IMI1 Final Project Report Public Summary

Project Acronym: ZAPI

Project Title: Zoonoses Anticipation and Preparedness Initiative

Grant Agreement: 115760
Project Duration: 03/2015 - 07/2021

1. Executive summary

1.1. Project rationale and overall objectives of the project

A zoonotic disease (or zoonosis) is an infectious disease that is transmissible from animals to humans. Zoonoses may be caused by different types of pathogens such as viruses, bacteria, fungi and parasites. Examples of zoonoses are Avian Influenza, Ebola, Rift Valley Fever, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), Salmonellosis, Anthrax...

Scientists estimate that more than 60% of human infectious diseases are originating from animals. As emerging infectious diseases are occurring at an increasing frequency in Europe and other regions of the world, as a consequence of several driving factors (e.g. climate change, travel, trade) it becomes crucial to develop new strategies to prevent very rapidly the spread of infectious diseases both in the animal and in the human populations. In addition to well-proven public health measures, such as implementation of quarantine, travel restrictions,…, the rapid development of medical intervention tools for animals and humans (for example ring vaccination schemes…) is also required to limit the disease spread.

Such a rapid development of animal and human medicine implies to accelerate all phases of the current chain of processes leading to efficient intervention strategies, without downgrading the quality of the work carried out to:

i) Identify the pathogen responsible of the disease
ii) Select the best available therapeutics for animals and humans (efficiency against the disease, possibility to produce large quantities under short timelines). The considered therapeutics are typically vaccines (e.g. non-infectious fragment of pathogens) for animals or humans, and neutralizing antibodies for humans…
iii) Test efficacy and safety of the therapeutics
iv) Produce the therapeutics
v) Obtain approval from regulatory agencies based on safety and efficacy results

To date, the average time to manufacture, in large amounts, a new therapeutic (vaccine or neutralizing antibody) against a known pandemic disease is of about 6 months. For newly emerging threats without a licensed therapeutic, such as the Severe Acute Respiratory Syndrome (SARS), the time required to develop and produce a safe and effective therapeutic is of at least a year and usually unknown as it depends on the nature of the infectious threat and on the current level of research and scientific knowledge for that threat.

The IMI (Innovative Medicine Initiative) funded Zoonoses Anticipation and Preparedness Initiative (ZAPI) Project aims at developing a methodology enabling swift responses to major new infectious disease threats in Europe and throughout the world, by producing targeted therapeutics (vaccines for animals and neutralizing antibodies for Humans) within 4 to 6 months after a disease outbreak will occur. The ZAPI program is the first true “One Health” project within the scope of IMI. It gathers world public and private experts from 5 countries, committed towards this common goal, in both animal health and human health fields. The consortium is headed by an EFPIA member, guaranteeing the practical relevance of the research conducted for industrial needs and its future use by industry. The ZAPI project benefits from the Public-Private Partnership model which contributes to improve mutual knowledge transfer and creates a high level of trust, as well as a common culture, between partners.

1.3. Overall deliverables of the project

In order to enable swift responses to major new infectious disease threats, the ZAPI project develops a general methodology that can be used for the rapid characterization of pathogens and the design and surge production of therapeutics (vaccines and neutralizing antibodies) against emerging

pathogens, in particular viruses. Moreover, the ZAPI project will propose to regulatory authorities and policy makers, recommendations in order to approve, in a sanitary emergency context, safe and efficient therapeutics developed using this methodology. To achieve these objectives, ZAPI works on representative models of currently emerging infectious pathogens (Bunyaviruses, i.e. Rift Valley Fever Virus (RVFV) and Schmallenberg Virus (SBV), and coronaviruses, i.e. Middle East Respiratory Syndrome Coronavirus MERS-CoV), to demonstrate the applicability of this approach toward future emerging viruses.

