llama antibodies[14] could prevent the coronavirus entering cells, and have begun preclinical trials in order to develop this as a potential treatment for COVID-19.

No programs using synthetic display libraries have progressed to clinical testing yet, emphasising that display technologies, whilst useful, cannot replace *in vivo* maturation in a pandemic situation[15].

Monoclonal antibodies are likely to be the first game-changing therapy against COVID-19, and are expected to be the first approved therapies specifically designed to counter coronavirus infections. As most large pharmaceutical companies have ample experience in manufacturing these kinds of medicines, their existing facilities can readily be converted to produce doses of a future COVID-19 treatment. The hope is that these therapies can serve as a bridge until widespread vaccinations are possible, as well as help people who cannot generate an effective immune response. Animal-derived antibodies have already shown some promising early results,[17] providing some immunity to the disease, or at least shortening the length of the illness — a valuable benefit to frontline health workers.

Section 5 – *In vitro* diagnostics (IVD)

Most clinical decisions made are supported or guided by the use of *in vitro* diagnostics (IVD). Therefore, the need for highly specific and precise IVD assays is obvious. One key element to these assays are antibodies with excellent specificities and affinities, used for the detection of target molecules with low abundance. The complexity of diagnostic markers is further increased, as often it is necessary to recognise one specific modification to a protein. To date, most of the high-quality antibodies used in IVD assays are developed by immunisation of animals, like mice, rabbits or sheep. It is currently unclear if it is possible to target all of the diverse and difficult targets with non-animal-derived antibodies, with the same end quality.

Due to the various diagnostic targets and the challenging requirements of antibody quality, the aim of each antibody development must be generation of the maximum diversity of antibodies, of the highest quality, to serve patient needs in the best possible way.

To achieve this, all available *in vivo* and *in vitro* methods of antibody generation are necessary to identify binders with outstanding quality for the development of highly specific and sensitive diagnostic assays. For these reasons, EARA/EFPIA are convinced that the existence of multiple methods for antibody generation - *in vivo* or *in vitro* methods - complement, but do not replace each other.

Section 6 - Animal welfare

The current consensus is that research with animals is justified where there are clear benefits for human and animal health. This decision is made after a harm/benefit analysis and the application of the 3Rs. While EARA/EFPIA support the continuous development of non-animal-derived technologies, we believe that new technologies still cannot fully replace animals.

The number of animals used in immunisation campaigns is limited to the number that is strictly necessary. Often, a mix of different antigens is used to immunise an animal, especially when immunising larger animals, to further decrease the number of animals used. This is also reduced by re-use of the animals, where this is possible. The current immunisation protocols, as approved by the animal ethics committees, make use of animal-friendly optimised adjuvants that do not cause discomfort, while still stimulating the desired immune response. These protocols do not cause discomfort beyond what is caused similarly by vaccination, or blood sampling of humans.

Additionally, technological advantages such as Next Generation Sequencing (NGS) and single B Cell sequencings, have allowed researchers to focus on deep mining of the animal immune-responses following initial characterisation of an antibody, and recovery of vast numbers of diverse antibodies as starting points for therapeutics. Thus, thousands of unique antibodies can be identified per animal, reducing animal usage from what was previously needed from hybridoma approaches. As few as 6-12 animals are sufficient to generate an antibody repertoire of thousands of unique antibodies against a particular target. Current practice includes advanced antibody sequencing methods at an early stage during the discovery process, which enables full extraction of the generated antibody response as well as recombinantly-produced antibodies during the functional assessment.

Section 7 – Socio-economic impact

Premature restrictions on the use of animal-derived antibodies for therapeutic purposes will have widespread socio-economic consequences, and there is a high likelihood that in the future patients worldwide will not have access to the best antibody-based medicines that would have otherwise originated from Europe (historically recognised as a powerhouse of innovative medicines)[31]. As this report outlines, nobody disputes the usefulness of synthetic libraries and alternative scaffolds, however, the EURL ECVAM Recommendation proposes that synthetic antibodies can immediately replace animal-derived antibodies for “all known applications” which, as have been outlined, is not currently the case.

