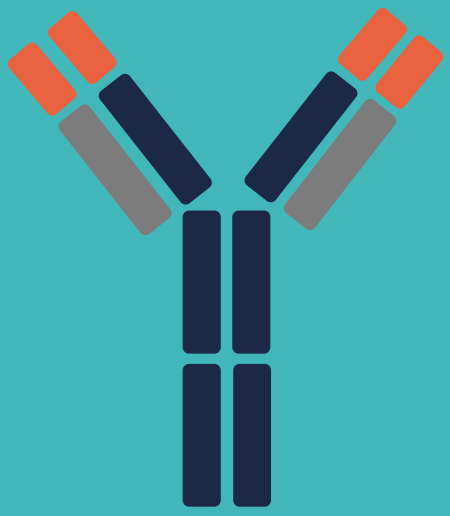
Immunisation of animals for the generation and production of antibodies

Opinion document in response to the EURL ECVAM recommendation on non-animal-derived antibodies



VIB

This opinion document has been compiled by VIB with input from experts from within VIB and experts from a number of Belgian biotech and pharmaceutical companies including Sanofi, involved in the generation and use of antibodies. For more information about VIB, see: www.vib.be.

1. Introduction

On 15 May 2020 the European reference laboratory for alternatives to animal testing (EURL ECVAM) published a recommendation on non-animal-derived antibodies[1]. It states *"that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications".* This recommendation is based on an ECVAM Scientific Advisory Committee (ESAC) opinion on the scientific validity of replacements for animal-derived antibodies. EURL ECVAM is of the opinion that synthetic recombinant libraries are a valid non-animal alternative, and considers that EU member states should no longer authorize animal immunisation "*where robust scientific justification is lacking"*.

Antibodies are generated and used for a wide range of applications in scientific research, in clinical diagnostics and as therapies to treat different diseases in humans and animals. Antibodies or antibody fragments also have wider applications beyond the biomedical field. They are for instance also being developed as biopesticides[2]. For the generation of these antibodies both animal and non-animal approaches exist.

Originally, the animal approach exists in injecting an animal with an antigen target, stimulating the immune system of the animal to produce high affinity and highly specific antibodies against this target. These antibodies are produced by B-cells of the immune system. Each B-cell clone produces a specific antibody. One can use 'polyclonal' antibodies, which is a mixture of antibodies, each produced by a different B-cell clone, and each possibly binding to a different site (epitope) on the antigen target or recognizing a different conformation of the target. One can also use 'monoclonal' antibodies: one specific antibody produced by one single B-cell clone, binding to one specific epitope. Selected monoclonal antibodies can be produced in larger quantities using hybridoma's[3] or by producing the antibody in a recombinant manner. These methodologies enable limitless production of the antibody and complete flexibility to engineer the antibody for any potential use. For selection of superior antibodies often the antibody gene repertoire, generated by immunisation of the animal is cloned into a laboratory micro-organism strain. In a next step phage display[4,5] is used to select the superior antibodies from the bulk. The gene coding for these antibodies can then be used to produce the antibody in large quanties in a recombinant manner.

Since a few decades mice can also be used to generate fully human antibodies. This is done by immunizing so-called Xeno mice in which the murine antibody genes have been functionally replaced by human antibody genes. Phage display is then also used to select the superior binders.

Non-animal derived antibodies are selected *in vitro* from large gene libraries stemming from either non-immunised (naïve) donor B-cells, or from synthetic universal antibody libraries. To generate a naïve library, the blood of a non-immunised animal needs to be used. But once the library is formed,

[1] EURL ECVAM Recommendation on Non-Animal-Derived Antibodies, JRC Science For Policy Report, 2020.
[2] See: www.biotalys.com
[3] Hybridomas are hybrid cell lines resulting from the fusion of the antibody-producing B-cell with immortal B-cell cancer cells
[4] Phage display is a technique where a library of antibody is expressed attached to the coat protein of phage and these phages are panned onto a surface on which the antigen is immobilized. High-affinity antibodies are enriched through the application of washing steps that remove the non-binders and low-affinity binders.
[5] Phage display technology is sometimes incorrectly suggested to constitute non-animal-derived library technology. This is not correct. A phage display library can result from the antibody repertoire produced from an immunized animal, or from the blood of a naïve animal, or from a synthetic antibody fragment library.

it can be used over and over again without further use of animals. Synthetic libraries are built by cloning gene fragments of antibody genes into a laboratory micro-organism strain. By using different combinations of antibody gene fragments, different genes are generated, each producing a different antibody or antibody fragment. Different approaches can be used to introduce variation in the antigen-binding variable regions of these fragments, including random synthesis using double-stranded DNA-triplets. Depending on the approach followed, the diversity of antibody fragments in these libraries can range from 10^9 to 10^{11}. For the selection of high-affinity binders from these libraries the same phage-display and panning methods are used as for gene libraries generated by hyper-immunised animals.