1.4. Summary of progress versus plan since last period

The ZAPI project, started on March 1st, 2015, has reached all its objectives including the **large scale manufacturing of veterinary vaccines and antibodies for human.**

Regarding the **production of ZAPI vaccines for veterinary species**:

- Selection and validation of 3 multimeric protein scaffold particles (MPSP), on which the immunogens can be linked to generate the final ZAPI vaccine, with further selection of 2 MPSPs for the medium and large scale manufacturing steps has been achieved
- Validation that coupling between immunogen subunits and MPSPs can be performed very reliably with the bacterial superglue system, using small and medium scale materials has been achieved
- Selection of E. coli expression for manufacturing MPSPs and definition of manufacturing process for producing MPSPs at small and medium scale has been achieved
- Selection (after extensive comparison with the baculovirus-based expression system) of the C1-based fungus expression system for generating high yields of the immunogen subunits, validated through the successful examples of SBV and RVFV selected subunits has been achieved
- Generation of specific antibody reagents and, besides the use of more classical analytical techniques, development of innovative analytical tools that can accurately monitor the quality of the ZAPI MPSP vaccine complexes and assess whether a vaccine batch is conform to quality specifications and can be released have been achieved
- Validation of the new analytical tools and assays with the small-scale batches and first medium scale batches has been achieved
- Manufacturing of medium scale batches for SBV and RVFV vaccine candidates and successful validation of these batches in cattle and sheep target species has been achieved
- Initiation of large scale manufacturing processes with the C1 expression system for vaccine subunits and *E. coli* for selected MPSPs. The process scale-up has been fully validated for the C1 fungus expression system and delivered very good amounts of purified immunogen subunits. For E. coli, although the upstream fermentation scale-up has been achieved to a satisfactory level, technical issues were encountered for the downstream process steps, due to the extremely large biomass that could not be handled properly with the available equipment. Despite these technical issues, there is a high degree of confidence for performing the complete large scale process, with a minimum number of steps, once process equipment will be sized adequately to produce GMP grade MPSPs.

Regarding the **production of ZAPI antibodies for humans**:

- Selection and validation of at least 1 lead antibody candidate per viral target
- An innovative expression system has been used successfully for the manufacturing of the key lead antibodies at small and large scale
- Antibodies produced using this innovative expression system displayed similar quality and *in vitro* potency characteristics as expected from conventional stable expression system, while enabling a gain of at least 6 months over the conventional platform process.
- Validation of antibodies expressed in the AZ system for their capacity to protect against viral challenges in animal models, either in a therapeutic (post-challenge) or prophylactic (pre-challenge) treatment regimen, both for medium scale batches and large scale batches.

- Full quality validation of antibody batches manufactured at large scale (200-liters) with the Transient Transfection Expression (TTE) system.

Regarding the aim to obtain a **regulatory framework for the rapid production of safe vaccines and antibodies in emergency situations**, the principle of platform technology was discussed with the EU Council, ENVI of EU Parliament, DG SANTE, EMA and National Regulatory Agencies. ZAPI proposed to EMA a core document, describing one backbone or platform, which could be accepted by legal authorities for multiple later insertions or additions of genes/antigens without new licensing of backbone component.
On the **veterinary vaccines part, t**he ZAPI core document is backed up by an annex proposing the requirements for vaccines manufactured by novel technologies, to support the facilitation of licensing, using the example of a Multimeric Protein Scaffold Particle (MPSP) produced at large scale within the ZAPI project. Both documents have been shared with EMA.
On the **Immunological Medicinal Products (IMPs) for human use** part, the core document has been shared with EMA.

1.5. Significant achievements since last report

The last period of WP3 activities has delivered very impressive results regarding the ZAPI vaccine aspects. Almost all initial objectives have been achieved and, in some cases, the ZAPI results were way above the initial expectations. The particle-display vaccines based on the coupling of a MPSP and a specific immunogen subunit was shown to be very efficient in inducing protection in target species (cattle and sheep). For the first time, it was demonstrated that a C1 fungus expressed antigen had a very good immunogenicity and was able to induce a high level of protection. The ZAPI-based MPSP complex vaccine (formulated in a commercial veterinary vaccine emulsion) can induce excellent protection when injected twice at low doses. The minimum protection dose observed in the 2 models is 10 µg but it could even be lower as no clear dose response was observed. Protection against challenge can be induced after a single administration (SBV cattle model) and this is a parameter to consider when facing emerging diseases in animal health. But it is not a rule since a one-shot immunization in the RVFV sheep model failed to protect.
In line with the surge capacity manufacturing objectives, manufacturing processes have been developed both for 2 different MPSPs (ALD-SC and E2-ST) in the *E. coli* system, and for 2 different immunogen subunits in the fungus C1 system. The ALD-SC manufacturing process was scaled up to medium and large scale. Upstream yields of at least 1000 mg / liter could be obtained for ALD-SC (but almost all this material was lost since the downstream equipment capacity was not large enough at the CRO facility). The very good yields observed at medium scale were confirmed in C1 for the expression of the 2 selected immunogen subunits, underlining the potential of the C1 expression system to deliver at least 100,000 doses per liter and likely more in the future. But the scale up at large scale revealed also issues not observed at the medium scale for the C1-SBV construct, indicating a need to use a C1 genetic background highly deleted from endogenous proteases.
Finally, an original set of highly innovative Quality Control methods has been developed for the future batch release of ZPI-based MPSP complex vaccines. These methods are using only biophysical tools (Asymmetrical Flow-Field Flow Fractionation (A4F), and quantitative Mass Spectrometry (qMS)) and are not dependent upon the generation of specific antibodies. Only 2 complementary techniques can provide the quality information needed to release the future batches of MPSP complex vaccines. This fulfils entirely the objective to have an *in vitro* only QC batch release system.