What would a world without animal-derived antibodies look like?

It is a statement of fact that animal-derived antibodies have emerged among the major class of therapeutic agents for the treatment of many human diseases, especially cancers, immunological, infectious, neural and metabolic diseases, ultimately providing a treatment for millions of patients[2].

What significant health benefits and scientific developments, from animal-derived antibodies have occurred since 1979? Here are four examples:

1. Herceptin, a monoclonal antibody used in the treatment of breast and colon cancers, was developed thanks to studies in mice, rats and hamsters. Around 3 million women have been

treated with Herceptin, and women with HER2-positive breast cancer now have among the highest survival rates, compared with all women with breast cancer [18].

2. Avastin, a recombinant monoclonal antibody used to prevent new blood vessel growth, a key factor in tumour development, is used to treat a wide range of cancers[19].
3. RoActemra, used in severe rheumatoid arthritis, is a monoclonal antibody developed in mice and has been shown to slow progression of the disease across different age ranges [20].
4. Ocrevus, is a therapy developed using mouse antibodies, taken by patients with multiple sclerosis, and has been shown to reduce the number of relapses the patient will experience by up to 70% [21].

To ignore the benefits of animal-derived antibodies, without readily proven available alternatives, would show a disregard for current and future public health needs, and could lead to a situation in which European citizens, and indeed the world, would no longer have access to the best medicines that science has to offer.

Restrictions on the use of animal-derived antibodies would threaten Europe's position as a global centre of innovation and life sciences research and development.

The life sciences sector operates globally, from basic research to product development and manufacturing. The European life sciences sector has become increasingly integrated into the global economy and the long-term success of Europe as a global centre of bioscience research brilliance in academia, and clinical research, is based on its ability to attract investment and to hold on to scientific talent.

It is highly probable that private research, seeing the consequences of the immediate restriction on the use of animal-derived antibodies, would seek other geographical locations for their investment, research and development.

The impact of this flight of research, investment and human capital would have a devastating impact on the ambitions to keep Europe at the centre of life science innovation.

Section 8 – Final conclusions

Any movement in the sector, from animal- to non-animal-derived antibodies, should be preceded by real evidence demonstrating that the technology is sufficiently mature to replace current practices. As has been highlighted, an immediate switch to non-animal-derived antibodies is not presently practical, for reasons of fit-for-purpose application in a regulated context, and due to the present technology and infrastructure being not set up for upscaling.

The biomedical sector needs to ensure the technologies to generate non-animal-derived antibodies develop and mature to a point where the scientific community can reach a consensus and agree that they are indeed capable of providing antibodies of the same quality and features as animal-derived antibodies, and at comparable costs. This point has not been reached yet.

EARA/EFPIA look forward to a continued constructive dialogue with the JRC and with competent authorities, to avoid any unintended consequences, while supporting scientific progress.

Section 9 - Detailed scientific case studies

1) Radioimmunotherapy antibody construct generation - Case study

In pretargeted radioimmunotherapy (PRIT), therapeutic antibody constructs which bind to a tumour-associated antigen on the one hand and to a radiolabelled compound on the other hand are used[23]. For generation of a binder directed against a metal chelate comprising DOTAM and a radioisotope of lead (PbII), both immunisation and phage display approaches were carried out. The antibody should be selective for metal chelates comprising DOTAM and ^{212}Pb or ^{212}Bi (^{212}bismuth, daughter radionuclide of ^{212}PB) as compared to other chelated metals, such as Cu- or Zn-DOTAM. For functionality of the final bispecific antibody construct, an extremely high affinity, in the pM to fM range, was required. *In vitro* antibody generation was carried out by phage display using standard protocols[24] and a fully synthetic library. For *in vivo* antibody generation, New Zealand White rabbits were immunised and antigen-specific B cells extracted from blood samples were used for PCR amplification of variable domains and subsequent recombinant expression of antigen-specific mAb[25].

