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Newsletter

3rd Issue February 2022

EDITORIAL

Welcome to the third issue of the ICRAD Newsletter!

The enormous consequences of the COVID-19 pandemic have reinforced the importance of a robust preparedness to react rapidly and timely to emerging and re-emerging zoonoses. We are confident that the 2nd ICRAD call "One health approach to zoonoses research and innovation" provides a substantial tool to contribute to the development of novel and improved instruments to understand and control zoonoses, including novel detection, intervention and prevention strategies.

The succesful use of mRNA vaccines against COVID-19 has enlightened and emphasised the crucial role of vaccines to control transmission and prevent severe disease and deaths caused by emerging pathogens.
However, the fast development of these modern vaccines was only possible due to preceding year-long research efforts to establish the suitable vaccine platform. This strongly supports the perception that appropriate funds for vaccine research remain of utmost importance to control infectious diseases. In the light hereof, this issue presents a global survey on research and innovation gaps within veterinary vaccinology to gain insight on the key gaps, priorities and barriers that need to be adressed in the future.

In addition, this issue provides 3 interesting contributions elucidating scientific and practical challenges related to the development and use of plant-based parasite vaccines, mRNA vaccines per se, and mRNA vaccines for fish, respectively.

Enjoy your reading.

Per Mogensen
ICRAD project manager

Jens Nielsen
ICRAD coordinator

Second Call for Transnational Collaborative Research Projects: "One Health Approach to Zoonoses Research and Innovation"

40 pre-proposals have been submitted
25 Topic 1
15 Topic 2

- Argentina: 2
- Belgium FNRS: 1
- Lithuania: 1
- Ireland: 5
- Estonia: 2
- Belgium FPS: 1
- France: 24
- Poland: 7
- Netherlands: 13
- Spain: 16
- Turkey: TUBITAK: 6
- Greece: 4
- Denmark: 6
- Sweden: 11
- Belgium FWO: 3
- Latvia: 1
- Germany: 11
- Italy: 11
- Russia: 2
- UK: 25

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Special focus on-

IMPROVED UNDERSTANDING OF EPIDEMIC AND EMERGING INFECTIOUS ANIMAL DISEASES - Research Area 1

MRNA VACCINES FOR FISH, STILL AN UNMET CHALLENGE

Authors:
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Aquaculture is a fast growing sector, and has emerged as the ideal solution to the decline of wild fish stocks. In 20 years, global aquaculture production has tripled, making it a key sector of the food industry[1]. However, the intensification of fish farming practices is not without consequences. Increased stocking densities typically leads to higher risk of infectious diseases, and losses can exceed 40% of total production for some species[2]. Although effective against a wide range of pathogens, antibiotic treatments face problems of drug resistance and safety[3]. In salmon aquaculture, they have therefore been replaced by vaccines against bacterial diseases, in a transition towards more sustainable practices that do not promote selection of multiresistant pathogens threatening public health[4]. Besides the considerable reduction of bacterial outbreaks by licensed vaccines, viral disease outbreaks remain a major constraint to the growth of aquaculture[5]. It is therefore essential to develop new versatile and effective vaccines for the prevention of future bacterial and viral diseases in aquaculture.

To date, the majority of vaccines available to prevent epidemics in aquaculture target bacterial pathogens. Formulated mainly with inactivated or attenuated micro-organisms and oil-based adjuvants, current vaccines often induce an insufficient immune response to viral agents[4,5]. The emergence of new nucleic acid-based vaccines offers a promising alternative for providing high protection against viruses that decimate aquaculture populations. Marketed since 2005 in Canada, the APEX-IHN vaccine (Elanco) is the pioneer DNA vaccine licensed for use in aquaculture to protect Atlantic salmon against infectious haematopoietic necrosis virus (IHNV)[6,7]. Reducing the mortality rate by almost 95% in treated individuals, this vaccine demonstrates the added value of using nucleic acids in vaccines[8]. More recently, in 2017, the CLYNAV vaccine (Elanco) was approved by the European Commission against salmon pancreatic disease (SPDV)[6]. Despite their ability to significantly reduce the infectious load and transmission potential of certain viruses, DNA vaccines face the apprehension that vaccinated fish are GMOs. The possible risk of integration into the genome of the vaccinated host has been shown to be very low, but DNA vaccinated fish is still considered as GMO by part of the public. mRNA vaccines provide an answer to these limiting factors.

