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Inside the Global Regulatory Dialogue

SANOFI PASTEUR's CARINE LOGVINOFF ON THE ADVANCEMENT OF HTS

At the session of the May CASSS European CMC strategy forum on the evolving use of next generation sequencing (NGS) to substitute for in vivo adventitious virus testing, Sanofi Pasteur Virology Analytical Expert Carine Logvinoff discussed the evolving regulatory environment for the testing, and the progress Sanofi Pasteur has made in developing its high throughput screening (HTS) methodology through interactions with regulators at CBER and engagement with international discussions and collaborations. Formatting changes and other minor edits have been made by IPQ for clarity and readability. The normal disclaimer that the presentation represents the views of the speaker and not necessarily that of his/her organization is not included.

Within Sanofi-Pasteur we have worked on the HTS for a number of years and notably regarding adventitious agent detection. I will briefly give a background regarding the testing package of adventitious agents prior to HTS. Then I will turn to our development regarding our HTS for adventitious agent testing, the validation, and how we approached the substitution of the *in vivo* test for adventitious virus with the HTS method.

I will briefly give you an overview of the evolution of the regulatory environment and how, along the past decade, we shared our progress on this technology.

A fundamental aspect of biological product safety is to assure absence of adventitious agents in the final product. Regarding the viral contaminant, they used to be tracked by a collection of tests expected to be, on the one side, broad spectrum, such as the *in vivo* tests in animals as well as the *in vitro* tests here written as the 'direct observation haemadsorption test.' But there are also the specific tests, such as the specific PCR, the *in vivo* antibody production test, or the 9CFR for porcine and bovine viruses, for example.

None of these tests are able to detect all viral contaminants, and that is the reason why it is the combination of all these tests that used to be applied on this prior to 2010. If I say 'prior to 2010,' it is related to the noisy entry made by HTS in the vaccine world, as mentioned by Emmanuelle [Charton, EDQM] in the introduction.

All this started with the publication of Joseph Victoria ***[link provided below]***, who used metagenomic and pan-microbial microarray on live attenuated vaccine and found porcine circovirus-1 (PCV1) sequences within rotavirus vaccine.

So this was extremely noisy in the field. FDA set up a VRBAC [Vaccine and Related Biologics Advisory Committee] meeting in May 2010. Of course, this paper highlighted the fact that the original testing package could miss viral contaminants. So FDA invited all vaccine manufacturers to consider any additional adventitious agent testing method to avoid these type of issues.

Sanofi-Pasteur's Exploration of NGS

At Sanofi-Pasteur, we made a commitment to accelerate the exploration of these new molecular testing methods, including HTS – although at that time we were also considering microarray and family PCRs.

Regarding the HTS for adventitious virus test that we developed, we considered that this technology is an effective and valuable tool for both identification and reduction of unknown adventitious virus. So from a safety perspective, that would allow filling the gap that the conventional testing package presented.

And also, within the 3R focus – so just to remind you the 3R correspond to the reduction, replacement, or the refinement of use of animals – we consider that HTS could help us to remove the *in vivo* test for adventitious agents.

Since 2009, we were exploring these technologies – first within research studies, characterisation tests, and investigation – and we were finally able to develop and implement it as an adventitious virus detection test that is now running under GMP.

Overview of HTS Test

Here is the overview of our test. So, I said we are running it under GMP – and I think we are not that many in the world who are able to do that for the moment. It has been quite a long achievement and a long journey.

We tried to optimize the pipeline in order to be able to suit any type of sample – so from cell bank, seed harvest, drug substance or product, whatever the sample is. The first step will consist of extracting the nucleic acid from this sample. We assessed different types of kits, and we had to be able to extract whatever nucleic acid is there. Single- or double-stranded DNA or RNA, whatever they are, the method has to suit any type of nucleic acid.

We have made a paper on the extraction as we set it up. ***[A link to the 2018 paper by "*Sathiamoorthy, Sarmitha et al*" *is provided below.]*** Several parts are fairly common second-strand synthesis. So whatever we extracted, we rated it as double-stranded DNA, so that this can enter into the sequencing tool.