From both antibody generation approaches, binders selective for ^{212}Pb- and ^{212}Bi-DOTAM with only low binding to other chelated metals (Zn, Cu) could be obtained. The most important requirement - very high target affinity - could only be accomplished by the *in vivo* generated antibodies. The rabbit-derived antibodies exhibited affinities up to 900 fM, whereas the phage display clones had affinities of only 100 pM. As the *in vitro*-derived binders were not competitive even after affinity maturation, the rabbit-derived monoclonal antibody PRIT-0128[26] was chosen as final lead and used for the development of the bispecific clinical candidate. This case report emphasises that immunisation of rabbits is still the best choice for generation of high-affinity antibodies against haptens. If only phage display had been available as antibody generation technology, the project would have been stopped at a very early preclinical stage.

2) Comparison of antibody generation techniques - Case study

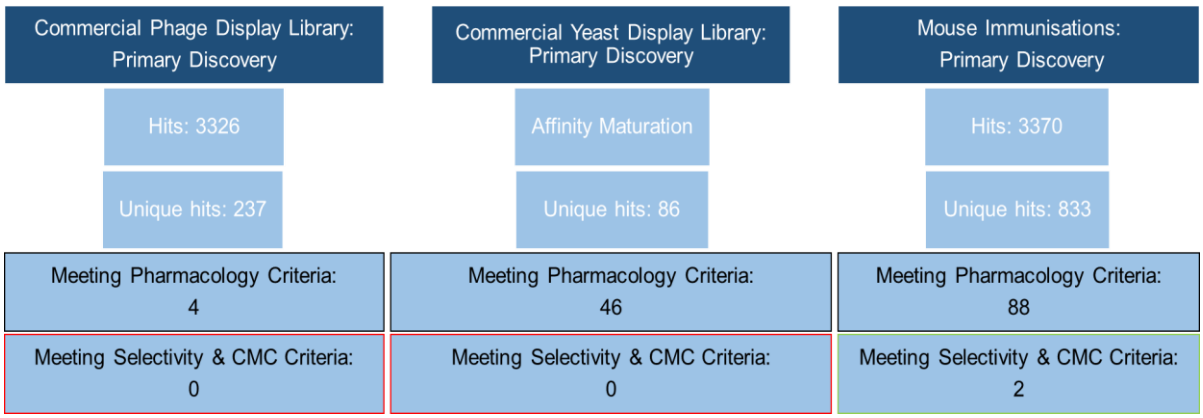
	Rabbit Polyclonal	Mouse monoclonal	scfv	VHH (immunised llama)	VHH (synthetic)
Number of animals	2	4	NA	2	NA
Library	NA	NA	Human (10^{10-12})	Llama (10^{7-8})	Camelid (10^{6-8})
Obtained clones	NA	62	102	43	10
ELISA	+++	+++	+++	++	+
WB	+++	+++	---	---	---
FACS	+++	+++	---	---	---
IHC	+++	+++	---	---	---
Duration (months)	3	10	16	12	6
Trials	1	1	3	3	2
Cost	Low (1-2 k€)	Moderate (6-8 k€)	High (20-25 k€)	Very high (35-40 k€)	High (20-30 k€)
Patent & library right fees	No need	No need	Up-front payment + royalties (If success)	No need	Up-front payment + royalties (If success)

3) COVID-19 neutralising antibodies - Case study

Comparison of nanobodies directed from the spike protein of SARS-CoV-2 can show the different potentials of immunisation and display technologies. Huo *et al* [27] immunised an alpaca with the RBD region (a fragment of the spike protein involved in the interaction with the human receptor ACE2). They obtained nanobodies that recognise RBD with a K_D of 5-10 nM. Wrapp *et al* [28] immunised alpacas with MERS-CoV S and SARS-CoV-1 S, and obtained nanobodies with a K_D of about 100 pM. One of these (VHH 72) cross reacts with SARS-Cov-2 S with a K_D of 1.15nM. Recently Xiang *et al* [29] also obtained nanobodies with an affinity in the picoM range from immunised llamas. Alongside this, Hanke *et al* [30] isolated nanobodies from a synthetic library comprising about $1x10^{10}$ independent clones. VHH H11 had a K_D of <1 µM and they needed to mutate this nanobody to obtain 2 mutants with a K_D of respectively 12 and 44 nM. These results show that an affinity maturation process is needed to obtain high affinity mutants, and that the affinity is lower, by at least one order of magnitude, to the one measured from nanobodies isolated from immunised animals.