Because of its great versatility, mRNA has indeed become a key player in the prevention and treatment of diseases[9]. mRNA vaccines have been particularly highlighted in recent months for their key role in the Sars-Cov-2 health crisis even if their efficiency seems transient and require repeated boosting. Significant progress in improving delivery methods and stabilizing mRNA has made them a very powerful and reliable tool for vaccination. If mRNA-based therapies are now a priority area of research, it is also because they have many advantages over other approaches. Firstly, mRNA induces strong immune responses. It does not integrate into the genome and is not infectious, thus guaranteeing safer administration than the injection of whole viruses or DNA[10]. Furthermore, the activity of mRNA is limited by its natural degradation in the body. This transient nature gives it real therapeutic added value by minimizing any undesirable immune responses[9].

However, the development of mRNA vaccines faces certain obstacles and questions: the cost of production, the storage of vaccines at low temperatures to limit the degradation of mRNA, the dose necessary to ensure effective protection, and the mechanisms of action of mRNA (induction of response, duration of the immune response, etc.).

In this respect, the NucNanoFish project, funded by Icrad, proposes to establish a nucleic acid platform using biodegradable nanoparticles for an efficient delivery of nucleic acid vaccines, through intra-muscular or oral/immersion routes, against well -known viral diseases of several European farmed fish species. Our research strategy is based on cutting edge research, both on fish animal models and viral immunity, and also on basic science regarding mRNA design and mRNA vaccine vehicles. For instance, one ICRAD partner has been focusing its efforts on two major areas of optimization since 2017: on the one hand, the improvement of mRNA sequences in order to make it more stable and to increase its potency, and on the other hand, the development of an innovative biodegradable vector able to protect and deliver mRNA to the cytosol of antigen-presenting cells (APC). We believe that altogether, such improvements would enable to elicit a better immune response at lower mRNA doses, subsequently reducing the overall cost of production.

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Untransiated regions (UTRs) play a crucial role in mRNA stability and translational efficiency. Due to their extensive long-life, the use of α and β-globin genes from either human or Xenopus laevis has been described for a long time as the standard approach to improve mRNA stability when considering vaccines development[11]. However, the capabilities of UTRs are not universal and vary according to the species or cell type targeted. It is therefore required to adapt UTR sequences to the target in order to get an appropriate mRNA expression. Here, we aimed to design a master vector with the property of enhancing mRNA potency in fish species. Several 5'UTR and poly(A) regions were assessed using in vitro and in vivo models and led to the identification of a promising mRNA sequence template. On figure 1, enhanced green fluorescent protein (eGFP) reporter gene was flanked by two different synthetic 5'UTRs with high ribosome loading (namely UTR2 and UTR4) while Poly(A) was fixed to its optimal length, ie 148 units. The different mRNA constructions where intramuscularly injected in zebrafish, either naked or formulated. Results showed a higher eGFP expression with UTR4 than with UTR2, confirming that the optimization of mRNA sequence is a key factor to enhance mRNA potency in view of vaccine application[12]. The importance of an adequate carrier was also highlighted, as transfection efficiency was much greater when mRNA was encapsulated in Lipid Nanoparticles (LNPs) than with its free form.