As part of WP4, the lead antibodies were generated at different production scales (medium and large scales). The anti-MERS-CoV antibodies were evaluated for neutralizing activity in an *ex vivo* assay and an *in vivo* mouse model. For MERS-CoV the results showed that all tested antibodies, independent of the scale of the production, were able to neutralise MERS-CoV in a pseudotyped virus neutralization assay. Results also showed that all tested mAbs provided protection when administered

therapeutically in a mouse challenge model. Similarly, for RVFV, two bispecific heavy chain-only human-llama chimeric antibodies generated at different production scales were assessed for potency by immunofluorescent, binding- and virus neutralization assays. Results confirmed that the potency quality of the antibodies was not impacted by the different production scales.
The developed expression platform for the production of GMP grade material was scaled up from 5L up to 200L. An initial run in a 50L single use bioreactor (SUB) was performed using MERS-7.7G6 as the model antibody. After that, runs at 'GMP' or industrial scale (200L SUB) were completed for each of the lead antibodies against MERS & RVFV viruses. The two lead antibody candidates of each model virus (MERS-CoV and RVFV) were expressed and purified. Analytical results showed consistent product quality attributes for both molecules. In addition to this, results were as expected for mAbs and mAb-like products and residual impurities assays confirmed clearance of host cell related impurities to appropriate levels. In conclusion, results demonstrated that the developed upstream and purification processes can potentially be used for generate GMP grade material. Subject to acceptance by the regulatory authorities the process can potentially be used to supply clinical material.
Analytical methods to determine product purity, consistency of Critical Quality Attributes (CQAs) and levels of residual impurities were developed and tested using the medium scale batches. The methods were applied to the large scale GMP-like material for the selected model antibodies (MERS 7.7 G6 and RVFV 107-104). Analytical results for the GMP-like material showed results consistent with the medium scale batches. No aggregation or fragmentation issues were observed for either molecules (under normal storage conditions). Product quality attributes were also found to be as expected for mAbs and mAb-like products and within typical criteria for material release.
Taken together the data generated in the last report period further supports the potential of using Transient Transfection Expression platforms to accelerate manufacturing where possible. The data shows that a Transient Transfection Expression platform can be optimised to generate high quality and consistent material with expected product quality attributes suitable for early clinical studies.

With the support of all workpackages, WP5 succeeded in forging proposals for regulatory processes more in line with the need of urgency associated with the occurrence of pandemics. The proposed regulatory process demonstrated to reached-out EU decision makers and members of the Commission, Parliament and Council, the high potential of novel methodologies and platform technologies for the development of vaccines and antibodies. As explained in previous reports, a very open dialogue with national regulators and EMA went along the whole ZAPI period. As a result, the platform approach and the principle of Platform Master File has been included in the **New Regulation REGULATION (EU) 2019/6 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC**
Importantly, EMA has published the draft Guideline on Data Requirements for authorisation of immunological veterinary medicinal products under exceptional circumstances and the proposals made by WP5 on the requirements for PfMFs are included. Also, in EMA's draft guideline in Data requirements for Vaccine Technology Platform Technology Master Files (vPTMF), the proposals made by WP5 on the requirements for PfMFs are included in full.