4) Monoclonal antibodies - Case Study

An example of an antibody generation program conducted against a cell surface target is shown below in the figure. Here, several methods were compared in a direct comparison with similar reagents in a library-based approach, in an attempt to target a cryptic epitope on a specific protein. High numbers of molecules were generated and screened and ultimately for this target, only the animal immunisation approach delivered molecules that had the desired pharmacology and attractive CMC properties, thus supporting the need for the use of multiple approaches for the successful prosecution of this important drug target.



5) Influenza vaccines - Case Study

Due to diversity of influenza virus subtypes and strains, within a given subtype driven by constant viral evolution, generation of antibody reagents for analytical assay development to support flu vaccines has presented some unique challenges. A key need is the creation of reagents that can differentiate antigenically related flu strains. Traditionally, strain-specific mouse mAb are used in immunological assays to achieve the differentiation. Companies have examined alternative technologies to differentiate flu viruses. For example, a nucleotide aptamer-based approach has been examined for critical reagent generation for certain flu strains. One of the main hurdles for the aptamer approach lies in the extensive candidate screening. To identify potential hits, randomly designed aptamer libraries in the order of thousands are needed to screen for a candidate that specifically binds the antigen with high affinity and has thus far resulted in a low success rate. This coupled with lack of

immune relevance to flu viral antigens make an aptamer approach less attractive. For another example, physically separating closely related key flu antigen regions based on mass difference using mass spectrometry (MS) has been tried with success. However, the MS approach still requires a flu virus antibody to establish the antigen integrity. At present, strain differentiative assays for flu vaccines remain dependent on antibodies, specifically mAbs that are generated through mouse immunisation. To explore alternatives to mouse mAbs and to reduce animal usage, ongoing efforts include deriving mAbs directly from target animal species after flu vaccination. This approach not only has potentials to produce highly immune-relevant (e.g. neutralising and high binding affinity) mAbs from vaccinated target hosts, but also avoid using mice for analytical antibody reagent generation.

Section 10 - Definitions

Antigen is the protein or other substance of interest that is recognised by the antibody.

Hybridomas are cells produced by the fusion between the B cells of an immunised animal and a tumour-like cell. By adding the tumour-cell, the B cell is now able to continue dividing infinitely and reproduce the antibody. This technique is used to generate stable clones of an antibody which can be used again and again after animal immunisation.

In vitro is used to describe experiments that take place in a test tube, in cells or in isolated tissues, outside of a living organism.

In vivo is used to describe experiments that take place within an animal.

Monoclonal antibodies are antibodies derived from a single B cell, often obtained through hybridoma technology. Each monoclonal antibody is encoded by a single DNA sequence and specifically recognises one epitope - the part of an antigen that is recognised by the antibody.

Phage-display is a technology for the development and production of non-animal-derived antibodies. It involves inserting the genetic sequences of a repertoire of antibodies, originally sourced from a human or animal, or designed *in silico*, into the DNA of a virus that solely infects bacteria, known as a bacteriophage. Antibodies are thereby produced on the surface of the bacteriophage, and those bacteriophages bearing antibodies that interact with a specific target molecule can be isolated from a large collection of bacteriophages. Thus, the DNA encoding antibodies binding to the target of interest can be isolated.

Polyclonal antibodies are produced by different clones of B cells, and they interact with the different epitopes of the same antigen. The mixture of antibodies found in the serum of an immunised animal is a polyclonal antibody.

Synthetic libraries are a collection of pre-constructed antibodies that can be screened using *in vitro* display technologies such as phage display.

