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Meeting Report

Zoonoses Anticipation and Preparedness Initiative, stakeholders conference, February 4 & 5, 2021



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ABSTRACT

The Zoonoses Anticipation and Preparedness Initiative (ZAPI) was set up to prepare for future outbreaks and to develop and implement new technologies to accelerate development and manufacturing of vaccines and monoclonal antibodies. To be able to achieve surge capacity, an easy deployment and production at multiple sites is needed. This requires a straightforward manufacturing system with a limited number of steps in upstream and downstream processes, a minimum number of *in vitro* Quality Control assays, and robust and consistent platforms. Three viruses were selected as prototypes: Middle East Respiratory Syndrome (MERS) coronavirus, Rift Valley fever virus, and Schmallenberg virus. Selected antibodies against the viral surface antigens were manufactured by transient gene expression in Chinese Hamster Ovary (CHO) cells, scaling up to 200 L. For vaccine production, viral antigens were fused to multimeric protein scaffold particles using the SpyCatcher/SpyTag system. *In vivo* models demonstrated the efficacy of both antibodies and vaccines.

The final step in speeding up vaccine (and antibody) development is the regulatory appraisal of new platform technologies. Towards this end, within ZAPI, a Platform Master File (PfMF) was developed, as part of a licensing dossier, to facilitate and accelerate the scientific assessment by avoiding repeated discussion of already accepted platforms. The veterinary PfMF was accepted, whereas the human PfMF is currently under review by the European Medicines Agency, aiming for publication of the guideline by January 2022.

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1. Introduction

Since the 1950s there has been a generalized complacency that eventually all infectious diseases can be controlled effectively, at least in humans in the western world. Despite this notion, during the H1N1 influenza virus pandemic in 2009, vaccines were not available in time to effectively control it. The Zoonoses Anticipation and Preparedness Initiative (ZAPI) was initiated to prepare for future outbreaks and to develop new technologies to accelerate development and manufacturing of vaccines. As the current SARS-CoV-2 pandemic showcases, emerging infections still catch us by surprise. Delays in vaccine availability will mean that vaccines typically arrive too late to affect the course of the first pandemic waves. Hence, a key principle of preparedness is to do as much work as possible before an emergency happens, so that the response can be decisive and efficient. For pathogens not yet encountered, platform technologies are needed that can rapidly produce prototype vaccines and, if successful, produce enough vaccine against the new pathogen. The ZAPI project was set up as an industrial manufacturing demonstrator for achieving surge capacity, both for vaccines and antibodies, based on a set of *a priori* constraints: no mammalian cells for vaccine production; no live vectored vaccines (i.e., no live genetically modified organisms); vaccine candidates based on soluble subunits; antibody lead candidates to be classical monoclonal antibodies or single-domain antibodies like VHHs (“nanobodies”); systems to achieve surge capacity (100 M vaccine doses) within 3–4 months after identification of candidates; and full *in vitro* Quality Control (QC) for batch release, according to the 3R EU guidelines [1]. To achieve surge capacity, an easy deployment and production at multiple sites is indispensable. This requires an easy manufacturing system with a limited number of steps in upstream and downstream processes, a minimum number of *in vitro* QC assays, and robust and consistent platforms. Three viruses were selected as prototypes for the ZAPI Project: MERS coronavirus (MERS-CoV), Rift Valley fever virus (RVFV), and Schmallenberg virus (SBV). While coronaviruses (including MERS-CoV) are well-known nowadays, bunyaviruses (including RVFV and SBV) are less well-known but are the largest group of viruses infecting mammals, including humans.

1.1. Antibodies for human therapeutic use

All three viruses contain surface glycoproteins that can elicit neutralizing antibodies: the RVFV Gn protein, the SBV Gc protein and the MERS-CoV spike protein. The structures of these proteins were elucidated during the ZAPI project, both from ZAPI participants and from groups outside the ZAPI project [2–4].