Selected antibodies can be further engineered to improve their affinity and other properties. In practice not only whole antibodies are used, also smaller parts such as Fabs, single chain variable fragments, nanobodies, and more.

2. Introductory comments to the ECVAM recommendation

The EURL ECVAM recommendation and underlying ESAC opinion are imbalanced and incomplete. They seem to be heavily built upon arguments related to certain disadvantages of animal-derived polyclonals and hybridomas, while ignoring their advantages.

- For polyclonals issues of low quality are raised, batch-to-batch variation, low specificity and high detection background. Hybridomas are criticized because they regularly still cross-react with other molecules or produce additonal heavy or light chains that interfere with specificity or sensitivity. It is stated that hybridoma cells can also die or lose expression of the heavy or light chain, creating the need to start over and re-validate. In important areas of scientific research hybridomas are however no longer widely used and have mostly been replaced by recombinant DNA technology, based on determination of the sequence of the selected antibody genes.

- The opinion also strongly focuses on certain advantages of the non-animal approaches. For instance the notion that universal libraries can be used again and again for each new target of interest. The opinion gives the impression to be inspired by discussions on low reproducibility of research findings caused by insufficient quality of animal-derived antibodies used in scientific research. Occasionally, batches of animal-derived antibodies are of insufficient quality. However, this is not an inherent result of their animal origin, but it is rather due to insufficient screening and lack of proper characterisation of the antibody properties, or use under inappropriate conditions for which they were not validated. Whether derived from animals or from man-made libaries, in both cases the screening and characterization is crucial and will determine the quality of the antibodies and their applicability for a specific purpose. Therefore, switching from animal-derived to non-animal-derived antibodies as such is not a guarantee that issues of quality will disappear. As compared to animal-derived antibodies, the use of non-animal-derived antibodies has so far been way too limited to allow any statistical conclusions that non-animal-derived antibodies do not suffer from the same problems as described for animal-derived antibodies. In other words, the challenges associated with the use of non-animal-derived antibodies still remain to be discovered after widespread use of these antibodies by non-antibody experts.

- The ESAC opinion is further imbalanced and lacking important information on the advantages of animal-derived antibodies and certain disadvantages of non-animal derived approaches. And even though the ESAC opinion and the ECVAM recommendation do not focus on the therapeutic applications, the main message of the recommendation does include therapeutics and states that animals should also no longer be used for therapeutic applications. The main arguments presented in the document are based on data obtained with the use of antibodies in scientific research and are way too easily extrapolated towards therapeutics. Animal-derived antibodies used as therapeutics are very well-characterized and do not suffer from any of the shortcomings listed in the document. This further demonstrates that disadvantages of animal-derived antibodies described in the document are not inherent to these antibodies but are the result of poor characterization. Next to that it should be recognized that the technology available today to ensure good selection and guarantee consistent quality in the generation and production of antibodies is much more advanced than a decade ago and that antibody producers implement these technologies which will contribute to the quality of antibody reagents in general.

The goal of this document is to provide additional information on the animal-based antibody generation approach and applications to remove the current imbalance and enable well-informed decisions on whether non-animal-derived antibodies are a valid alternative for the use of animal-derived antibodies. This document will also provide information on the possible societal consequences of a blunt ban on the immunisation of animals for the generation and production of antibodies.

3. A technical comparison: animal-derived versus non-animal-derived antibodies

3.1. The number and quality of generated antibodies

For most applications it is of utmost importance to dispose of a large panel of monoclonal antibodies to identify the very best (affinity, solubility, stability, low immunogenicity, epitope targeting) molecule for a particular application. For diagnostic and therapeutic purposes usually high-affinity antibodies are required.

There are distinct differences between the number and quality of antibodies that are selected / generated against antigens when starting from (i) man-made naïve human or animal antibody libraries and synthetic libraries, or (ii) when using immunised animals. From naïve libraries one can select one, a few or maybe a handful of interesting binders that often do not meet the stringent criteria for developability into a therapeutic or diagnostic product, whereas from immunised (outbred) animals large numbers of top quality antibodies can be identified, which recognize a diverse set of epitopes across the antigen surface, rendering this approach much more useful for commercial product development (see for instance Basilico et al, JCI 2015). This is because the immune response in the animal provides a very selective natural environment in which highly functional antibodies are selected for in a very efficient way.