Recombinant antibodies are produced by cloning antibody genes. This technology involves recovering the antibody genes from the source cells, amplifying and cloning the genes into a vector, introducing the vector into a host, and achieving expression of adequate amounts of functional antibody.

Section 11 - References

1 Abéngozar MA, de Frutos S, Ferreiro S, et al. Blocking ephrinB2 with highly specific antibodies inhibits angiogenesis, lymphangiogenesis, and tumor growth. Blood. 2012;119(19):4565-4576. doi:10.1182/blood-2011-09-380006

2 Lu, R., Hwang, Y., Liu, I. et al. Development of therapeutic antibodies for the treatment of diseases. J Biomed Sci 27, 1 (2020). https://doi.org/10.1186/s12929-019-0592-z

3 Kaplon H, Muralidharan M, Schneider Z, Reichert JM. Antibodies to watch in 2020. MAbs. 2020;12(1):1703531. doi:10.1080/19420862.2019.1703531

4 Sioud M. Phage Display Libraries: From Binders to Targeted Drug Delivery and Human Therapeutics. Mol Biotechnol. 2019;61(4):286-303. doi:10.1007/s12033-019-00156-8

5 Jain T, Sun T, Durand S, et al. Biophysical properties of the clinical-stage antibody landscape. Proc Natl Acad Sci U S A. 2017;114(5):944-949. doi:10.1073/pnas.1616408114

6 Kaleli NE, Karadag M, Kalyoncu S. Phage display derived therapeutic antibodies have enriched aliphatic content: Insights for developability issues. Proteins. 2019;87(7):607-618. doi:10.1002/prot.25685

7 Rossant CJ, Carroll D, Huang L, et al. Phage display and hybridoma generation of antibodies to human CXCR2 yields antibodies with distinct mechanisms and epitopes. MAbs. 2014;6(6):1425-1438. doi:10.4161/mabs.34376

8 Ayoub MA, Crépieux P, Koglin M, et al. Antibodies targeting G protein-coupled receptors: Recent advances and therapeutic challenges. MAbs. 2017;9(5):735-741. doi:10.1080/19420862.2017.1325052

9 Hutchings CJ. A review of antibody-based therapeutics targeting G protein-coupled receptors: an update. Expert Opin Biol Ther. 2020;20(8):925-935. doi:10.1080/14712598.2020.1745770

10 Hansen J, Baum A, Pascal KE, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science*. 2020;369(6506):1010-1014. doi:10.1126/science.abd0827

11 Baum A, Fulton BO, Wloga E, et al. Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science*. 2020;369(6506):1014-1018. doi:10.1126/science.abd0831

12 VIB.VIB ACHIEVES IMPORTANT MILESTONE IN THE DEVELOPMENT OF A COVID-19 DRUG. March 2020. Accessed 26 October 2020. https://vib.be/news/vib-achieves-important-milestone-development-covid-19-drug

13 VOA news. Alpaca Helps to Make COVID-19 Vaccine June 2020. Accessed 26 October 2020. https://learningenglish.voanews.com/a/alpaca-helps-to-make-covid-19-vaccine/5459364.html

14 EARA. Llama antibodies could help combat Covid-19. May 2020. Accessed 26 October 2020. https://www.eara.eu/post/llama-antibodies-could-help-combat-covid-19

15 Antibody Society. The COVID-19 Biologics Tracker. Accessed 26 October 2020 https://www.antibodysociety.org/covid-19-biologics-tracker/

16 Regeneron. REGENERON'S REGN-COV2 ANTIBODY COCKTAIL REDUCED VIRAL LEVELS AND IMPROVED SYMPTOMS IN NON-HOSPITALIZED COVID-19 PATIENTS. September 2020. Accessed 26 October 2020. https://investor.regeneron.com/news-releases/news-release-details/regenerons-regn-cov2-antibody-cocktail-reduced-viral-levels-and?utm_source=POLITICO.EU&utm_campaign=3971733469-EMAIL_CAMPAIGN_2020_10_23_02_05&utm_medium=email&utm_term=0_10959edeb5-3971733469-189512705

