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**To** European Commission; Directorate-General for Health and Food Safety Biotechnology unit (SANTE.E.3)

**Date** 13 of May, 2025

**Subject** Joint open letter from COGEM and ZKBS on the environmental risk assessment of new vaccine developments based on self-replicating mRNAs and viral replicon systems

Dear Ms. Sacristan Sanchez,

The Netherlands Commission on Genetic Modification (COGEM) and the German Central Committee on Biological Safety (ZKBS) would like to draw the attention of the European Commission (EC) to a recent decision on medicinal products based on viral replicons and self-amplifying mRNAs (samRNAs).

In January 2025, the EC granted authorisation for placing on the market of a samRNA vaccine against COVID-19 (Kostaive) following a positive opinion from the European Medicines Agency (EMA) in December last year.[a,b] The vaccine consists of an RNA construct derived from the vaccine strain TC83 of Venezuelan equine encephalitis virus (VEEV) and packaged in lipid nanoparticles. In the vaccine the VEEV structural genes have been replaced by the SARS-CoV-2 spike protein open reading frame. The viral RNA polymerase mediates the expression and amplification (replication) of the RNA construct itself inside the host cell. This and other products based on viral replicon particles are currently under development for numerous medical applications.

To the best of the committees' knowledge, an environmental risk assessment (ERA) under guideline EMEA/CHMP/BWP/473191/2006[c] was not conducted before the approval of Kostaive. COGEM and ZKBS are both of the opinion that an ERA of self-propagating molecules such as samRNAs and similar

a Arcturus Therapeutics, Inc. (2025). European Commission approves CSL and Arcturus Therapeutics' KOSTAIVE®, the first self-amplifying mRNA COVID-19 vaccine. https://ir.arcturusrx.com/news-releases/news-release-details/european-commission-approves-csl-and-arcturus-therapeutics (accessed 13 May 2025)

b European Medicines Agency (EMA). Kostaive. https://www.ema.europa.eu/en/medicines/human/EPAR/kostaive (accessed 13 May 2025)

c European Medicines Agency (EMA). Environmental risk assessments for medicinal products containing, or consisting of, genetically modified organisms (GMOs) – Scientific guideline. https://www.ema.europa.eu/en/environmental-risk-assessments-medicinal-products-containing-or-consisting-genetically-modified-organisms-gmos-scientific-guideline (accessed 13 May 2025)

medicinal products currently under development is imperative, as specific risks to third parties and the environment can arise from those medicinal products, for example from complementation or recombination events.

An additional consideration is that, depending on the RNA construct used, the use of these medicinal products may lead to the formation of virus-like vesicles (VLVs). Notably, VLVs are also being developed as vaccines. These VLVs can spread in the body, although it is currently unclear whether they can also be transmitted to third parties. These viral replicons are usually regulated as GMOs under directives 2009/41/EC and 2001/18/EC. For a detailed explanation of why an ERA is considered necessary for viral replicon constructs, including samRNAs packaged in lipid nanoparticles and similar drugs, please consult the appendix attached to this letter.

COGEM and ZKBS call for consultation and coordination between the EC and the EU Member States on the status of samRNAs and for transparency on the criteria underlying their regulatory status. The committees urge the EC to develop procedures to ensure that possible environmental risks are assessed and that such medicines are approved in a robust process that takes the points described in detail in the appendix into consideration.

To ensure an appropriate ERA and a robust authorisation process, it will be necessary to develop guidelines for an ERA of self-propagating molecules. These guidelines should include consideration of the likelihood of recombinant organisms being formed after application of those medicinal products. COGEM and ZKBS recommend that the competent authorities of directives 2001/18/EC and 2009/41/EC be involved, as they have a decade-long experience in risk assessment of GMOs and interpreting the definitions laid down in the GMO directives and would be aware of possible inconsistencies in enforcement. As advisory committees on biosafety, the COGEM and ZKBS also offer to contribute to this process. Both committees have considered viral replicon systems and issued several detailed opinions on the topic in recent years.[d,e]

This approach would ensure that novel vaccines and therapeutics currently under development would be approved safely and without risks to third parties and the environment. It would also prevent regulatory discrepancies in different fields of GMO regulation that could lead to legal uncertainties for applicants and companies. More importantly, organisms to be classified as GMOs would not be authorised without having undergone an appropriate ERA. In the committees' opinion, both points are essential to ensure the public's trust in and acceptance of medicinal products developed using modern biotechnology.

Yours sincerely,

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d ZKBS (2021). General position statement of the ZKBS on frequently carried out genetic engineering operations based on the criteria of comparability: Genetic engineering work with RNA viruses derived from minigenomes, replicons and virus-like particles for introduction into human or animal cells. https://zkbs-online.de/fileadmin/user_upload/Downloads/Stellungnahmen/Viren/EN/RNA_viruses_minigenomes_replicons_virus-like_particles_2021.pdf ref. 45310.0118

e COGEM (2022). Generic downscaling of containment requirements for work with viral replicons derived from alphaviruses and flaviviruses. https://cogem.net/en/publication/generic-downscaling-of-containment-requirements-for-work-with-viral-replicons-derived-from-alphaviruses-and-flaviviruses/ COGEM advise CGM/221223-01

Professor Thomas W. Vahlenkamp
Chair of ZKBS

Professor Sybe Schaap
Chair of COGEM

# Appendix

This appendix accompanies the joint open letter from the Netherlands Commission on Genetic Modification (COGEM) and the German Central Commission for Biological Safety (ZKBS) expressed in the open letter. In the letter they address their concerns regarding the approval of viral replicons and self-amplifying RNAs (samRNAs) as medicinal products without prior environmental risk assessment (ERA) of specific aspects resulting from the nature of these medicinal products.[1]

**1. Description of Kostaive**

In January 2025, the European Commission (EC) granted marketing authorisation for a vaccine against COVID-19 following a positive opinion from the European Medicines Agency (EMA) in December last year.[2,3] This samRNA vaccine, Kostaive, is derived from the vaccine strain TC-83 of Venezuelan equine encephalitis virus (VEEV), from which the structural genes have been deleted and replaced by the spike open reading frame from SARS-CoV-2.[4] The samRNA contained in lipid nanoparticles is applied to humans.