Even though they can be retrieved in a relatively fast way, the limited number of hits from naïve or synthetic antibody libraries are often not optimal and need to be improved by very tedious and time consuming iterative in vitro maturation processes that do not provide guarantees on the desired

outcome. These processes have become more sophisticated over the years, but cannot compete with the natural in vivo maturation.
In vitro maturation makes use of technologies such as light chain shuffling methodology and complementarity determining region (CDR) targeted mutagenesis are applied. The CDRs are the hypervariable regions in the antibody that form the antigen-binding region. With these approaches it is possible to improve the affinity properties of these antibodies, but this can for instance lead to a decrease in antibody stability (Julian MC et al, Protein Eng Des Sel, 2015, and Julian MC et al, Sci Rep 2017) as well as possible lower expression yields, lower solubility and increased immunogenicity issues, which in turn will lead to unreliable behaviour in the final application and impact its developability. Antibodies obtained after animal immunisation seem to suffer less from these shortcomings as the hyperimmunisation of the animal will naturally favour and proliferate those B cells producing better performing antibodies at the expense of B cells with weaker antibodies.

3.2. Complex targets

Active immunisation of animals is especially superior to selection of antibodies from artificial, man-made antibody libraries when good quality antibodies are needed for a complex target, such as multicomponent assemblies, membrane proteins and highly dynamic proteins. This is because during immunisation these complex antigens can be presented to the host immune system in their native conformation. Specifically for the generation of antibodies against proteins or protein complexes that span the cell membrane such as ion channels or receptors, animal immunisation with whole cells that express these proteins is highly superior to selection of antibodies from libraries. Review of the scientific literature also confirms that therapeutic antibodies and antibody fragments targeting such ion channels and receptors are mainly derived through animal immunisation (Hutchings et al, MAbs 2019; Akli Ayoub et al, MAbs 2017; Hutchings C., Expert Opinion on Biological Therapy, 2020). Also, antibodies such as Rituximab and Herceptin, which both also target a receptor, have been obtained by immunisation with cells expressing these targets, followed by screening of the antibodies on cells and cell-based assays (Stohl W, Protein & Cell, 2018).

An important example where animal immunisation has made a true difference is the production of nanobodies coming from llamas that were immunised in Belgium against the highly mobile G-Protein Coupled Receptors (GPCR). These nanobodies enabled the researchers to fix the GPCR in one specific conformation helping to elucidate the structure of these very important receptors, thereby creating more in-depth understanding of how these receptors function. GPCRs are an important class of highly dynamic proteins that play a crucial role in how cells sense signals from their environment and which trigger cellular responses. As a result, new paths for the development of novel drugs and therapies have been opened. In 2012 Robert Lefkowitz and Brian Kobilka received the Nobel prize in Chemistry for their work on the GPCRs.

A comparison between antibodies against GPCRs derived from animal immunisation and antibodies from non-immune libraries showed that these two types of antibodies are functionally distinct. While all antibodies from non-immune libraries partially inhibited the target function, the antibodies derived from immunisation were full inhibitors (Rossant et al., 2014, mAbs 6, 1425-1438). Moreover, the antibodies derived from immune and non-immune libraries targeted completely different epitopes demonstrating the benefit of using different antibody sources.

3.3. Physiochemical properties of antibodies

There are important differences between the physiochemical properties of synthetic antibodies and antibodies that have been generated and have matured *in vivo*. This has consequences for the 'developability' of these antibodies. Lonberg (Nat Biot, 2005) stated that in vivo generated antibodies have favorable biophysical properties. During natural *in vivo* affinity maturation there seems to be a counterselection against antibodies that tend to aggregate. "There is also evidence that CDR3 sequences are selected for attributes that may be more important for properties such as stability and aggregation than for simple molecular recognition." It explains why lengthy lead optimization procedures are needed for bringing leads selected from naïve and synthetic libraries into the clinic. Also Almagro and colleagues conclude this in their review in Frontiers in Immunology from 2018: "This is particularly important for therapeutic antibodies generated *via* phage display, related enriching technologies, and humanized antibodies where the selection process proceed *in vitro* without the filters imposed *in vivo* that tend to select well-behaved molecules when used as therapeutics."