The conventional approach was to produce monoclonal antibodies (mAbs) against the viruses using wild-type mice. Alternatively, transgenic (“H2L2”) mice designed to produce human-rat chimeric antibodies that can be reformatted to fully human antibodies, and camelids (llamas and dromedary camels) engineered to produce heavy chain-only antibodies (HCAbs) and VHHs. HCAbs are devoid of light chains and have long complementary-determining regions with unique epitope binding properties, allowing them to recognize and bind with high affinity to epitopes not recognized by conventional antibodies. VHHs (sometimes referred to as nanobodies, due to their small size), are the antigen-binding domains of HCAbs that can be produced in bacteria and yeast in large amounts, at low cost. Furthermore, VHHs can be used as building blocks for the generation of multifunctional complexes. To test the antiviral activity of VHHs, *in vitro* neutralization assays were developed, in which viruses were incubated with the VHHs and with susceptible cells. Neutralization was screened for large numbers of VHHs using innovative virus neutralization tests (VNT), suitable for high-throughput analysis with automated read-out. Importantly, the same assays can be used to screen natural antibodies from human patients’ sera.

Studies with bunyaviruses demonstrated that neutralization of RVFV

and SBV is most efficient when combining VHHs targeting different viral glycoprotein subdomains [5]. These findings stimulated the development of multivalent complexes, in which VHHs are covalently linked to scaffolds through an innovative technology known as “bacterial super-glue” [6].

The most promising VHH combinations were subsequently genetically fused, yielding monospecific or bispecific constructs. The bispecific constructs were efficacious in preventing death of RVFV infected mice, especially when given prophylactically (6 h before virus challenge, 100% survival), and to a lower extent when given therapeutically (18 h after virus challenge, 60% survival). The effect was highest when VHHs targeting different regions on the Gn protein were combined, leading to a strong synergistic effect.

For SBV, as wildtype mice are not susceptible, interferon receptor knock-out (IFNAR^{-/-}) mice were treated with monospecific or multi-specific nanobody complexes one day before viral challenge. Prophylaxis with monospecific complexes led to 80% survival, whereas all mice survived when inoculated with multispecific complexes, demonstrating that combinations of VHHs targeting different antigenic sites result in very potent neutralization activity.

Antibodies against MERS-CoV were derived from dromedary camels that were first vaccinated and subsequently challenged with MERS-CoV. These dromedary camels developed exceptionally potent virus-neutralizing antibody responses [7]. Several MERS-CoV-specific VHHs were identified. *In vitro*, these VHHs efficiently blocked virus entry at picomolar concentrations, binding the receptor binding domain (RBD) of the viral spike protein with exceptionally high affinity. Furthermore, camel/human chimeric HCAbs with the camel VHH linked to a human Fc domain (HCAb-83) had an extended half-life in serum and protected mice against a lethal MERS-CoV challenge [7].

Initially a broad panel of functionally diverse, fully human antibodies was generated from immunized H2L2 mice. Eight human mAbs were developed that target functionally distinct domains of the MERS-CoV spike protein, interfering with the three critical entry functions of the MERS-CoV spike protein: sialic acid binding, receptor binding and membrane fusion. Neutralizing antibodies were shown to target multiple, non-overlapping epitopes within the receptor binding domain as well as in the fusion subunit of the spike protein. Passive immunization with these neutralizing antibodies protected mice from lethal MERS-CoV challenge [8].

Apart from MERS-CoV specific antibodies, antibodies with remarkable cross-reactivity were identified, that can be useful to pre-empt future epidemics [9]. The broadly reactive antibodies were shown to target the stem-helix region of the spike protein, which is conserved across betacoronaviruses. The antibodies provided protection in mice against MERS-CoV challenge, demonstrated by reduced weight loss, reduced pneumonia and pulmonary virus replication [9].

Generally, industry uses transient expression platforms to rapidly generate material during the early stage of drug development. To date gram per litre expression levels have been reported [10–12], however for resupply, a repeat transfection is required. To ensure process consistency, phenotypic characterization of the host cells and optimal process conditions were carefully assessed. The transient transfection process was assessed for process robustness at 5 L scale and then finally at 200 L scale to demonstrate scalability.