During the last ZAPI period, COVID complicated further outreach to political decision makers, EU institutions and global stakeholders. This was well compensated by the participation of stakeholders from all over the world at the February 2021 virtual stakeholders meeting, by the organization of the CEPI/IABS/ZAPI webinar and the publication of its conclusions and by the participation at the Dyadic webinar. Intense dialogue with EU institutions, including the European Investment Bank to consider an EU infrastructure, based on ZAPI knowledge and expertise, to assure the EU sovereignty on preparedness and vaccine and antibody production has been started, are ongoing. Unfortunately, the EU does not have yet the funding mechanisms in place (like the HERA EUROPEAN HEALTH EMERGENCY PREPAREDNESS AND RESPONSE AUTHORITY) to go forward quickly. HERA: getting ready for future health emergencies (europa.eu).

With regards to ZAPI's coordination and management, appropriate organizational structures and processes ensuring ZAPI's compliance to the ZAPI contractual document (IMI Grant Agreement (GA) and the ZAPI Project Agreement (PA)) have been set up. Those structures and processes help monitor the Scientific and administrative coordination of the project, the maintenance of the contractual documents, and the project's impact, ethical and intellectual property management. A 5 months no-cost global extension amendment request has been submitted and approved. This additional delay has allowed ZAPI industrial partners to produce industrial scale batches using ZAPI's methodology, and therefore demonstrate that this methodology is able to produce targeted therapeutics within a few months after a disease outbreak occurs.

1.6. Scientific and technical results/foregrounds of the project

ZAPI has achieved major accomplishments in the characterization, development and expression of immunogens and antibodies against the three target viruses, in view of the production of vaccines and neutralizing reagents.

The first period has resulted in the identification of key immunogens for the three target viruses using *in silico* prediction and different epitope mapping technologies. A majority of these key immunogens consist of relatively large domains or subdomains of viral proteins.

The second period has resulted in significant advances in the selection of key immunogens for each of the target viruses, which have been expressed using the four systems listed above and characterized. Small animal immunogenicity studies have been performed and allowed confirming the immunogenicity of the selected key domains. Additionally, the experiments proved the functionality of antigens expressed using different non-mammalian systems.
In parallel, Period 2 resulted in major advances in the characterization and development of highly potent neutralizing antibodies against the three target viruses. This achievement validates the ZAPI antibody discovery pipeline. In addition, a protective HCAb against MERS-CoV has been developed, showing protective efficacy in the mouse model, representing an alternative avenue for the production of ZAPI neutralizing reagents.

The third period has resulted in the following major advances:

- A MERS-CoV isolation and sequencing protocol was established and used to show that no mutations occur within the direct spike-receptor interface region.
- Some optimizations have been made (optimizations of the SpyT/SpyC vaccine platform, the C1 expression platform, of the BV replicon and of the YFV-17D replicon).
- The development of two MERS-CoV vaccine candidates by using reverse genetics which are attenuated, genetically stable and provide full protection upon challenge with wild-type virus.
- Partners have demonstrated that human antibodies targeting different structure/function domains of the MERS-CoV S protein are protective in vivo. They also have demonstrated that RVFV nanobody complexes are protective in vivo. And finally, they have demonstrated that antibodies and nanobody complexes (bispecific antibodies) protect IFNAR mice against a lethal challenge with SBV.

The fourth period has resulted in the following major achievements:

- Efficacy studies in small animal models and in target species confirmed the immunogenicity of all selected key immunogens for each target virus. The display of immunogen subunits on MPSPs improves immunogenicity but the benefits are limited for antigenic domains.

- Significant progress has been achieved for various manufacturing processes and associated tools for larger scale production of the selected ZAPI vaccine components.
- Harbour Biomed and University of Utrecht partners filled in a patent on Antibodies and antigen-binding fragments that recognize the spike (S) protein of Middle East respiratory syndrome coronavirus (MERS-CoV)
- The human mAbs MERS-CoV specific generated, targeting different domains and functions of the MERS-CoV spike glycoprotein. These mAbs were evaluated in a small animal model (mice). Two mAbs were selected for further characterization: mAb 7.7G6 binding the globular domain of S protein and mAb 1.6c7 binding to the stem domain of S protein. The results indicated that both mAbs showed protection when provided as a therapeutic treatment either before or after infection.