Not only is stability an important parameter for therapeutic antibodies to deliver reproducible efficacy, robust manufacturing processes and superior protein-kinetics and protein-dynamics profiles, but properties like thermal stability, solubility, specificity and bioavailability are also very important. *In vivo* matured antibodies are mostly superior in these properties (Jain, T. et al, Proc Natl Acad Sci USA, 2017; LU, R.M., J Biomed Sci, 2020; Baker, M., Nature 2015).

3.4. Therapeutic antibodies on the market

A comparison of FDA approved 'fully human' monoclonal antibodies published in 2017 shows that almost three quarters of these antibodies are derived from transgenic animals, where about one quarter stems from phage display selections from non-immune libraries (see figure 1 below). Phage display selections from non-immune libraries and the use of transgenic animals to generate human antibodies have been developed and implemented at about the same time and are both employed by the pharmaceutical industry. These numbers therefore suggest an almost 300% higher success rate for the animal immunisation route than starting from artificial, man-made antibody libraries to deliver a successful therapeutic antibody.

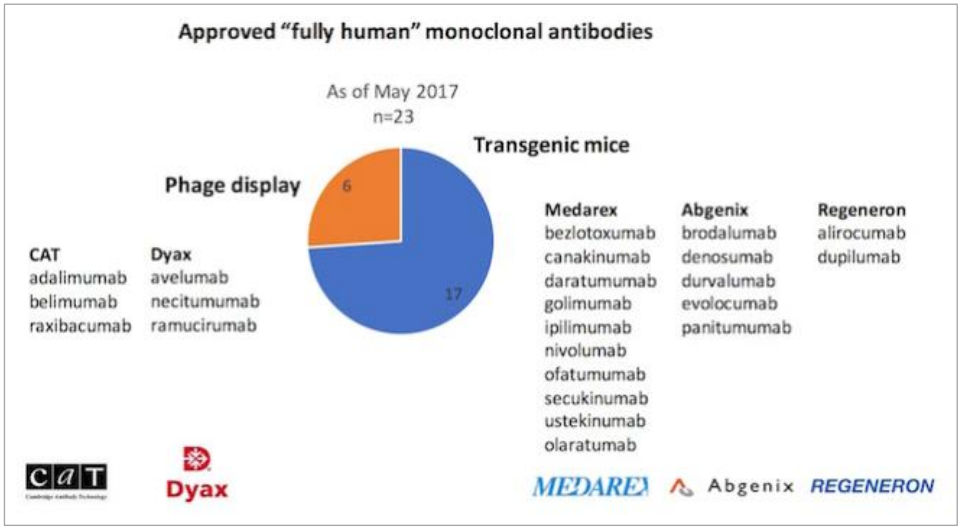


Figure 1: comparison of the origin of approved 'fully human' monoclonal antibodies.
Source: https://lifescivc.com/2017/05/human-antibody-discovery-mice-phage/

More recent data numbers show that US FDA approved antibodies are mostly derived from animal immunisation (90% in the past five years). Display selection technologies of artificial, man-made libraries have and still are yielding beneficial drugs, but the success rate from panning of displayed synthetic libraries is significantly less than antibodies from animal immunisation, dispite the widespread use of display techniques in the pharmaceutical industry (Lu, R.M. et al, J Biomed Sci, 2020; Kaplon, H et al, MAbs, 2020; Sioud, M., Mol Biot, 2019).

4. Impact

4.1. Scientific research

Antibodies are very widely used in scientific research. Traditionally, (polyclonal) antibodies have mostly been used as a tool to visualize the presence of proteins on blots, or as affinity reagents to purify or eliminate specific proteins from a mixture. But antibodies, and especially antibody fragments have become an extremely versatile research tool. They can be used in a wide variety of fields, such as immunohistochemistry, immunomodulation, intracellular expression (intrabodies) and biosensor-applications. They can also be used for expression profiling, physical mapping of proteins, protein-protein interaction studies, functional and structural analysis and (in)activation of toxic or reporter genes. Depending on their use, the requirements for properties like affinity, specificity, solubility, etc, may vary.

The multiplicity of targets is theoretically infinite and new proteins, protein mutants and variants are continuously discovered. These protein targets for which antibodies are developed range from very simple (for most sequential epitopes) to structurally very complex (certain conformational epitope targets). This generates a need to have the ability to use different platforms that can produce the necessary quality antibodies. In scientific research it is very important to maintain the ability to raise high quality antibodies against targets of varying composition or highly dynamic targets. Naïve or synthetic libraries will not be able to guarantee this.