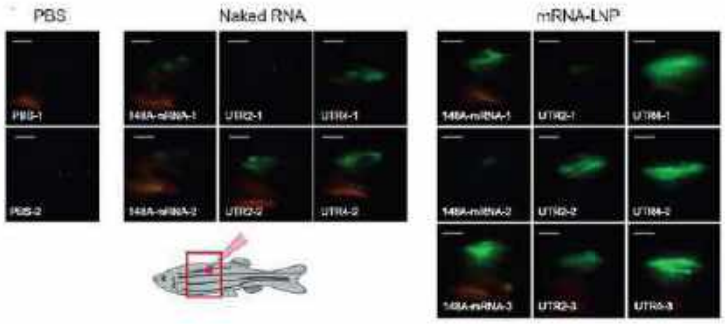

Figure 1. 8-month-old zebrafish (n=2 for negative control as well as naked mRNA, n=3 for mRNA-LNP) were intramuscularly injected with either 10 µL of naked mRNA (20 ng/mL) or 10 µL of mRNA-LNP complexes (200 ng of mRNA), respectively. eGFP expression was observed with a Leica fluorescent stereomicroscope on anesthetized animals at 48 h post-injection. The site of injection and the location where the picture was taken (red square) are presented in the drawing. Scale bar, 2 mm.

In parallel, the Nucnanofish consortium7 has been working on the development of a versatile nucleic acid delivery platform, which would reduce the payload of mRNA needed by improving first internalization and then endosomal escape in cells of interest, such as APC. While LNPs comprising four lipids (including an ionizable one) are to date the gold standard for mRNA vaccination platform and used as reference in our studies, we have designed an innovative hybrid vector. The latter, depicted in figure 2 and named LipoParticles (LP), is based on biodegradable poly(lactic acid) nanoparticles coated with lipid bilayers. The use of a rigid core, whose added value compared to liposomes has been evidenced in vitro previously13, allows not only the stabilization of final assemblies and a promising transfection efficiency in vitro but also the encapsulation of adjuvants such as Nod2 immunomodulators14 that can foster protective responses. The potential of this nanocarrier to trigger an immune response will be shortly evaluated in vivo in two species of farmed fish (rainbow trout and common carp), using two viral models for which preliminary data exist, the SVCV (Spring viremia of Carp Virus) and VHSV (Viral Hemorrhagic Septicemia Virus), before extending the approach to other fish viruses.

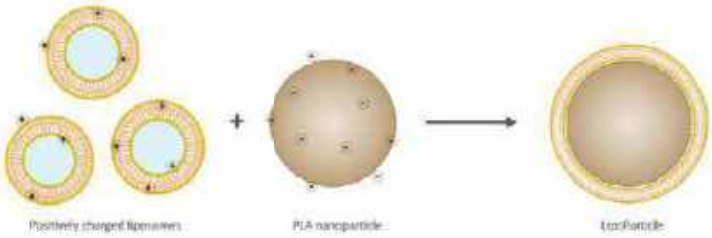

Figure 2. LipoParticles preparation from cationic liposomes and negatively charged PLA nanoparticles.

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Special focus on-

VETERINARY VACCINOLOGY

Biotechnology and Biological Sciences Research Council

RESEARCH AND INNOVATION GAPS WITHIN VETERINARY VACCINOLOGY

Key findings on Veterinary Vaccinology Gaps identified by the Global Veterinary Vaccinology Research and Innovation Landscape Survey Report.

In July 2021, UKRI-BBSRC (BBSRC), in consultation with other animal health funders, conducted a survey on the global veterinary vaccinology landscape to gain insight on the key gaps, priorities and barriers that need to be addressed in the future. This survey was launched to inform the refresh of the BBSRC Veterinary Vaccinology strategy[1].

122 responses were received from 42 countries. The majority of respondents worked in academia, major disciplines included Vaccinology, Immunology, Virology, Molecular Biology and Bacteriology.

Key research and innovation gaps identified from the survey include:

- **Understanding fundamental immunology** remains a priority including the nature of protective immunity and host-pathogen interactions and hence understanding of correlates and surrogates of protection. There is a need for advanced standardised technologies as an enabler to be able to detect and measure correlates/surrogates of protection.

- More research and innovation are required to **understand mucosal immunology and vaccinology** and the ability to deliver vaccine to these sites.

- Immunological methods and technologies act as enablers for studying immune responses in all veterinary species and thus vaccine development. Future research should consider **collaborative work using *in vitro* tools alongside *in silico* tools**. Overarching databases that coordinate knowledge of development, availability, distribution, and exploitation of lab reagents in collaboration with commercial partners will be key.

