COMMISSION BRUXELLOISE DE L'EXPÉRIMENTATION ANIMALE

3R 3V

BRUSSELSE COMMISSIE VOOR DIERPROEVEN

Advice of the Brussels Commission on animal experiments (25/08/2020)

Report "EURL ECVAM Recommendation on Non-Animal-Derived Antibodies"

The development and production of monoclonal and polyclonal antibodies and other affinity reagents still involve animals, despite the availability of non-animal technologies. In accordance with the legal requirements of Directive 2010/63/EU on the protection of animals used for scientific purposes, **animals should not be used in procedures where a non-animal alternative exists and where the same or higher level of information is obtained as in animal procedures.** Therefore, the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) has mandated its Scientific Advisory Committee (ESAC) to assess the available evidence and issue an opinion on the scientific validity of antibodies and non-animal antibody affinity reagents produced using animal-free technologies. Taking into account the available evidence, ESAC adopted an opinion on the suitability of existing animal-free technologies to produce affinity reagents of equal or superior quality (purity, activity, specificity, affinity, stability, reproducibility) to those provided by antibodies produced by conventional animal-based methods. In addition, ESAC has commented on the scientific benefits of using non-animal affinity reagents and assessed whether there are production and/or application scenarios for which they are not suitable and antibodies derived from animals are still needed. This "science-for-policy report", which can be found via the following link https://publications.jrc.ec.europa.eu/repository/bitstream/JRC120199/jrc120199pdf.pdf , describes the EURL ECVAM recommendations on non-animal antibodies developed on the basis of ESAC advice and the detailed report of the working group.

This issue will be on the agenda of the next NCP meeting with the European Commission, including a discussion on concrete steps to be taken to ensure compliance with the obligations of the Directive.

As a preparation for this meeting, the competent authority asked our advice on this report and how Belgium could impose the corrective measures? What concrete steps could be taken to ensure that the Brussels Capital Region complies with the obligations of the Directive?

A meeting online was scheduled on Tuesday the 25th of August 2020, in order to produce a common point of view of the Brussels Commission on Animal Experiments.

Participated: Vera Rogiers (VUB), Catherine Vroonen (UCB), Blaise Descampe (GSK), Fadel Tissir (UCL), Alban de Kerchove (ULB) & Stefan Roels (DGZ).

COMMISSION BRUXELLOISE DE L'EXPÉRIMENTATION ANIMALE
3R 3V
BRUSSELSE COMMISSIE VOOR DIERPROEVEN

Report as a summary of the discussions:

Global remarks:

- A clear distinction must be made between monoclonal Abs and polyclonal Abs.

  The ECVAM report has separate comments on polyclonals and on monoclonals. The criticism on monoclonals is basically criticism on hybridoma's and the fact that they sometimes produce additional heavy or light chains the interfere with specificity or sensitivity. The problem with the ECVAM report is that the way these problems can be solved have no relation with the origin of the antibody. Because both multiclonals and recombinant production of antibodies can be done with antibodies derived from immunized antibodies. These solutions are not limited to using naive or synthetic libraries

- One also has to take into consideration the different factors that play an important role in the choice of suitable Abs: the quality needs (including affinity, epitope, propensity to immunogenicity, post-tranlational modifications, nonspecific interaction) for the methodology involved ((Western blotting, ELISA, Immunohisto-chemistry, chromatin Immunoprecipitation,…) and the complexity of the target protein.

- General biased statements should be avoided e.g. "that most animal-derived antibodies are of "questionable quality" and that "non-animal derived reagents are better and that they will improve reproducibility of results".

- The question was raised whether among the authors of the report enough experts in immunology with experience on the bench or in cutting edge methods using mAbs were involved.

Proposals made:

- Every application should be separately analysed by experts with experience on the bench before global conclusions could be made.

- The application of monoclonal and polyclonal antibodies should be treated separately.

- If a constructive plan must be proposed, then it should (for instance) first consider mAbs. Both polyclonal and monoclonal antibodies are and will continue to be required; they are used for different purposes. Polyclonal antibodies need in vivo production (even if some people may argue that you can mix monoclonal antibodies).

  Extra budgets and extra time should be foreseen. At least 4-5 years are needed not only to save running projects but also to compare the results of the same experiments carried out with mAbs from libraries with those coming from animal production. Only when the situation is clear for mAbs, polyclonal Abs could be considered.

- All statements made should be supported by peer reviewed references.

- In order to be a true alternative, next to the qualitative requirements, the non-animal-derived antibody libraries must be accessible and affordable for the whole life science sector and not for a happy few who can afford to purchase or license the proprietary tools (vectors, libraries, ...) and screening technology offered by providers and antibody catalogue companies.

