Round table conclusions

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Current knowledge and actions for new approach methodologies

NAMs or new approach methodologies has gained more attention over last few years to reduce and replace animal's models by *in silico*, *in chemico*, *in vitro* or *ex vivo* approaches. A complementary use of NAMs with *in vivo* models can help to reduce the number of animals used and confirm the relevance of *in vitro* models without completely removing animal's models. Indeed, the classical protocol is still testing *in vitro* and confirm the hypothesis *in vivo*. However, *in vitro* and *in vivo* models should not be systematically compared if we want to induce this change and find new alternatives methods.

A modification of this animal-based testing paradigm cannot be easily accepted especially in some areas such as toxicology, where it seems more difficult to move to the *in vivo* testing methods. In other fields, changes were easily made, for example, the cosmetology area replaced the animals by *in vitro* models for skin testing.
We can also note that some drugs are now validated without *in vivo* models thanks to *in silico* methods and artificial intelligence. However, not all tissues and organs are similar, with different regulatory pathway, and some tests are more difficult or impossible to replace for now.

NAMs for better science

To be sure to have the most relevant model and to build the link between *in vitro*, *in vivo* models and the patients, biomarkers are essential to ensure that they all answer the right biological question and verify the useful of the models, whether *in vitro* or even *in vivo* models that already exist. Indeed, animal models are sometimes developed

without even understanding completely the pathology in patients and questioning about their pertinence.
The academic sector has then a role to play in increasing knowledge about pathologies mechanisms to develop and imagine more robust models. Nevertheless, it is always difficult for the scientific community to agree on the right biomarker, and the validation of the biomarkers can also take time. A holistic approach could make everyone agree for the most relevant biomarker and help building multi-disciplinary models.

Post-translational modifications are always possible and can make the use of a biomarker difficult. It is also often rare to have a single biomarker for a pathology, it is often a set of biomarkers that will reflect the metabolic pathways. However, the practical advantage of using biomarkers should be noted: if the function of the biomarker is shown in an *in vitro* model, it will quickly and easily validate the model and prove its relevance. Every model has its own limit, but it is important to know them and to use the right model to answer the right question. For example, in immunotherapy, there is a lot of different animals' models with their limits and no agreements on the "right" one. To overcome the limits of those different models, it is important to develop a standardized *in vitro* approach that can answer specific question.

More transversality between the different sectors is needed to move faster. The effectiveness of a study or even the relevance of the model must often be revalidated. Sharing the results can make possible a better use of NAMs where it is possible and where it has already been shown to replace animal models, even if the field was initially different but the use remains close. For example, a consensus between different laboratories or industrials would also make it possible to agree on the relevance of a single model rather than wasting time developing several similar ones.
However, between different fields, the method for answering a scientific question may be different, making dialogue also difficult. For example, the needs and approach will not be the same between a biologist and a physicist in the design of an alternative model. Beyond the different sectors, it is also necessary to bring together the "pro" and "anti" alternative models' people to demystify NAMs and reassure those who work with animals. Complementarity is also necessary to develop more relevant models, giving more answers than a single model that is sometimes incomplete.

Regulatory and political aspects of NAMs

This transversality and coordination between the different domains and sectors will allow us to gather as much data as possible on what is possible or not in terms of animals' models replacement. Regulatory agencies also have an essential role to play as they can put pressure to limit animal model use and find alternative issues. Here too, dialogue is essential to access as much information as possible before making decisions and applying. Regulatory agencies must exchange information with the various players involved to keep up with the progress. Nevertheless, even if the data are present, it can take up to 10 years for a NAM to be validated at the regulatory level and replace an animal model. Besides, differences within a regulatory agency can make this process longer. If an agency cannot agree internally, convincing another agency to change can be more challenging.

The different sectors also have their regulatory agencies and their own needs in terms of validation. For example, medical devices and chemistry do not respond to the same

regulatory agencies. These differences between the different regulatory agencies in different fields mean they do not move at the same speed.
The cosmetics industry was the first to show the way, followed by the food industry and, finally, the pharmaceutical industry, which is the most important user of animals because the regulations are more difficult to change, even if a new direction is taken. Indeed, the concepts of security and safety are not the same between those sectors, sometimes making changes in product validation methods difficult.

Another challenge is the global aspect of commercialization: a manufacturer wants to be able to market his product everywhere. However, regulatory agencies do not have the same guidelines between different countries. If some countries do not follow the new recommendations, the effort is useless, and we are going backwards. This makes dialogue essential at the global level in order for this limit not to become a brake. Quite the reverse, if countries take the lead, others can follow their steps by seeing that it can work.

The cost of change

Transversality between sectors and domains is again important because everyone will have a role to play in the cost of this change. First, there is the financing of the development and validation of NAMs. A company can spend a large sum of money on developing a new method to be used by other industries that have not financed anything. That is why the government will have to participate and help fund this research that benefits the community. If we take the example of COVID-19, decisive political action has made it possible to unlock significant funds and accelerate vaccine research and validation. Similar action may be possible for NAMs.

Similarly, European directives banning research on animal testing in cosmetics have pushed manufacturers to fund research of alternatives. Significant expenses would not have been covered without the government's pressure.
It will also be necessary to fund communication on those alternative models in the industrial and governmental sectors and to the general population. For example, some animal rights organizations, like People for the Ethical Treatment of Animals (PETA), uses part of its donation money to raise awareness.

A human cost will also be present in training new staff and supporting those who used to work with animals to evolve their skills. This communication part is essential to bring together this duality between those working with animals and those seeking to develop NAMs. Linking these two parts can make it possible to move faster. It would also be crucial to communicate with those who find it hard to move from animal models to look for new pertinent models and to raise their awareness. Unfortunately, these communication and awareness-raising actions often target an audience already interested in using NAMs, such as this congress or PETA's actions that target a public already aware of the animal cause.