- The veterinary vaccinology community has stressed the need to use **appropriate model systems** for developing and testing vaccines. Suitable use and access to animal models to assess safety is required, specifically in field settings. One response quoted:

"There are no equivalents to B or T cell knockout mice for livestock animals. Often profound differences in the repertoire and function of immune system components exist, such that findings do not always translate across species."

- A key priority for the future is the need to **establish and characterise pipeline of 'plug and play' platforms of vaccine platform technologies.** These need to be efficacious, economically suitable, scalable, rapid to produce and regulated. Data analytics, bioinformatics, and genomic databases will play a key underpinning role in supporting technology platform development.

Key collaboration, capability and capacity gaps identified in the survey include:

- The nature of veterinary vaccinology research and innovation requires collaborative working. Coordinated response and knowledge exchange is needed between **academia, regulators, and industry** to understand the vaccine development landscape.
More support is needed in promoting a **One Health approach** by aligning veterinary and human vaccine research and innovation. One response quoted:

"More consideration should be given to public health benefits and how these can be valued."

- There is a need to support and maintain capacity in veterinary vaccine research across the entire vaccinology research and development career track.

- The Covid-19 pandemic has highlighted the priority of strengthening **emergency research capacity, manufacturing, and infrastructure** (inc. containment facilities) in veterinary clinical practice for emerging epidemic and epizootic diseases and that capacity is required for large scale vaccine field trials.

[1] https://bbsrc.ukri.org/documents/1506-veterinary-vaccinology-strategy/

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Key recommendations for funders:

- Funding basic research on understanding **immunology and host-pathogen interaction**
- Invest in basic research on **novel vaccine technology platforms**
- Multi-disciplinary groups:
  - Promote a **One Health** working with human vaccinology
  - Bridge knowledge gaps between regulatory bodies, academia, and industry so that innovative ideas for vaccine development can progress smoothly from bench to animal testing to large scale production.
- Provide training and development in an environment which develop vaccines to commercialisation
- Foster international collaboration

Looking forward:

Veterinary vaccinology research and innovation has made significant headway in the last five years through the success of being a global collaborative community. The survey has provided priorities for future research and innovation within the field spanning the veterinary vaccinology pipeline. The results of this survey will be used to help inform the refresh of the BBSRC Veterinary Vaccinology strategy and provide a framework for future research and innovation activities.

The Global Veterinary Vaccinology Landscape Survey Report will be published in 2022 alongside the Veterinary Vaccinology strategy 2022-2027.

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Special focus on-

ARE PLANT-BASED EXPRESSIONS SYSTEMS THE SOLUTION FOR WORM VACCINES?

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Ruud Wilbers – Laboratory of Nematology, Wageningen University

Gastro-intestinal worms are worldwide, both in humans and animals, among the most common pathogens. It is estimated that around 1.4 billion people are either infected or exposed to gastro-intestinal worms worldwide. In the veterinary field, basically every animal is infected at a certain time-point in life. Both in humans and animals, control of worm infections relies almost entirely on treatment with anthelmintics. However, with the increasing incidence of anthelmintic resistance, immunological control of worm infections through vaccination is often put forward as the most rational and cost-effective alternative (1,2).

Progress in this area, however, has been disappointing. At this moment, there are two commercial vaccines on the market that protect animals against nematode infections, i.e. *Huskvac* against the lungworm *Dictyocaulus viviparus* in cattle and *Barbervax* against the sheep abomasal nematode *Haemonchus contortus*. Importantly, both these vaccines are based on material directly derived from the parasites. The *Huskvac* vaccine is based on irradiated infective larvae whereas the *Barbervax*® vaccine is based on a crude antigen mixture extracted from the gut of adult *H. contortus* worms. This type of approach is unfortunately not applicable for most nematode species as it is practically impossible to obtain large quantities of worm material. For this reason, since the early 1990s, a large number of subunit vaccines have been evaluated against a range of gastro-intestinal nematodes. Unfortunately, very few of these vaccines induced sufficient levels of protection to consider further commercial development. This is particularly the case for recombinantly produced antigens.