Results obtained during the fifth and last period resulted in the following major achievements:
For the veterinary vaccine part:

- The particle-display vaccines based on the coupling of a MPSP and a specific immunogen subunit was shown to be very efficient in inducing protection in target species (cattle and sheep).
- The development of manufacturing processes for 2 different MPSPs (ALD-SC and E2-ST) in the E. coli system, and for 2 different immunogen subunits in the fungus C1 system. The ALD-SC manufacturing process was scaled up to medium and large scale.
- The development of an original set of Quality Control methods for the future batch release of ZAPI-based MPSP complex vaccines. These methods are not dependent upon the generation of specific antibodies.

For the human antibodies part:

- The generation of the lead antibodies at different production scales (medium and large scales).
- The development of suitable upstream and purification processes for supplying GLP grade material.
- The development of analytical methods to determine product purity, consistency of Critical Quality Attributes (CQAs) and levels of residual impurities.

According to these major results, a transient expression platform can be optimised to generate high quality and consistent material with expected product quality attributes suitable for early clinical studies.

In conclusion, on the antibody pipeline, ZAPI provided the opportunity to explore the suitability of a transient expression platform to generate clinical grade material. A manuscript is in preparation describing the output. Moreover, the ZAPI project enabled the scale-up of different antiviral antibody formats (conventional antibodies and llama-human chimeric bispecific heavy chain only antibodies). Finally, the ZAPI project provided preliminary information about the potential and developability of bispecific heavy chain only antibodies.
For the vaccine pipeline, the general conclusion is that the ZAPI project has produced a new way to design highly efficient vaccines, based on the particle-display of well-defined immunogen subunits. An optimal decision tree has enabled first to identify readily soluble subunits. Then, after comparison of different expression systems to produce the selected subunits, to propose the new C1 fungus system as the system of choice for surge capacity manufacturing since it could deliver very high yields with a simple downstream process. The incorporation of the recently developed bacterial superglue technology has been a critical step to finalize the initial particle-display concept. This technology actually provided an overall flexibility that was explored in the ZAPI project with several candidate prototype vaccines. Remarkably, it was determined that the Multimeric Protein Scaffold Particle (MPSP) used to display the selected immunogen was neutral to the efficacy, and the easiest MPSP were selected for the large scale manufacturing process. Manufacturing various MPSP complex

vaccines through the coupling of a MPSP and a subunit was very robust, and very consistent. Using these ZAPI MPSP complex vaccines in vaccination-challenge studies in target species, it was demonstrated that a solid protection could be induced even with doses as low as 10 µg and it is suspected that the MPSP complex modular vaccine platform could allow even lower doses. The combined data generated from studies in various target species indicate that the particle-display platform is particularly potent end efficient. Similar data have now been published by other teams using similar particle-display vaccine systems. But the ZAPI project is the first to demonstrate protection efficacy in target species models against highly virulent challenges. This can be considered as an important pioneering work, and opens new avenues for the design of human and veterinary vaccines in the future.
Finally, a set of innovative Quality Control techniques has been developed, allowing to implement a full in vitro QC system for MPSP complex vaccine batch release. This is another key achievement for the ZAPI project, fulfilling the EU guidelines regarding animal welfare and application of the 3R rules.

Major results have been published in renown peer-review journals, including the following:

For the vaccine part:

1. Rodon, J. et al. 2019. Blocking transmission of Middle East respiratory syndrome coronavirus (MERS-CoV) in llamas by vaccination with a recombinant spike protein. Emerging Microbes & Infections, 8:1, 1593-1603, DOI: 10.1080/22221751.2019.1685912
   DOI: 10.1080/22221751.2019.1685912

2. Okba, N. M. A. et al. (2020) Particulate multivalent presentation of the receptor binding domain induces protective immune responses against MERS-CoV. Emerging Microbes & Infections 9(1): 1080-1091 DOI: 10.1080/22221751.2020.1760735

3. Wichgers Schreur, PJ et al. Vaccine Efficacy of Self-Assembled Multimeric Protein Scaffold Particles Displaying the Glycoprotein Gn Head Domain of Rift Valley Fever Virus. *Vaccines*. 2021; 9(3):301. DOI: 10.3390/vaccines9030301