We are using Illumina – Sebastiaan explained the principle of Illumina. Out of this we get a data file that is then analysed through our Phylo-ID software that we developed internally to do the evaluation, based on the phylogenic approach. From this we can conclude if a sample is clean of viral adventitious agent, or if it requires follow up investigation.

This technology allows us to detect nucleic acid, but it does not tell us if it is a naked nucleic acid or if it is part of a live particle. That is why we need a follow-up investigation that could be partly bioinformatic based or lab experimental based.

Method Validation

As this test has to be built as a safety test, we have to consider it under a validation umbrella. With such a complex technology, we have broken down the validation approach into different pillars:

● On the left, the sample prep includes everything that is related to the sample. We do the nucleic acid extraction, how we index every library, the library preparation, the controls we put in, the sample flow and the personnel flow. ● We also looked at the validation of the equipment from the sequencing perspective, as well as the supporting laboratory equipment. ● The software I already mentioned – Phylo-ID. ● The computing platform that is necessary for the large data – there are millions and millions of data to analyse and to house, so this takes a huge storage space – and we also need the computing power to do the analysis. ● Finally, the analysis and the interpretation of the signal and of the validation.

So this is for the validation part. As I said, we had in mind in the development of this technology to substitute it for the *in vivo* test for adventitious agent. We followed the new chapter within the **European Pharmacopoeia**, Chapter 5.2.14.

This approach says that the implementation of new methods as substitutes for *in vivo* 'requires a comparison of the specificity (breadth of detection) and the sensitivity of the new and existing methods. For this purpose, an appropriate panel of representative, well-characterised model viruses should be used to assess the ability of the new method to detect viruses that are, or are not, detected by the *in vivo* methods, and to determine if the sensitivity is at least equivalent to the sensitivity of the *in vivo* methods.'

This is a really nice approach, but it might be difficult to assess the sensitivity of broad detection methods such as the *in vivo* ones. And especially when there was no standard for these methods. However, we took the benefit of a study conducted by the **NIH** and Rebecca Sheets, which was pivotal for us ***[link provided below].***

Within this study, the NIH assessed the sensitivity of *in vivo* and *in vitro* tests against 16 viruses across nine viral families. Eleven of these viruses were tested *in vivo* as well as *in vitro* and only six were detected. On the right part of the slide you can see the sensitivity of the test *in vitro* and *in vivo*. The longer the bar is, the more sensitive the assay is.

I will just mention that there are only a few of the model viruses that were nicely detected *in vivo* and these viruses are the ones that suit really well with the animal model that we are using. For example for flu, they were assessed in eggs – and for VSV, it was both the mouse and the eggs.

We wanted to link our HTS data to this *in vivo* data. So the idea was to reproduce the virus type that was used by NIH and to assess this virus within our HTS approach. That is what we have done and here is the result that we obtained on the right part of the slide.

You have the sensitivity that we observed within the HTS assay. So we were able to go down to 10^2 genome copy per ml for some viruses. But at least for all of the viruses, we were able to detect them at 10^4 copy per ml.

We translated the limit of detection that was observed *in vivo* into the genome copy number in order to be able to do the comparison. As you can see, we were exactly equivalent to the *in vivo* data, except for the viruses that were really well suited to the animal model that I have already mentioned, like VSV and flu.

We then continued the work and validated the test as a limit test, spiking these model viruses at 10^4 per ml. We could show that we are reproducibly able to detect these sixteen viruses. We also confirmed the specificity of the assay and the breadth of detection.

With that we fully validated our HTS detection assay. We demonstrated the sensitivity using the panel of viruses that was developed by NIH and that provided us with the opportunity to streamline our testing package with the replacement of the *in vivo* adventitious agent test.

International Regulatory Developments around NGS

Within the same time, the regulatory environment evolved. The first one to evolve was on the **WHO TRS** [Technical Report Series] part – with notably the cell substrate TRS, which was the first one to mention the potential use of these new alternative technologies. That was in 2010, published in 2013. The next TRS on live attenuated vaccines also introduced this possibility of using HTS.