The failure to induce protection is often explained by the inappropriate folding of the peptide backbone and/or the lack of glycosylation on these recombinant antigens. Many nematode antigens indeed carry N- and/or O-glycans on their peptide core and some of these can be highly immunogenic. Bacterial expression systems are not able to perform complex glycan modifications and the glycan decorations that occur in eukaryotic expression systems, like yeast and insect cells, often show little resemblance to the glycans naturally found on nematode proteins.

However, over the last two decades plants have emerged as a versatile alternative expression system for the production of recombinant (glycol)proteins. Plants offer several advantages

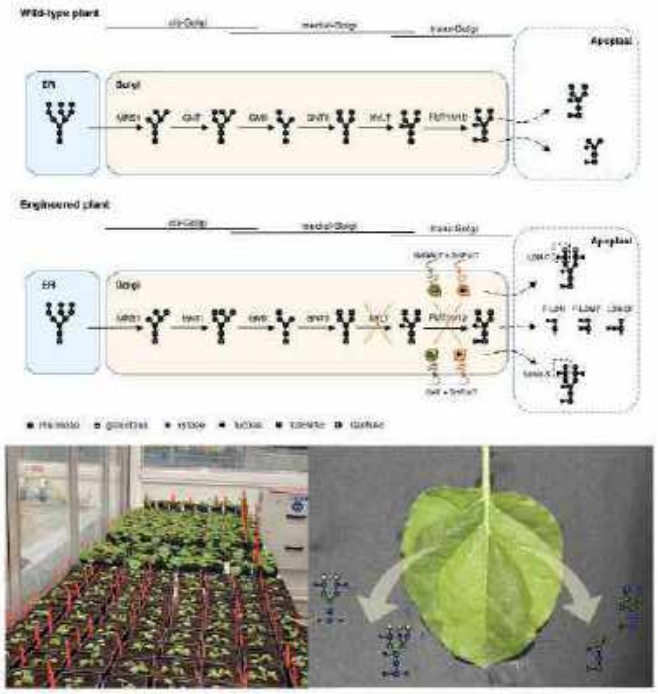

Figure 1 – Glyco-engineering of recombinant proteins in plants. Plants have emerged as a versatile production host of biopharmaceutical proteins that is quick, flexible, cost-effective and scalable. Many biopharmaceuticals are decorated with sugar structures (called glycans), which can vary in sugar composition between different organisms. Hence, producing a biopharmaceutical might require engineering of the production host in order to obtain the most effective drug. As illustrated above, the attachment of glycans to proteins (glycosylation) is initiated in the endoplasmatic reticulum (ER). At this stage the glycan is still immature and mainly consists of mannose residues. However, when a secreted proteins passes through the Golgi several modifications occur in a highly coordinated process. Plants generally add very simple glycan structures on their proteins, which offers an easy starting point for glycan engineering (glyco-engineering). With glyco-engineering we are able to remove plant specific enzymes that are responsible for undesired glycan modifications (indicated by red crosses) or introduce glycan modifying enzymes from different organisms (indicated with yellow and red cartoons) alongside your protein of interest.

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over more traditional expression systems in terms of speed, scalability and production costs. Furthermore, the risk of contamination with mammalian pathogens is low when using plant-based expression systems. Three major expression strategies are currently used for recombinant protein production: 1) transient expression in contained greenhouses, 2) stable transgenic plants either contained in greenhouses or open fields, and 3) plant cell suspension cultures. Especially the transient expression system (most common in the tobacco variety Nicotiana benthamiana) now also offers opportunities to fight emerging pandemics. The scalability and speed of this expression system allows for the production of millions of influenza or COVID-19 vaccine doses in a month (3, 4). Also, in the fight against helminth parasites plants are starting to play a role. For example, an antigen of the human hookworm Necator americanus was expressed at high levels in Nicotiana benthamiana plants and has entered phase I clinical trials (5). More recently, plants have been exploited for the production of recombinant helminth glycoproteins with a defined N-glycan composition (see figure 1). Recombinant glycoproteins from the human parasite Schistosoma mansoni were produced in N. benthamiana plants with a native glycan composition by adapting the post-translational machinery of the plants (6). The outcome of these studies show that it is now technically possible to synthesise helminth glycoproteins with a tailored glycan composition. This development will now fuel new developments in recombinant helminth antigen production with tailor-made N-glycans with, hopefully, improved vaccine efficacy.