4. Aebischer, A. Development of a Modular Vaccine Platform for Multimeric Antigen Display Using an Orthobunyavirus Model. *Vaccines* 2021, *9*, 651. DOI: 10.3390/vaccines9060651

For the Antibody part:

5. Widjaja, I. et al. 2019. Towards a solution to MERS: Protective human monoclonal antibodies targeting different domains and functions of the MERS-coronavirus spike glycoprotein. Emerg. Microbes Infect. 8, 516-530. DOI: 10.1080/22221751.2019.1597644

6. Wichgers Schreur, PJ et al. Multimeric single-domain antibody complexes protect against bunyavirus infections. eLife 2020;9:e52716. DOI: 10.7554/eLife.52716

7. Wang, H. et al. A conserved immunogenic and vulnerable site on the coronavirus spike protein delineated by cross-reactive monoclonal antibodies (2021) Nature Communications 12 (1715). DOI: 10.1038/s41467-021-21968-w

1.7. Potential impact and main dissemination activities and exploitation of results

The only way to face unexpected viral outbreaks is to develop our capacity to execute an « immediate and decisive intervention ». This strategy raises a dilemma for industrial manufacturing of (human or veterinary) vaccines and therapeutic antibodies:

- How to react very fast and at the same time not chase false alerts for « non events » / self-resolving outbreaks in order to avoid investing huge levels of resources (skilled staff is limited) with high risk to fail?
- How to address exponential needs while manufacturing capacity increase can only be « low arithmetic » (2x or 3x)?

There are 3 different time periods for a vaccine / antibody development:

- Scientific time
- Technical & Industrial time
- Regulatory / Registration time

The ZAPI's challenge was to decrease these 3 timelines to be effective against an outbreak that spreads around the world in a few weeks or a few months.

Reducing scientific and technical timelines for the vaccine pipeline:

ZAPI partners showed that it was possible to reduce the **Scientific time** by accelerating the definition of the immunogen subunit, thanks to the increasing global knowledge on what is the subunit target to use, depending on the virus families. In this respect, *in silico* screening, NexGen sequencing technologies, and learnings from veterinary vaccines (especially the ones that are able to induce protection in different animal species) can be leveraged for the design of human vaccines).

ZAPI partners, have identified immunogenic subunit domains for all 3 virus models included in the ZAPI project:

- Gn for RVFV
- Gc for SBV
- RBD for MERS. The RBD has been mapped based on the predicted location and structure of the RBD of 2 other betacoronaviruses, MHV and SARS-CoV.

Antigens produced in different expression systems were coupled to a MPSP Multimeric Protein Scaffold Particle using the Spy-Tag / Spy-Catcher bacterial superglue. Animal trials showed that the Antigen-MPSP complexes provide a solid protection. Animal trials also demonstrated that there is a much better immunogenicity for the MPSP complex compared to the mixture of uncoupled MPSP and subunit.

ZAPI partners showed that it was possible to reduce the **Technical / Industrial Timeline** by using a robust expression platform with very high yields and a short cycle time. These parameters can actually limit the impact on manufacturing plant footprint, allowing to produce a very large number of doses in a few months, such as the Dyadic C1 fungus system. The selected expression systems and manufacturing processes do not require highly specialized production sites and highly trained staff to be implemented at large scale.

Reducing scientific and technical timelines for the antibody pipeline:

In addition to vaccines, therapeutic antibody intervention is also a critical tool in the responses to a pandemic. This could be the first line of treatment or the only effective treatment for a percentage of the population who are immunocompromised or/and unable to generate a vaccine-induced immune response. Rapid selection of the lead candidates and rapid manufacturing strategies are therefore required to support a therapeutic antibody intervention during a pandemic response. ZAPI partners successfully isolated neutralising antibodies and designed novel bispecific antibody molecules in addition to the conventional antibody formats. The format of choice was related to the complexity of the virus or the best identified strategy to enable an effective antibody-mediated virus neutralisation.