For purification, the traditional column process was replaced by membrane-based filtration, allowing the process to remain robust in a shorter time. The time required for a typical viral clearance study will delay the response time during a pandemic. However viral clearance can be claimed based on prior data on viral inactivation, anion exchange and viral filtration. Process performance of six different 5 L batches showed a recovery higher than 70% and purity close to 100%. At the same time, the host-cell protein levels were well below the 100 ng/mg threshold, typical of mAb products which further demonstrates the suitability of the transient expression process.

Normal release testing would require approximately 21 days, which

could be reduced to 7 days by removing the physicochemical assays and relying on data obtained during the development phase. Furthermore, testing of bioburden could potentially be accelerated with ScanRDI, flow cytometry-based testing (Biomérieux, www.biomerieux-industry.com/products/scanrdi-rapid-and-sensitive-detection) that provides results in just 90 min, and with sterility testing using the Celsis Advantage system (Charles River, www.criver.com/products-services/qc-microbial-solutions/microbial-detection) that reduces time of analysis from 14 to just 5 days. However, these methods still require further validation.

A spike protein binding assay was selected to compare the potency and stability to a reference standard, through a MesoScale Discovery technology. Several factors were optimized such as spike protein concentration, incubation time, detection antibody concentration and optimization through a mAb dilution series.

During scale up, the process was optimized to maintain consistent yields and productivity by addressing vessel geometry, heat transfer, mass transfer and vessel fluid dynamics. Addition of the transfection complex was a challenge, as time and mixing are critical factors to achieve expression.

Product quality attributes were consistent over different batches and at different scales and were within typical criteria for material release. The relative binding activity was similar at the different scales, indicating functionality was comparable.

Long-term stability of mAb purified from the 200 L scale production runs was tested. Purified mAbs were stored at a concentration of 50 mg/mL in a standard buffer for 6 months at $-80\text{ }^{\circ}\text{C}$, $5\text{ }^{\circ}\text{C}$ (stress storage condition) and $25\text{ }^{\circ}\text{C}$ (accelerated stress condition), and stability was tested at 1.5, 2, 3, 4.5 and 6 months. Differential calorimetry was used to assess the thermal stability of the molecules, while dynamic light scattering was used to investigate propensity for self-association. Additionally, samples were tested for visible and subvisible particles as well as size exclusion chromatography for assessment of purity. A recommended storage condition of $-80\text{ }^{\circ}\text{C}$ was proposed based on data generated, although a more suitable storage condition will be required under pandemic conditions.

1.2. Vaccine by design methodology for veterinary vaccines

Viral attachment and entry of SBV are mediated by the envelope glycoproteins Gn and Gc, of which Gc is targeted by neutralizing antibodies. The locations of the epitopes on the glycoproteins were identified by analyzing the reactivity of a set of mAbs and antisera against recombinant SBV glycoproteins [13]. One of two *N*-glycosylation sites was shown to be essential for interaction of Gc with a subset of Gc-specific mAbs. The epitopes could be grouped into two clusters, as revealed by fine mapping using chimeric proteins. Combination of disulfide bonding and epitope mapping generated a structural model of the SBV Gc *N*-terminus [14]. X-ray crystallography studies showed the projecting spike of SBV as the major target of the neutralizing antibody response, and generated X-ray structures of the spike protein in complex with two neutralizing antibodies [2]. Immunization of mice with the spike domains elicited virtually sterilizing immunity [2], whereas immunization with "Gc Amino"-encoding DNA plasmids, or "Gc-Amino" expressed in a mammalian system, conferred protection in up to 66% of the animals [15]. A multivalent antigen containing the covalently linked Gc domains of SBV and the related Akabane virus protected mice and cattle against SBV challenge infection [15]. The same domains of other orthobunyaviruses can be used as immunogens, showing the transferability of the methodology within the same viral genus.