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Special focus on-

mRNA VACCINES & ZOONOSIS

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Environmental factors play a key role in the zoonotic transmission of emerging pathogenic viruses amongst other infectious agents as mankind is constantly disturbing wildlife's ecosystems by cutting down forests to build human settlements or by catching wild animals for food, which deprives the viruses of their natural hosts and gives them opportunity to infect humans (da Silva et al, 2021)

Through coevolution of infectious agents such as viruses a relationship is developed between them and host species, restricted by genetic adaptation that leads to a species barrier. During the event of spill-over of such infectious agents in new host, severe disease may occur due to the lack of adaptation to the new host (Wrong et al 2009). Bats and birds are the main animal reservoirs of infections for viruses such as coronavirus from which they can be transmitted to other animals that live near with humans (Woo et al 2012). Genetic mutations and acquirement of new genes or modification of existing ones i.e., those encoding the spike protein (S) or Receptor Binding Domain (RBD), may occur leading to adaptations that enable them to cross species barriers (Qu et al., 2005; Wu et al., 2012). The bats are considered as the most prevalent factor for newly emerging viral infections due to several reasons. They are mammals and actually a very diverse order (1240 species corresponding to 20% of all the known mammalian species on the planet) (Han et al., 2020). They live up to twenty years, they live in large communities in very close proximity to one-another making the passing of infectious diseases withing their communities very easy. In addition, their immune system seems to enable virus persistence (Chan et al., 2013). They have in their peripheral blood higher number of T-cells than B-cells leading to the lack of adequate amounts of neutralizing antibodies, suggesting that the control of viral infections in the bats may occur via unknown mechanisms adapted by their immune system which needs to be elucidated (Baker et al., 2013; Banerjee et al., 2020).

The immune system is expected and has been proven to offer protection against widespread infections that may be lethal. Vaccine development is a key component in the prevention of widespread life-threatening diseases across species barrier. It is proven to be effective in controlling polio, yellow fever, measles, and human influenza viruses (Pardi et al., 2018). Traditional vaccines stimulate an antibody response by injecting into the body antigens in various forms, i.e. specific proteins, whole attenuated or inactivated infectious virus or bacteria, or a recombinant antigen-encoding vector (harmless carrier with an antigen transgene). These antigens are prepared and grown outside the body (Batty et al., 2020). Therefore, they may be subjected to unintended and even undetected modulations. Furthermore, traditional vaccines require the production of pathogens, which, if done at high volumes, could increase the risks of localized outbreaks of the pathogen at the production facility.

In the 1990s, there was enthusiasm for DNA-based gene therapy to prevent and treat human disease without the need to encounter all the above-mentioned difficulties. The messenger RNA (mRNA), a single-stranded RNA, that is complementary to one of the DNA strands of a gene was considered also attractive for quite a few reasons. It is made in the cell nucleus and then exported to the cytoplasm where it is taken up by immune system cells such as dendritic cells through phagocytosis and the encoded pathogen's antigens are synthesized by the dendritic internal machinery (ribosomes) (Pardi et al., 2018). The synthesized protein molecules stimulate an adaptive immune response to create antibodies that precisely target that particular pathogen. In the meanwhile, the fragments of mRNA are degraded by the body within a few days after introduction. Since the mRNA fragments are translated in the cytoplasm do not affect the body's genomic DNA which is in the nucleus of the cell (Park et al., 2020). However, when research on this aspect started in the late 80's serious problems were faced. The molecule of mRNA was found to be highly unstable and extremely hard to work with, almost impossible to be delivered intact into a foreign body and even if that was ever succeeded it elicited very strong inflammatory response. It took decades of hard work with dead ends, rejections, and dogged persistence before in vitro-transcribed (IVT) mRNA expressing therapeutic proteins was succeeded. (Dolgin 2021).