Polyclonal antibodies are used very broadly. They are very versatile and can be produced rapidly at low cost, which are important advantages. They are often used as a detection reagent throughout the scientific investigation. Replacing polyclonals by monoclonals or multiclonals would result in significantly longer waiting times before the reagent can be generated and delivered and would also be much more costly. This is not desirable in scientific research for a simple detection reagent. Cost, flexibility and especially speed are important issues in scientific research. Any delay in research leads to delay in patent applications and in bringing medicines to patients.

Animal-derived antibodies have contributed and still are significantly contributing to important scientific breakthroughs, and the GPCR example is already given above. But there are many thousands of other examples, and this is logical given the versatility of how antibodies and antibody fragments can be used in scientific research. A screen of current scientific literature will show that most of the antibodies used in research come from the immunisation of animals.

Given their versatility and widespread use in scientific research it is very important that antibodies and antibody fragments remain available to the research community at low cost and with short delivery terms. Access to high quality antibody tools should also remain guaranteed. Having to fully switch to library-derived antibodies, would deprive the scientific community of a very important

antibody source. That can only be justified if the alternative is able to provide high quality antibodies that are fit for purpose on reasonable terms. This starts with access to the 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. In addition, most of the synthetic antibody libraries comprise a theoretical number of variants which exceeds by orders of magnitude the number of varients that can be screened in phage display or even in ribosome display selections. Consequently, every selection round will lead to a new set of affinity reagents and it is impossible to know whether you have the very best binder of the library. Therefore, after having isolated a potential good candidate, additional tailoring will be performed to improve potency This also requires access to the screening, maturation and other tools that are associated with the generation of high quality antibodies. If these conditions cannot be met, having to fully switch to non-animal-derived antibodies will have very serious negative consequences for scientific research.

4.2. Applications for regulatory purposes

Antibodies are used in different ways for regulatory purposes. They are for instance used to support pre-clinical and clinical studies (as biomarker reagents, PK, ADA, etc...). The European regulatory bodies currently only refer to positive control antibodies generated by immunizing animals, indicating that they are less familiar with antibodies from synthetic libraries for these purposes. The EMA guidelines even state that if human sera are not available, the use of animal sera is the only option. . In the end what counts is that the antibodies are well characterised, stable and that there is batch-to-batch reproducibility[6]. Stability is the product of the combined biophysical properties. As already indicated above, animal-derived antibodies generally have favourable biophysical roperties.

4.3. Diagnostics

Also in diagnostic applications the antibodies should be well-characterized, stable and should have batch-to-batch reproducibility. And of course, high affinity, very high specificity and low or no background detection are of utmost importance. This will determine the quality of the diagnostic tool.

Antibodies derived from immunised animals have been able to meet these criteria and are therefore widely used for generating specific and sensitive diagnostic tools. As a result this has allowed the development and clinical use of well-performing diagnostic tests to screen, diagnose and monitor a very wide array of diseases. Non-animal-derived antibodies have not been able to significantly penetrate this field. This is because the generation and selection of well-performing non-animal-derived antibodies for clinical diagnostic tests has proven to be very disappointing. The non-animal-derived antibodies often have too low affinity and poor specificity.

[6] See for the requirements:
https://www.fda.gov/media/70858/download
https://www.fda.gov/media/119788/download
https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf

4.4. Therapeutics

One of the most important aspects for therapeutic monoclonal antibody development is that at the start of the campaign a diverse set of target-specific antibodies is created that covers a diverse set of epitopes on the target surface with sufficient affinity and that can then be triaged on the intended functional drug performance in cell-based assays. Further, important developability criteria need to be fulfilled by a future antibody drug that include cross-reactivity with the pre-clinical species version of the antigen (for tox and mechanistic studies), as well as suitability for large scale manufacturing. The approach taken by most drug development companies is therefore to use robust antibody generation platforms that deliver multiple high quality antibody candidates and/or use several platforms in parallel. In vivo platforms for monoclonal antibody generation have been shown to be most suitable in this respect indicated by their aforementioned success rate, with in vitro platforms potentially providing complementary repertoires but not being able to replace in vivo platforms for therapeutic antibody generation.

Polyclonals also still today have a very important therapeutic application area, that is to combat envenoming. In South and Middle America, Africa, Middle East and South-east Asia scorpion and snake envenoming cause severe health and economic problems, especially in the rural areas. It is therefore considered a poverty disease. Although far from optimal, the polyclonal antibody fragments obtained from hyper-immunised horses are still the only valid treatment option, despite all the efforts that have been undertaken to substitute these therapeutics by monoclonal antibodies or by antibodies from non-immune antibody libraries. Even carefully selected mixtures of the very best monoclonal antibodies from hybridomas or non-immune antibody libraries are less effective in treating the envenomed victims. Therefore, a total ban on animal immunisation will deprive these patients from possible life saving treatments.