For example, conventional antibody formats were suitable to protect against MERS-CoV in *in vivo* studies, but llama-human bispecific heavy chain only antibodies were more effective against RVFV
Highly potent antibodies were successfully screened using established tools. Transient expression platform was used to rapidly generate gram amounts to facilitate *in vitro* and *in vivo* studies as well as enable the selection of the lead candidates. Transient expression platform was also evaluated for its suitability to rapidly generate GMP grade material compared to the conventional stable cell line platform. Even though there are limitations to the use of transient platform to enable commercial supply, the outcome of the evaluation suggests transient expression can potentially be used to produce GMP quality material where required (*manuscript in preparation*).

The reduction of the regulatory pipeline, can, potentially achieved via the use of:

- Quality by Design
- An established platform

The **Platform Master File (PfMF) concept** proposed by ZAPI partners allows to accelerate the licensing procedure. The PfMF must show that 1) the final product manufactured with this platform is inherently safe for target species and the environment, and 2) the manufacturing process is consistent and robust.

The principle of platform technology as proposed by ZAPI in its Platform Master File has been accepted by EMA for completing the annexes of the New Regulation on Veterinary Medicines *(EMA advice to the European Commission, EMA/CVMP/351417/2019 Committee for Medicinal Products for Veterinary Use).* The principle of platform technology as proposed by ZAPI to EMA in its Platform Master File for immunological medicinal products (IMPs) is currently being discussed.

Intense exchanges with EU Commission and EU Parliament resulted in the Fast Track procedure being covered by *article 25 « Applications in exceptional circumstances" of the New Regulation on Veterinary Medicinal Products*, which should be applied in 2022.

Finally, discussions on a **regulatory framework for the rapid production of safe vaccines and antibodies in emergency situations**, resulted in the ZAPI approach being used for urgent development of some COVID-19 vaccines.

All ZAPI's **ZAPI results were presented at the ZAPI Final Stakeholders Conference held on February 4th and 5th 2021**.

ZAPI's methodology is already being taught in at least 2 **teaching courses**: VAXIN LIVE Masters course http://live.univ-lyon1.fr and the Infectious Diseases One Health Master programme https://www.infectious-diseases-one-health.eu

All partners have clearly benefited from the ZAPI Project:
The great interactions and collaborations within ZAPI consortium Partners led to the setting up of additional projects, some of which were funded by H2020, IMI, National funding and CEPI. In particular, some outcomes of the ZAPI project are included and funded in IMI2 CARE project (therapeutic antibodies against SARS-CoV-2 outbreak).
The two scientific SMEs of the ZAPI consortium have developed quite well**.** Dyadic has been able to further develop and its C1 technology which was used for the industrial scaling up of the vaccine candidates in the final stages of the ZAPI project, and for the development of second generation of COVID-19 vaccines. Harbour Biomed has filed for a patent application for the therapeutic anti-MERS-CoV monoclonal antibody which is now being tested in phase 1 clinical trials against the COVID-19 (MANCO project supported by H2020).
The industrial partners improved their respective manufacturing processes.

The **ZAPI methodology** has set the key principles for achieving the surge capacity needed during « war time » by training and evaluating practical ways to be effective during « peace time ». **The industrial training has to be maintained and improved in the coming years by:**

- **expanding this approach to other targets**
- **refining the process for establishing a solid platform for our future**

Moreover, the concept of a "ZAPI facility" should be developed as when facing a pandemics / panzootics you will need to rapidly reallocate well trained staff on a new topic, and mobilize multiple facilities around the world.

1.8. Lessons learned and further opportunities for research

There have been multiple advantages related to Public-Private Partnership consortium for the EFPIA industrial partners:

- Risks and resources are shared with public partners, which avoided industry to be the only funding partner for initiatives of scientific and industrial interest.
- It saved resources and budget for EFPIA partners.
- The high-quality research and scientific capacity provided by the consortium would not be available directly to the industry as it is provided by the project framework.
- There is a competitive advantage for the participants, since they will be first to exploit the results of the project, and to have access to the databases and reagents produced.
- The consortium created added value from the scientific and technical data that were generated.
- The public private partnership allowed to explore and develop different options at the same time.

Moreover, the EFPIA partners included in the project are complementary and capable to cover all the key aspects from the development to the implementation of the different achievements expected from the project.

Finally, this public private partnership allowed each party to understand better the constraints of the other party. This is illustrated for example by the definition of key factors for selecting an expression system by a selection committee composed of public consortium and EFPIA members, which is based on the specific industrial expertise of the EFPIA members for the development of pharmaceutical products.