Overall, the advantages of mRNA vaccines over traditional vac-

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vaccines are many. They include the ease of design, the speed and lower cost of production, the induction of both cellular and humoral immunity, and the lack of interaction with the genomic DNA. The mRNA is translated in the cytosol, so there is no need for the RNA to enter the cell nucleus, and the risk of being integrated into the host genome is averted, since there is no need to carry such nucleus entering mechanisms(Verbeke et al., 2019). Because some viruses (i.e., Retrovirus) have mechanisms to be imported into the nucleus, where single-stranded RNA can use reverse transcriptase to make DNA from the RNA, there is misinformation implying that mRNA vaccines could alter DNA in the nucleus. mRNA in the cytosol is very rapidly degraded before it would have time to enter the cell nucleus. No cell culture, biohazard materials, and complex purification procedures are required. Another advantage is that mRNA vaccines do not induce vector immunity, thus not interfering with subsequent vaccinations.

The *in vitro* transcribed mRNA has the same structural components as natural mRNA in eukaryotic cells. It has a 5' cap, a 5'-untranslated region (UTR) and 3'-UTR, an open reading frame (ORF), which encodes the relevant antigen, and a 3'-poly(A) tail. By modifying these different components of the synthetic mRNA, the stability and translational ability of the mRNA can be enhanced, and in turn, the efficacy of the vaccine improved. The mRNA nucleotides can be modified to both decrease innate immune activation and increase the mRNA's half-life in the host cell (Kariko et al., 2008). For example, pseudouridine, 2'-O-methylated nucleosides can be incorporated to mRNA to suppress immune response stimulation to avoid immediate degradation and produce a more persistent effect through enhanced translation capacity (Figure 1).

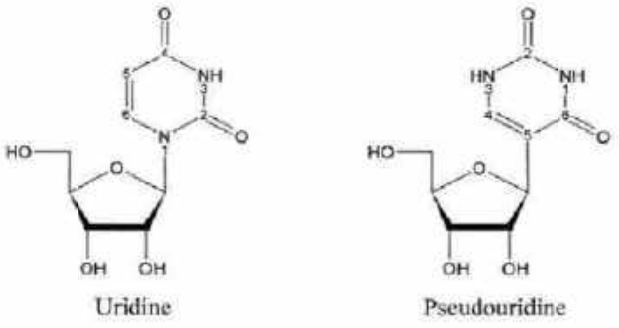

Figure 1. Chemical structure of uridine and pseudouridine with numbering of selected atoms in the pyrimidine rings. Figure 1 - uploaded by Finn Kirpekar https://www.researchgate.net/figure/A-Chemical-structure-of-uridine-and-pseudouridine-with-numbering-of-selected-atoms-in_fig5_11004070.

Replacing rare codons with synonymous codons frequently used by the host cell also enhances protein production. The open reading frame (ORF) and untranslated regions (UTR) of mRNA can be optimized for different purposes (a process called sequence engineering of mRNA), for example through enriching the guanine-cytosine content or choosing specific UTRs known to increase translation. An additional ORF coding for a replication mechanism can be added to amplify antigen translation and therefore immune response, decreasing the amount of starting material needed (Pardi et al, 2018; Jeeva et al., 2021). Because mRNA vaccines are not constructed from an active pathogen (or even an inactivated pathogen), they are non-infectious. Also since the antigens are produced inside the cell, they stimulate cellular immunity, as well as humoral immunity (Kramps & Elders 2017).