4.5. Other application areas

What is seen in the therapeutic area, also counts in other areas where antibodes are being deployed. For instance, in the development of antibodies as protein-based biocotrol agents to treat plant pests and plant diseases, the antibodies generated through animal immunisation warrant a higher success rate and shorter development time. If animal immunisation would no longer be allowed, the development time will prolongue with at least two or three years, with no guarantee that the most important and critical parts of the plant pest or infectious organism could be hit.
Europe has the ambition (European Green Deal, EU Farm-to-Fork strategy) to significantly reduce the use and risks of chemical pesticides. In order to achieve that goal access is needed to the best and most efficient technology platforms, which includes animal immunisation.

5. Antibodies, better antibodies, best antibodies

A good antibody is an antibody that is fit for purpose. Depending on the antigen target and its application area, the requirements for the antibody will differ. For diagnostic, and even more so for therapeutic applications, the quality requirements are quite strict.

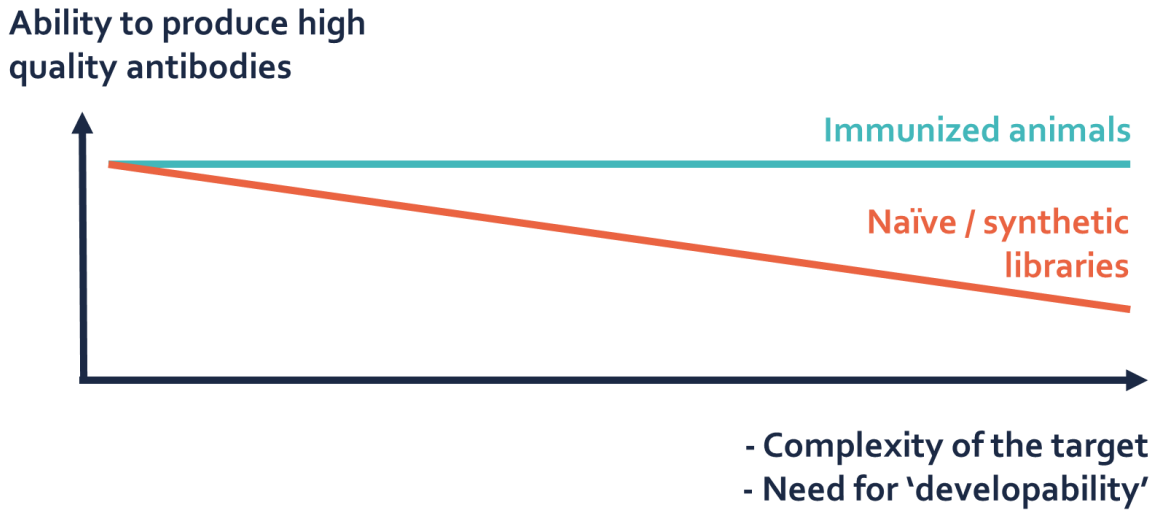


Figure 2: the relation between the ability of libraries and immunised animals to produce high quality antibodies and the complexity of the target and/or need for developability

Libraries derived from naïve B-cells and synthetic libraries are able to produce antibodies that are fit for purpose, especially for the more 'easy' targets and in cases where the requirements for 'developability' are not very stringent.

But for complex targets (e.g. snake venom) and when the requirements for developability are high, immunised animals have a much higher ability to produce quality antibodies than naïve or synthetic antibody libraries. To access the most potent antibodies it is important to maintain the possibility to immunise animals for the generation and production of antibodies.

6. Animal welfare aspects

Animal welfare is an important issue, and the life science sector fully supports the responsible use of animals in compliance with the applicable legislation and the three R's principle (Replacement, Reduction and Refinement). The use of animals for the generation of antibodies is subject to strict housing and care rules, veterinary oversight and a strict ethical committee approval process.

The three R's and animal welfare are taken into account in different ways. The number of animals in immunisation campaigns is limited to the amount that is strictly necessary. Often a mix of different antigens is used to immunise an animal, especially when immunizing larger animals, to further significantly decrease the number of animals used. The number of animals used 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 optimized adjuvants that do not cause discomfort, while on the other hand stimulating the desired immune response. These protocols do

not cause discomfort beyond what is caused similarly by vaccination or blood sampling of humans. Any discomfort is very limited.