Fold recognition identified four possible arrangements of both RVFV glycoproteins in the virion envelope. These models proposed that the ectodomain of Gn forms the majority of the protruding capsomer and that Gc is involved in the formation of the capsomer base [16]. Antigenic regions within Gn were mapped with Pepscan™, identifying six continuous and three discontinuous epitopes. Eight subdomains of the

Gn glycoprotein were expressed and one domain showed high expression levels and was well recognized by neutralizing mAbs. This domain, Domain VII, corresponded with a subdomain within the Gn crystal structure referred to as "head domain" (Gn_{head}) [17]. The antigen was genetically fused to a SpyCatcher (SpyC) at the *N*-terminus, and produced by a recombinant baculovirus, in insect cells. Three self-assembling multimeric protein scaffold particles (MPSPs) with C-terminal SpyTags (SpyT) were produced in *E. coli*: lumazine synthase (LS), aldolase (ALD) and E2. Combining the SpyC-linked Gn head domain with SpyT-linked MPSPs resulted in particles displaying the antigen at the surface. Efficacy of the candidate vaccine was initially evaluated in the RVFV mouse model. Partial protection was achieved in the absence of adjuvant, whereas full protection was achieved in the presence of adjuvant. The neutralization titers in the mice correlated with protection [18].

Vaccination of young dromedary camels with a modified vaccinia virus Ankara (MVA-S) expressing the full-length Spike protein of MERS-CoV significantly reduced infectious MERS-CoV excretion from the nose [19]. The identification of dipeptidyl peptidase 4 (DPP4) as the MERS-CoV receptor facilitated the subsequent characterization of the RBD in the S1 region of the MERS-CoV spike protein. Vaccination with the S1 region reduced MERS-CoV excretion after challenge of animals. Using the SpyT/SpyC system, the RBD was coupled to LS MPSPs and used to immunize rabbits. This resulted in neutralizing antibodies, which could prevent the production of infectious virus in the rabbits. The technique can also be used to couple on the same particle multiple RBDs from different but related viruses [20].

1.3. Scale up for manufacturing MPSPs and antigen subunits

The choice of MPSPs for antigen display was based on their ability to self-assemble, and their high stability. Initial studies were done at small scale, from which the aldolase-SpyC and E2-SpyT were chosen to proceed with upscaling. The process was taken from small scale in shake flasks to medium scale - 5L fermenters. For the ALD-SC MPSP, a simple downstream processing program is needed: sonication for cell lysis, centrifugation, heat treatment, a second centrifugation step, size exclusion chromatography, and endotoxin polishing. The E2-SpyT could not be purified after heat treatment, so the downstream process relied on ion-exchange chromatography.

1.4. Antigen subunit production in baculovirus and C1 fungus expression systems

Using insect cells for expression, some issues were encountered: with the Gn head domain of RVFV, decreased expression was noted with increasing seed passages, whereas the harvest time of the Gc-Amino domain of SBV needed optimization, as the antigen was unstable in the culture supernatant. Although the baculovirus expression levels were 8–10 times lower than expected, the medium-scale production was found to be robust and reproducible. Yields were not improved using protease inhibitors. Until stability studies have been performed, the product should be stored at $-80\text{ }^{\circ}\text{C}$.

For the C1 fungus expression platform, the initial aim was to achieve expression of at least one antigen for each of the three viruses, to produce stable antigen subunits, and to reach expression levels of at least 100 mg/L. Three antigens were produced and secreted into the medium, MERS-CoV RBD, SBV Gc-Amino and RVFV Gn_{head}. However, initially, the expression levels were relatively low, and the stability was limited. Further optimization was done by knocking out nine proteases in the C1 fungus genome, which led to a maximum yield of 1800 mg/L, 300 times higher than the baculovirus system.

1.5. MPSP/antigen subunit coupling step and quality control system

A broad range of conditions were tested to find the optimal

conditions for coupling, showing that this can be done overnight at room temperature. The coupling kinetics for ALD-SC + ST-SBV and E2-ST + SC-RVSV were similar.