A serious problem that needed to be tackled was that for a vaccine to be successful sufficient mRNA must enter the host cell cytoplasm to stimulate production of the specific antigens. Naked mRNA is ineffective in entering the cells since it is unstable and easily destroyed by RNAases in skin and blood and its size and negative charge prevents it from crossing the cell membrane by simple diffusion due to electrostatic repulsion. Another major step in innovating RNA technologies was the encapsulation of its molecule in lipid nanoparticles (LNP) to facilitate their delivery into the cells (Buschmann et al., 2021). Liposome-encapsulated mRNA was shown in 1993 to stimulate T cells in mice. The following year self-amplifying mRNA was developed by including both a viral antigen and replicase encoding gene. The method was used in mice to elicit both a humoral and cellular immune response against a viral pathogen (Pascolo 2004). The next year mRNA encoding a tumor antigen was shown to elicit a similar immune response against cancer cells in mice (Kallen & Theß, 2014). The first clinical phase 1 studies using modified mRNA vaccines in LNP were against influenza virus H10 and H7 hemagglutinin (HA) during the years between 2015 and 2018, resulting in 100% seroconversion. The success of COVID-19 mRNA vaccines has proven that RNA technology, as a new platform, is safe and effective for commercial production (Figure 2).

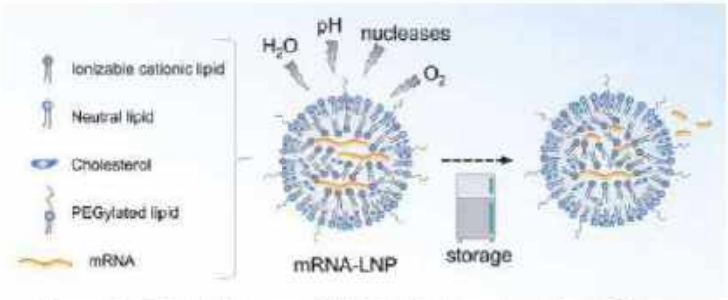

Figure 2. mRNA-lipid nanoparticle COVID-19 vaccines: From: Schoenmaker L, Witzigmann D., Kulkarni J.A., Verbeke R., Kersten G., Jiskoot W. and Crommelin D.J.A. (2021). Structure and stability. Int. J. Pharmaceutics 601: 120586 https://www.sciencedirect.com/science/article/pii/S0378517321003914

The short-term reaction of the body to the vaccine (called reactogenicity) is similar to that of conventional, non-RNA vaccines. However, adverse reaction to mRNA vaccines could be scored occasionally by individuals susceptible to an autoimmune response (Pardi et al., 2018). To minimize this, mRNA sequences in mRNA vaccines are designed to mimic those produced by host cells.

There have been rapid developments in safe and effective mRNA vaccines for zoonotic infections in the past year. mRNA vaccines are in development for several other potential pandemic zoo-

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notic infections, including Ebola virus, rabies virus, Zika virus, HIV-1, Chikunguya virus and influenza. Although the development of viral variants of influenza has occurred at a pace that has been too rapid for effective vaccine development, there may be hope for the control of pandemic avian influenza by the combination of improved and rapid viral genotyping and the rapid development and mass production of mRNA vaccines (Feldmann et al., 2019). The initial response to the epidemic of SARS-CoV-2 was too little and too late and resulted in the pandemic that we currently face (Bailey et al., 2018; Mascola & Fauci 2020). The hope lies in rapid and effective vaccination programs that include mRNA vaccine technology. Living with viruses of high pathogenicity will only be possible with effective vaccination programs. New outbreaks from zoonotic viral infections, such as coronaviruses and influenza viruses, require early detection and control to prevent the development of future pandemic and endemic diseases.

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Second Call

Second Call for Transnational Collaborative Research Projects: "One Health Approach to Zoonoses Research and Innovation"

Timeline

Submission of research project proposals	
1st October 2021	Launch of the co-funded call
First step: submission of pre-proposals	
15th December 2021, 15:00 CET	Deadline for pre-proposal submission
31st March 2022	Communication of eligibility check and evaluation outcomes to the research project coordinators
Second step: submission of full proposals	
15th April 2022	Full-proposal submission open
30th June 2022, 15:00 CEST	Deadline for full proposal submission
1st November 2022	Communication of the evaluation outcomes and the funding recommendation to the research project coordinators
National/Regional grant agreements	
1st quarter 2023	Start of research projects
Funded research project monitoring (estimated)	
4th quarter 2024	Mid-term report
3rd quarter 2026	Final report

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