Animal use beyond the generation of the antibody

When it comes down to the principles of replacement and reduction, and a discussion whether or not non-animal-derived antibodies constitute a valid alternative one should consider the animal use that is required throughout the entire drug development process, rather than only the initial immunisations. Animal experiments are amongst others necessary for determing the safety of novel compounds before administering them to humans. When the failure rate of non-animal-derived antibodies is higher and their biophysical properties make them less 'developable', the total number of animals used to develop a product will certainly be higher for non-animal-derived antibodies than for animal-derived antibodies. The important message here is that one should realize that for important applications non-animal-derived antibodies do still require the use of animals and these numbers are orders of magnitudes larger than the amount of animals that is required to generate a potent antibody through immunisation.
It is also a misconception that animals used for immunisation are suffering and killed afterwards. Indeed laboratory mice and rats are sacrificed to generate hybridomas, however the vast majority of other animals such as chicken, goats, sheep, cattle, horses, dunkeys, llamas and alpacas that regularly serve for antibody production are not euthanized after the immunisation process.

7. Accessibility of high quality antibody libraries and tools

In order to be a true alternative, next to the quality requirements, the non-animal-derived antibody libraries must be accessible and affordable for the life science sector, and the resulting antibodies must be affordable for the end-user. Such libraries should also be suited for the purpose, i.e. suitable for a specific research target, and should allow to screen large-enough collections with the required sequence and structural diversity. Ideally there should be unrestricted access to the libraries, the know-how of generating them and/or the (financial) capacity to methods, platforms and tools required for the generation and screening of such libraries and to technologies required for in vitro maturation of selected antibodies.

Pharmaceutical companies with a biologicals development platform generally can afford to purchase or license the propietary tools (vectors, libraries, …) and screening technology offered by providers and antibody catalogue companies (e.g. Abcalis, Abcam, Bio-rad antibodies, Invitrogen, …). Moreover, in contrast to smaller companies and research institutes, larger pharmaceutical companies have the budget to tap into the technology service provider's platforms, or the operational capacity to generate their own libraries. The setup of proprietary platforms for generation and screening of synthetic or naïve antibody libraries is reflected in an IP portfolio proving that this is a reality[7]. These proprietary safeguards, however, preclude the availability of such technology platforms or libraries to smaller companies, start-ups, academic or research

[7] Examples here include Morphosys AG, with a patent portfolio on synthetic or recombinant antibody technology (EP3642227A1, EP3423578B1, EP1979378B1, EP2528944B1), Ylanthia^{å} Fab library (EP2640742B1, EP2435568B1) and proprietary optimization technology (Slonomics^{å}), as well as Novimmune SA (EP2513312B1), Genentech (EP1513879B1), Medimmune, among other big Pharma, as well as companies with proprietary library and screening technology (e.g. specific Phase display) such as Zumutor biologics (EP2528944B1).

institutes with limited operational and licensing budget, making the latter dependent on service providers.

The currently known technology platform service providers[8] own proprietary tools, and/or proprietary screening technology and/or proprietary libraries, or other process IP, which comes with a heavy price, thereby limiting the access of the existing high-quality platforms to companies which can again afford such an investment.

For academic groups and companies with limited budget these commercial service providers are in fact restricting the availability of synthetic or naïve antibody libraries, either through financial barriers or by restricting the use of their proprietary tools, as well as by retaining knowhow (kept as trade secret), thereby further blocking the scientific community from improvements and innovation in the field. This mainly serves to ensure commercial service providers of their competitive position, though with limited availability. For instance, in the field of naïve antibody libraries, the tools to generate customized naïve humanized libraries are not disclosed as to maintain a proprietary position in a particular technology.

Finally, also a number of research institutes within Europe are developing improved technologies for generating synthetic or naïve antibody libraries, for instance to obtain more stable non-animal-derived antibodies, hold patents on these methods and synthetic antibody libraries (e.g. CNRS, INSERM: EP3066120B1), resulting in another FTO hurdle for research institutes and companies with limited licensing budgets.

In conclusion one can state that a mixture of secret and proprietary tools and methods exist in the field of synthetic and naïve antibody library screening and development, creating a threshold for their use. The availability, being based on financial capacity and thus dominated by corporate structures, hugely limits the options on non-animal antibody development for small biotech and academia, which on the longer term will inevitably negatively affect further innovation in this area.

8. Societal and socio-economic impact

Antibodies in general and animal-derived antibodies in particular have created very important health benefits. Important therapeutic biologicals are animal-derived antibodies (Rituximab, Herceptin, ….) and they have worldwide resulted in a very significant number of QALYs (Quality Adjusted Life Years) added. Also in the current COVID crisis different animal-derived antibodies show great promise for prophylactic or therapeutic treatment. As for therapeutic antibodies in general, the majority of the antibodies that are now being investigated and developed against COVID-19 which are not patient-derived, are animal-derived.