For the analytical QC system, the objective is to define the identity, quantity and quality of the two components and of the final vaccine complex. For the two components, this is relatively straightforward; the MPSP (first component) quality can be assessed as a homogeneous population of self-assembled particles, and the antigen subunit (second component) can be assessed by ELISA with virus neutralizing mAbs. For the MPSP-subunit complex, the amount of subunit needs to be quantified and quality assessment is based on the absence of free subunit in the final product; the coupling ratio also needs to be checked. Two techniques are used for QC of the MPSP-subunit complexes, asymmetrical flow field-flow fractionation (A4F) and quantitative mass spectrometry (qMS). A4F can provide information on the particle population homogeneity and the particle complex average molecular weight, both of which can be used to indirectly determine the coupling ratio. qMS can determine the exact identity and quantity of both the MPSP and subunit and can thus provide insight in the direct coupling ratio. Combining the two techniques provides a full picture for the quality of the final MPSP complex product.

1.6. *In vivo* efficacy

The candidate vaccines were validated in target species. The efficacy of the RVSV candidate vaccines was tested in the lamb model. RVSV G_nhead, coupled to LS was used and formulated with adjuvant. Whereas lambs vaccinated with coupled antigen were completely protected from the challenge virus infection, lambs vaccinated with uncoupled antigen were not. In a follow-up trial, three different MPSPs were compared side-by-side. All three MPSPs induced neutralizing antibodies and prevented fever and viremia, with no differences in efficacy. Therefore, the MPSP selection can be based only on the performance of the manufacturing process.

For SBV, LS was used as the MPSP and two candidate subunits were compared, the Gc Head (GcH) produced in cell line S2, and the Gc Head-Stalk (GcHS), produced in C1. Calves were vaccinated at day 0 and day 14 and challenged at day 35. The level of neutralizing antibodies was higher in the GcHS construct, but both constructs were equally efficient in inducing sterile protective immunity against the challenge. No viremia occurred and no viral RNA could be detected in tissue samples of calves immunized with GcH or GcHS.

1.7. Remaining questions

When an animal is vaccinated, there is never a pure antibody or cellular response but a balance between antibody and cellular responses, with many parameters that are not controlled. A particle-based vaccine may induce better cell and antibody mediated immunity and immune memory than a standalone subunit. Due to the flexibility of the system, it is possible to combine the target immunogen with known strong immunogens, resulting in an immune enhancing effect by multimeric presentation of immunogens.

However, several questions remain open:

Is there a need for two subsequent administrations, or is one dose sufficient? This depends, among other factors, on the type of challenge, the epidemiological situation and the breadth of protection needed. In the field, one administration may provide a sustained level of immunity for a sufficiently long time. Consequently, the level and breadth of protection required will need to be addressed on a case-by-case basis.

Should the vaccine be given with or without adjuvant? Adjuvants are generally broadening the immune response and dose sparing. Therefore, vaccines given without adjuvant usually need higher amounts of antigen, which would be disadvantageous for the manufacturing capacity.

What is the best adjuvant? Not all types of adjuvants work well with particles. Some combinations work very well, others do not, and some

combinations may only work well in certain species.

Does vaccination with saturated, partially decorated or weakly decorated particle complexes lead to different responses? This particle effect needs further evaluation as we enter a new field in vaccinology.

There is a clear general positive effect of the presence of particles in the vaccine formulation. This is because particles are drained very fast and effectively to lymph nodes. Playing with particle display and superglue capacity opens possibilities to co-present immunogen subunits from different pathogens, including variants.

Other questions in the context of therapeutic antibodies:

How to screen and select neutralizing antibodies rapidly?

How to identify antibodies that are non-neutralizing *in vitro*, but may still be potent *in vivo*?

How to screen and select optimal combinations of antibodies for *in vivo* neutralization?

In terms of surge manufacturing capacity for antibodies, the transient transfection expression is quite efficient, although there is a limit, especially for the plasmid DNA supply. Facing something like COVID-19 in the future may require the need to implement multiple strategies in parallel (transient expression, CHO pools, CHO clones), or to follow alternative systems, like the C1 fungus expression system. These antibodies may have a short half-life, but if the objective is to treat patients in ICU, a single perfusion may improve prognosis. This is a new way of thinking on how to use antibodies.