Thoughts:

- In view of animal welfare issues: one should realize that for important applications non-animal-derived antibodies do still require the use of animals.
- A high number of clinical tests and fundamental research protocols have been standardised and validated based on well-defined Abs derived from animal use. If that work needs to be redone using non-animal derived Abs, this means not only extra work but also extra budget to come to "the same" tests that actually work well. This will be very expensive and not very motivating for companies and research institutes.
- The technology is already present for more than 30 years. However, still today it only represents a small part of the Abs market (<1 %), pointing to problems in realistic applicability
- Whatever the use of hybridoma or B cell cloning and genetic engineering to allow affinity maturation, the result is that a limited number of animals is now used for immunization and that indeed, current technology allows significant reduction and refinement.
- In-house experience: no good experience in the efficiency of non-animal derived antibodies (many antibodies do not work as expected: differences in quality and differences depending on the techniques for which they are used e.g. Western blots, ELISA, immunohistochemistry, Immunoprecipitation, etc)
- The stability of synthetic antibodies is not always optimal (cfr precipitation)
- For the production of vaccines, polyclonal Abs (animal derived) are today a necessity. When a vaccine is ready, safety tests have to be carried out using 1000 Abs. This is not possible using mAbs as producing these by recombinant methodology would be extremely time consuming. Using less Abs (e.g.10) would result in regulatory failure. Additionally, the potential impact on the release of vaccines against seasonal diseases (eg flu) and the regulatory impact for the release of all vaccines if methods are changed should also be considered.
    - ✓ As an illustrative example of a potential limitation of antibodies of non-animal origin is the so-called HCP (Host Cell Protein) test that aims to protect the patient by evaluating the residual quantity of host cell proteins. (However, there is already lobbying to use orthogonal methods based on the assumption that immunoassays are not catching all proteins)

      This test currently uses antibodies of animal origin. Host cell proteins are present in very large numbers at the start of the vaccine production process and the vast majority are eliminated during purification. In this specific case and in particular for vaccines currently marketed,

the replacement of antibodies of animal origin by antibodies of non-animal origin would require the artificial construction of a reagent similar to the product of animal origin. However, the proportion of the antibodies directed against these HCPs obtained in animals cannot be quantified with precision, and the composition of the replacement reagent cannot therefore be defined. This means that in this case the substitution could lead to a reagent exhibiting limits in its ability to recognize certain type of host cell protein and pose a potential risk to patient safety.

The risk for commercial vaccines is to lead to the development of a reagent that is not equivalent in its capacity to recognize certain proteins from the host cell and not to be able to quantify them correctly, thus inducing a potential risk for the safety of the patient.

- ✓ Potential counter-productive effort: An efficacy test known as the potency test is, as its name suggests, a test that guarantees the efficacy of a vaccine, namely whether the vaccine indeed induces protective antibodies or immune reaction able to protect the patient against the target disease. Usually these efficacy tests are carried out using laboratory animals (mainly mice) and our objective is to replace these tests in mice (*In vivo*) by a non-animal test (*in vitro*). At the present time, the authorities' expectation is that the alternative (*in vitro* ELISA test) must show equivalence or superiority over the animal elicited protection. To demonstrate equivalence, the antibodies used must have very specific characteristics linked to show their relevance in functional activity (they must be effective to protect against the disease) and capable of showing degradation of the vaccine. This assessment is essential for public health. Recent experimental results have shown that methods which do not use laboratory animals are not always able to provide antibodies with the necessary characteristics. There is a risk of not identifying antibodies that meet the requirements of the authorities, namely their ability to demonstrate protection against the disease and to identify degradation of the vaccine. This situation would then lead to the use of the potency test on animals, the latter being done all over the commercial life cycle of a vaccine, a thousand times more animals will be consumed than if animals had been used only for antibody production.

- For nanobodies: initial comparative studies *in vitro – in vivo* resulted in the success of *in vivo* techniques and the failure of *in vitro* ones (see Ablynx – VIB). Nanobodies are indeed very important and their role in current scientific research and products in development should be emphasized, but as such one could also generate and use synthetic nanobody libraries. But these libraries suffer from the same disadvantages as any other synthetic libraries, namely that the nanobodies have not been derived from in vivo matured camelid Abs and are inferior in quality in terms of developability.

- For companies and research groups, a ban on animal immunization means that Europe becomes much less attractive for doing R&D, leading to animal immunizations 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. This would have a significant impact on the competitivness and attractivness of many labs, with an impact on employment on the regulatory acceptance of clinical diagnostic tests, vaccines,… since analytical packages will need to be submitted and reviewed, and not only by European health authorities

Conclusion :

- If one wants to talk about replacing animal-derived antibodies by non-animal derived antibodies one has to:

  ➔ Consider independently monoclonal and polyclonal antibodies

  ➔ Recognize that the production of pre-existing monoclonals does not require animals, and thus does not warrant replacement by non-animal derived affinity reagents

  ➔ Recognize that evaluation of Abs quality depends on the application in which they will be used, that the variety of these applications is immense, and that no general recommendation can be easily made with regards to this variety

  ➔ Consider to start replacement with one group of antibodies only, and only when necessary (e.g. monoclonal)

  ➔ Organise well designed comparative studies in which Abs (both animal derived and non-animal derived) against the same epitopes are evaluated and compared in different methods and uses and this by different institutes. This will take years and a budget that is yet to be found for each study. Only then will we have a clear answer on the reliability and quality of non-animal derived antibodies in comparison to the animal derived ones. As mentioned earlier, these should be evaluated and compared using the same techniques. This will take a lot of work, time and budget, for replacing Abs of which we already know that they work and which have been validated and incorporated in a huge corpus of scientifically peer-reviewed articles. The question remains if this is really necessary and if at all feasible.

  ➔ Be aware that for important applications, such as therapeutic antibody development, non-animal-derived antibodies do still require the use of animal immunisation, and that making rapid and ill-considered decisions on the replacement of animal-derived by non-animal derived antibodies could implicate safety tests that provoke more animal suffering than before.

  ➔ Support creation of EU funded center of excellence for non-animal antibody development, assessment and use.