On the **European Pharmacopoeia** side, as already mentioned, there was this creation of the 5.2.14 chapter that was really helpful. But there was also a really big revision of the cell substrate chapter as well as the 2.6.16 chapter on the test for extraneous agents [in viral vaccines], which changed the approach regarding the testing package.

Now the testing package has to be based on the viral risk assessment, and within this chapter the new technology can now be presented as an alternative to *in vivo* tests for example.

Progress Sharing through Meetings and Collaborations

All along this first decade, we have shared our progress broadly. First, we were quite active in sharing this at various conferences.

I will not give the details of the conferences, but maybe will just mention the last **IABS** [International Alliance for Biological Standardization] meeting of November 2019 [held at the University of Ghent], for which a congress paper is to be published at the end of this month. All the presentations will be available to everyone and I think it was a really important meeting – both for the technology aspect and the interface with the regulatory authorities.

We were also quite active as part of an interest group and collaborative studies. Since the beginning with the Victoria paper mentioning about the PCV contamination of the rotavirus vaccine, FDA has formed a working group with different vaccine manufacturers, including CROs, also academic labs. The intent of the group was to evaluate new technologies for viral safety.

The group has conducted collaborative studies, including spiking ones, to obtain data to fill the gaps both technical and bioinformatic regarding standardization of HTS. We have been a member of the **AVDTIG** [Advanced Virus Detection Technologies Interest Group] since its formation and we are co-leading with our colleagues some sub-groups like those on sample prep and the bioinformatic follow-up investigation.

We were also part of another collaborative study that was conducted by **WHO** on some reference standards. Most of this is also shared through our publications, internal publications regarding our Phylo-ID tool, or how we prepare our sample. There is also the paper from the AVDTIG group that is mentioned here, the report from the past IABS meeting, some preliminary evaluation of the technique, also against qPCR.

Sharing with Health Authorities

These are the broad sharings that we have done during this past development. There was also some more specific sharing that we did with health authorities, especially with CBER.

I said that we considered that these tests could substitute for the *in vivo* tests for an extraneous agent, and we proposed that in early 2017 for one of our new vaccine projects. We proposed that to CBER, and CBER was quite positive with this proposed replacement, and invited us to have any type of technical meeting as we felt the need.

Actually, we had the opportunity to meet a few times with CBER on this technical discussion. The first one was mainly to present our strategy and to make sure that we would have the support of CBER on this approach. That was in 2017. Last year we had technical discussions, just prior to an IND submission, to align on what should be submitted and the level of data to be submitted for the IND.

More recently, last month, we also exchanged on some challenges we are facing with a specific matrix. These were open and extremely fruitful discussions where we have exchanged ideas. This ensures mutual confidence of the HTS test and also meeting CBER expectations.

The Present View

With that, I would like to say that we have successfully developed a test that allows agnostic detection of viral contaminants. This HTS test for viral extraneous agent substitutes for the *in vivo* test. We did that as per the 5.2.14 chapter approach. We applied this test on our animal cell bank and viral seed. And through the continuous and diverse exchange with health authorities during the development and the validation, we ensured that the expectations and compliance were met.

I will finish with how we approach testing for viral contaminants these days. For our new vaccine, we are not considering *in vivo* tests anymore, because we consider that these can be substituted by adventitious virus. And when we run HTS, we do not need to have specific PCR anymore because its sensitivity is close to that of HTS, from what we know. We also replaced the 9CFR for porcine and bovine viruses where they were needed.

We are also considering in a trial aspect to replace the HAP, the MAP and the RAP [hamster, mouse and rat antibody production] tests where they were needed, by HTS. With that, I want to show you who we want to thank over this long development that is still evolving.

LINKS:

These papers addressing virus control testing were referred to by Logvinoff in her presentation:

- Detection of Minority Variants, Joseph Victoria, *et al.,* 2010
- Recovery of Viral Nucleic Acids, Sarmitha Sathiamoorthy, *et al.,* 2108
- Adventitious Virus Assays, Rebecca Sheets, *et al.,* 2014
- Candidate Reference Material, Edward Mee, *et al.,* 2016