In terms of surge manufacturing capacity for vaccines, the platforms applied in the ZAPI project are very flexible. They become almost “open-source” as the only proprietary system used is the bacterial superglue system. It is possible, if needed, to multiply the manufacturing sites, since the process is very simple and easily transferrable from site to site. This will enable a global supply. The very high C1 yields are creating the need to rethink how to manufacture MPSPs. In order to alleviate the discrepancy between the antigen subunit yield in C1 fungus and the MPSP yield in *E. coli*, it is expected that the *E. coli* expression process can be improved and MPSPs can be stockpiled in advance. Further, more efficient systems remain to evaluate for the expression of MPSPs (including currently available, efficient *E. coli* systems, or alternative bacterial systems).

The new QC approach for ZAPI-based vaccines is in line with the vaccine by design approach. This is implemented in the human pharma industry, but it will be completely new in the veterinary field. This is enabled by the fact that we have a biophysical object – the particle complex – that can be described precisely. Theoretically, this will allow a “reagent-free” QC system.

Policy and regulatory aspects.

There are lessons to be learned from the COVID-19 pandemic. We need to prepare during inter-pandemic periods, to advance platform technologies and try to advance vaccines that can be developed up to market authorization for the pathogens that can cause major outbreaks.

The good experience that has resulted from the current pandemic is the development of vaccines at an unprecedented speed. The development of new technologies in the vaccine field has the potential to change the world of vaccinology; to deliver vaccines very fast in the context of an outbreak. Therefore, it is important to consider that certain platforms may be more appropriate than others in the context of an outbreak situation.

This leads to the regulatory appraisal of new platform technologies. Data available for a certain platform technology could be used in respect to new emerging pathogens. In this pandemic, it was possible to avoid having to repeat all the pre-clinical studies that would normally be required for approval, as data from pre-clinical studies with the platform (e.g., mRNA vaccines) were already available. This process must be streamlined further, without cutting corners, having all the evidence to ensure a vaccine is safe and effective.

The Platform Master File (PfMF) is part of a licensing dossier intended to accelerate access to market of new products, particularly in urgencies like pandemics, and to facilitate and accelerate the scientific

assessment by avoiding repeated discussion of already accepted platforms. After the regulation (EU) 2019/6 was published, there were implementation and delegation acts. A first proposal for a PfMF was created, including some changes for the Annex II of the Regulation Veterinary Medicinal Products. EMA used this proposal as a recommendation to the Commission. In 2020, due to the success of the Veterinary PfMF, a proposal for a PfMF covering antibodies and vaccines for human use was sent to EMA. The EMA used the following definition for Vaccine Platform Technology: Collection of technologies that have in common the use of a “backbone” carrier or vector that is modified with a different antigen or set of antigens for each vaccine developed using the platform. It includes, but may not be limited to, protein-based platforms (virus-like particles), DNA vaccine platforms, mRNA-based platforms, replicons (self-amplifying RNA) and viral and bacterial vector platforms. A positive evaluation shall result in a certificate of compliance to the European legislation for the Platform Technology Master File. The submission of the PfMF shall comply with relevant guidance published by the Agency.

The PfMF approach was to propose definitions of platforms and comparable immunological (veterinary) medicinal products (I[V]MPs) manufactured by novel technologies, restricting the proposals to the quality part of the dossiers, based on the experience gained within the ZAPI project. But increasingly, discussions are ongoing in the human field on how much data from the safety and pre-clinical part should be added to the PfMF as well. Scientific requirements for platform derived I (V)MPs were also proposed, which may be relevant for other modern technology derived products.

The PfMF is the part of an I(V)MP Marketing Authorization Application which describes the platform technology. The same approved PfMF can be used for formulating monovalent and/or combined vaccines or mAb preparations of a given manufacturer. The PfMF certificate will be issued by the EMA to the applicant. It will be valid for all combinations it was approved for, or it may be extended. The PfMFs approved by EMA should then be used as standalone dossiers. In the relevant final product licensing dossier, a cross-reference to the PfMF should be sufficient. However, the detailed administrative approach is up to EMA and the authorities to decide on.