17 Gray T, Bradbury A, Dubel S et al. Reproducibility:bypass animals for antibody production. Nature correspondence 2020. doi.org/10.1038/d41586-020-01474-7

18 UCLA Health. Herceptin has saved countless women's lives and earned UCLA's Dr. Dennis Slamon the Lasker Award. Accessed 26 October 2020. https://www.uclahealth.org/physiciansupdate/herceptin-has-saved-countless-women-s-lives-and-earned-ucla-s-dr-dennis-slamon-the-lasker-award

19 Bevacizumab (Avastin). June 2020. Accessed 06 November https://www.drugs.com/monograph/bevacizumab.html#

20 Jones G, Panova E. New insights and long-term safety of tocilizumab in rheumatoid arthritis. Ther Adv Musculoskelet Dis. 2018;10(10):195-199. Published 2018 Oct 7. doi:10.1177/1759720X18798462

21 Multiple Sclerosis News today. 4 ways Ocrevus can improve your life. August 2017. Accessed 26 October 2020. https://multiplesclerosisnewstoday.com/2017/08/14/4-ways-ocrevus-improve-life/#:~:text=In%20addition%20to%20slowing%20the,(a.k.a.%20less%20brain%20fog).

22 EFPIA. Manifesto - Building a healthier future for Europe. Accessed 26 October 2020. https://www.efpia.eu/manifesto/

23 Goldenberg DM, Chang CH, Rossi EA, McBride WJ, Sharkey RM. Pre-targeted Molecular Imaging and Radioimmunotherapy. Theranostics 2012; 2(5):523-540. doi:10.7150/thno.3582.

24 Silacci M, Brack S, Schirru G, Mårlind J, Ettorre A, Merlo A, Viti F, Neri D. Design, construction, and characterization of a large synthetic human antibody phage display library. Proteomics. 2005 Jun;5(9):2340-50. doi: 10.1002/pmic.200401273.

25 Seeber S, Ros F, Thorey I, Tiefenthaler G, Kaluza K, Lifke V, Fischer JA, Klostermann S, Endl J, Kopetzki E, Pashine A, Siewe B, Kaluza B, Platzer J, Offner S. A robust high throughput platform to generate functional recombinant monoclonal antibodies using rabbit B cells from peripheral blood. PLoS One. 2014 Feb 4;9(2):e86184. doi: 10.1371/journal.pone.0086184. PMID: 24503933; PMCID: PMC3913575.

26 Patientscope WIPO IP Portal. WO2019/201959 - Antibodies for chelated radionuclides. October 2019. Accessed 26 October 2020.

27 Huo J, Le Bas A, Ruza RR, et al. Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2 [published correction appears in Nat Struct Mol Biol. 2020 Oct 15;:]. *Nat Struct Mol Biol*. 2020;27(9):846-854. doi:10.1038/s41594-020-0469-6

28 Wrapp D, De Vlieger D, Corbett KS, et al. Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies. *Cell*. 2020;181(6):1436-1441. doi:10.1016/j.cell.2020.05.047

29 Yufei X, Sham N, Zhengyun X, et al. bioRxiv 2020.08.24.264333; doi: https://doi.org/10.1101/2020.08.24.264333

30 Hanke, L., Vidakovics Perez, L., Sheward, D.J. et al. An alpaca nanobody neutralizes SARS-CoV-2 by blocking receptor interaction. Nat Commun 11, 4420 (2020). https://doi.org/10.1038/s41467-020-18174-5

31 VIB. OPINION: DO NOT BAN ANIMAL IMMUNIZATION FOR ANTIBODY PRODUCTION. November 2020. Accessed 13 November 2020 https://vib.be/news/opinion-do-not-ban-animal-immunization-antibody-production

European Animal Research Association - www.eara.eu

European Federation of Pharmaceutical Industries and Associations - www.efpia.eu

AnimalhealthEurope - www.animalhealtheurope.eu

European Animal Research Association

efpia European Federation of Pharmaceutical Industries and Associations

animalhealth europe