The clinical diagnostic industry relies today mainly, if not solely, on animal-derived antibodies. This has allowed the development of highly sensitive and specific diagnostic tools, with recent examples

[8] Examples of such commercial technology service providers include Adimab, with its Platform on humanized synthetic antibody libraries for production in yeast (EP3124497, EP2193146, EP3336225 & EP2593594), selling Technology Service including the rights for using their exclusively build libraries; or Iontas (EP3277810B1), Specifica (WO2020/14143A1, Bradbury et al.), Proteogenix (LiAb-VHH MAX™ library), Hybrigenic services (EP3066120B1), Neoclone , and Biorad HUCAL â technology antibody platform (originally developed & owned by Morphosys AG: Knappik A et al. 2000. J Mol Biol. 296:57-86; Prassler J et al. 2011. J Mol Biol. 413:261-78.

in the diagnosis and follow-up of the COVID disease, complemented with related infectious and pulmonary markers. Non-animal antibody service providers have tried to penetrate this field, with very limited success. This is particularly true for difficult antigens, such as e.g. steroids, for which equivalent performances are far to be reached.

There still is a very large volume of antibodies that are being generated for basic research. The financial cost to replace polyclonal antibodes for basic research with non-animal-derived antibody generation will be very high. As this is substantially funded by governmental sources this represents a poor use of taxpayers money. Having to use the naïve and synthetic libaries will significantly increase both the delivery times of these reagents, their costs and put European research at a competitive disadvantage to the rest of the world.

Even though both non-animal-derived and animal-derived antibodies make it to the market as products, and both constitute good products, the majority of these antibodies are and are likely to remain animal-derived. And as shown above, there is good reason for this, because the animal approach has a much higher probability of resulting in a developable product. The more complex the target is, the more this is valid. For some targets the non-animal approach may not be successful at all. When it would no longer be possible to immunise animals for the development and production of antibodies, it will be much more difficult, if not impossible to develop antibodies of superior quality for important targets. This creates a genuine risk of losing out on potential benefit for society.

There is a genuine risk that a full ban on the use of animal immunisation for the generation of antibodies leads to a situation where European patients may no longer have access to the best antibody based medicines. Because antibodies are a growing group of medicines for both widespread and rare diseases, such a negative effect should not be underestimated.
For companies a ban on animal immunisation means that Europe becomes much less attractive for doing R&D, leading to animal immunisations moving to other parts of the world, followed by important R&D moving away from Europe, especially R&D from larger players that have more global activities. Smaller European companies with more local activities cannot take similar decisions and will see their business negatively affected.

9. Additional comments to the ECVAM recommendation

Some members of the ESAC panel have a financial interest in non-animal-derived antibody technology, which creates a serious conflict of interest. It is not clear how this conflict of interest has been managed and how bias has been prevented.

10. Conclusion and recommendations

The technology to retrieve potentially useful antibodies from non-immune, man-made libraries has been developed over the last three decades. The technology is still improving, however, for a variety of applications, including scientific research, diagnostics, medicinal and other products, they cannot fully compete with the antibodies derived from immunised animals. On many occasions, the animal derived antibodies are produced faster, easier and at more affordable cost. For other applications and for some types of targets, the animal derived antibodies require less downstream engineering or they can simply not be replaced by antibodies generated without an animal immunisation step.

For these reasons it would be counterproductive to have a general and blunt ban in Europe on the use of animals for immunisation. Such a ban in Europe will first lead to a move of this activity to other parts of the world, thereby retarding the immediate access to a number of newly developed research reagents. A ban on the use of antibodies derived from animal immunisation may also lead to European citizens and patients no longer having access to the best products.

The immunisation step causes only a very marginal distress to the animals, and with the exception of (transgenic) mice to generate hybridomas, the immunised animals should not be killed.

Antibodies obtained after animal immunisation are, on average, of better quality than those antibodies where animal immunisation has been avoided. Therefore, the drop-out of inadequate antibodies from non-immune libraries is larger than that from immunised animals. So, in the end in the development of a therapeutic antibody less animals (primariliy mice and monkeys) are needed for the pre-clinical selections when starting from antibodies derived from immunised animals.

Animal immunisation for the development and production of antibodies should remain possible, as a ban on immunisation would result in substantial potential societal benefits being lost. Governments are strongly advised not to support such a ban.