The veterinary PfMF is a stand-alone part of the marketing authorization application dossier for a biological veterinary medicinal product, which contains relevant information on quality, safety and efficacy concerning the platform technology, which is part of this veterinary medicinal product. The standalone part may be common to one or more biological veterinary medicinal products platforms. Safety and efficacy need to be demonstrated for a final product (mock-up or ready for licensing) and not necessarily for the platform alone, but this is up to the authorities. The human PfMF is similar (see Table 1).

For mAbs, relevant sections of existing Monographs and Guidelines should be taken into account with special attention to:

- Expression system: description and characterization of host cell line including cell banks, characterization and testing of cell lines, backbone of expression vector (without the transgene/monoclonal antibody genes).
- Raw materials/starting materials e.g., cell culture media components, chromatographic matrices, etc.
- Description of analytical methods for drug substance and drug product. Lot release assays.
- Description of generic manufacturing process

Regulation (EU) 2019/6 on veterinary medicinal products replaces Directive 2011/82/EC, with the overall aim of achieving a “better regulation” in the EU. It provides for a modern, innovative and fit-for-purpose legal framework, it gives incentives to stimulate innovation and to increase the availability of veterinary medicines and it strengthens the EU action to fight antimicrobial resistance. The regulation was approved in 2018 and published in January 2019, with a 3-year

Table 1

Comparison of Platform Technology Master Files for veterinary and human medicinal products.

Veterinary PfMF	Human PfMF
Detailed description of the expression systems	Detailed description of the expression systems
Recommendation of the nature of the immunogenic epitope(s) to be represented in the expression system	Recommendation of the nature of the immunogenic epitope(s) to be represented in the expression system or vector platform
For material of biological origin, the existing relevant test requirements provided in EU and Ph. Eur. Provisions	
Detailed description of the expressed construct	Detailed description of the expressed construct
Genetic stability of the platform	Genetic stability of the platform elements
Physical/chemical/biological stability of the platform	Physical/chemical/biological stability of the platform
Safety of the platform	Safety (preclinical test) of the platform
In process control tests and control of final platform at final product testing	In process control tests and quality control strategy of final platform at final product testing
Proposal for addition of scientific requirements in Annex II	

implementation period, in which secondary legislation has to be developed. In January 2022 the legislation becomes applicable.

The Title IV in the Annex – Requirements for marketing authorization applications for particular veterinary medicinal product – contains reference to vaccine antigen master file, vaccine platform technology and multi-strain dossier, which are all interconnected. Advice and recommendations on implementing measures under Article 146(2) of Regulation (EU) 2019/6 on veterinary medicinal products can be found on the Commission website and on EMA website. The Annex will be published, after which it is formal.

The concept paper was released for consultation on the January 29, 2021. Stakeholders were requested to comment on the concept paper until the end of March and the discussion on the guidelines in the Immunologicals Working Party will take place in May 2021. In July, the draft guideline will be adopted by the CVMP, which will then go out for a new round of consultation until October. The expected date for adoption by the CVMO and publication of the guideline is in January 2022, in time for the application of the new regulation.

2. Conclusion

In terms of new models and setting new standards, innovative platforms for accelerating the definition and manufacturing of immunogenic vaccine components have been developed along with pertinent animal models. The modular approach for vaccine design and manufacturing can now be used in future pandemics – ZAPI was set up as a preparedness project and has delivered as such. The industrial partners were engaged from the beginning and are using the outputs: large-scale manufacturing of vaccine candidates for veterinary use and antibodies for human use have been taken up. Full *in vitro* QC systems have been developed, which contributes to reduction of the use of animals for scientific purposes.

In the regulatory practice, having a Platform Master File that is accepted for multiple use by the regulators, the progress in the regulation of veterinary medicinal products, the IMP for human use, all adds to the value of what has been created in the ZAPI project.

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