**2. Self-amplifying mRNA and viral replicon systems**

samRNAs are able to amplify (i.e. replicate) their own genetic material (RNA molecules) within a host cell. samRNA vaccines produce a viral replicase that synthesises new RNA copies from the original mRNA molecule. These vaccines also contain a gene of interest (transgene), namely the antigen needed to evoke an immune response. A key advantage of these vaccines over the original Moderna and Pfizer non-self-amplifying mRNA vaccines used in the COVID-19 pandemic, is that lower quantities of mRNA are required for vaccination to achieve the same immune response.[4,5,6]

The samRNA vaccines currently under development – including the above-mentioned Kostaive and its viral replicon particle (VRP) vaccine platform − are obtained by genetic modification of alphaviruses. The alphaviral genome is a single stranded RNA with positive orientation that can be directly translated into viral proteins, and is thus infectious for susceptible cells. The viral non-structural proteins mediate the replication of the viral genome which is then packaged into new viral particles.

samRNA vaccines are constructed by deleting the genes from the alphaviral genome that code for the proteins that make up the virus particle (the structural proteins). The gene of interest (transgene) is inserted at the position of the deleted genes. The remaining viral genome retains the genes that encode the proteins required for replication of viral RNA in the cell, along with all the viral regulatory sequences needed for RNA amplification and production of the protein(s) of interest.[a] Proteins encoded by samRNAs are therefore capable of synthesising new RNA molecules in host cells, but (in theory) no new viral particles are formed. For vaccine delivery, samRNAs are often formulated as lipid bilayers like lipid nanoparticles (LNP).[4,6,7] Alternatively, the samRNA molecule can be encapsidated in a particle consisting of viral proteins to form VRPs.[8] VRPs are produced by providing the missing structural genes *in trans* to package the viral genome inside the structural proteins. Following infection, the viral replicase is expressed, resulting in amplification and thus possible persistence of the viral genome in the cells. However, new viral particles created with either LNP or VRP systems cannot in principle be assembled and spread between cells due to the absence of the required viral

a. Regulatory sequences include the untranslated regions on either side of the RNA-molecule, the 5' cap structure and the 3' polyA tail as well as a subgenomic promoter for the expression of the protein(s) of interest. The regulatory sequences and the replicase sequence cover approximately 65% of the alphavirus genome.

structural genes. As a result, only a single-round infection can take place. Nevertheless, under certain conditions, which are specified below, the traits deleted from the samRNA may be compensated for by the presence of related viruses in vaccinated persons (via recombination or complementation). Furthermore, the choice of transgene used may influence the safety and nature of the samRNA.

*Restoration of virus particle formation by functions provided by viruses infecting vaccinated persons*

While samRNAs in general cannot produce virus particles, their deleted traits can potentially be restored by complementation or recombination, enabling them to spread. If, after vaccination with a samRNA, genes similar to the deleted sequences are present in the same cell, the deleted characteristics may be compensated for. This complementation scenario can occur if the vaccinated individual is simultaneously infected with a related virus. In such cases, the samRNA genomes could be packaged in viral structural proteins and form virus-like particles (VLPs), which may spread to other cells. In the newly infected cells, complementation would again be necessary for the formation of new VLPs.

The deleted traits in the samRNA genome can also be restored by recombination with related viruses, which could lead to the formation of a modified virus that can spread from cell to cell without the need for further complementation. The transgene would be removed by recombination.

*samRNAs may spread via infectious viral-like structures*

In addition to the complementation and recombination scenarios described above, which can result in the spread of samRNAs, certain transgenes can also (partially) compensate for the deleted functions of the samRNA genome. For alphavirus-derived samRNAs, it has been shown that infectious virus-like structures (virus-like vesicles, VLVs) can be formed if structural genes from other viruses are inserted. These infectious structures incorporate the samRNA molecule and enable the infection of other cells and the dissemination of the samRNA within the body of the vaccinated individuals. In some cases, VLV formation is deliberate (i.e. when VLVs are the active vaccine component).[9,10,11,12,13,14,15] However, the formation of VLVs can also occur unintentionally, raising concerns about unintended consequences of using these samRNAs. It has been reported that infectious VLVs have been formed when using samRNAs containing envelope proteins from several medically important virus families (*Rhabdoviridae,*[9,11,12,13,16,17] *Retroviridae,*[18] *Filoviridae*[14] and *Coronaviridae*[19]). As viruses from the family *Togaviridae* are highly similar to alphaviruses, VLV formation is very likely if open reading frames that code for envelope proteins from this virusfamily are present in the samRNA construct. Capsid-independent VLV formation has been shown for Semliki forest virus and Sindbis virus systems.[20,21]

It is currently unknown whether individuals can shed these infectious viral structures and so unintended exposure of third parties may occur. The potential formation of infectious VLVs introduces additional complexities as these structures may unintentionally facilitate the spread of the samRNA genome.

*Effect of samRNAs can be enhanced by additional integration of immune-inhibiting proteins*

Several transgenes can be inserted into a samRNA using a second subgenomic promoter, or by employing regulatory sequences like internal ribosome entry sites (IRES) or 2A cleavage signals. Certain transgene combinations within one samRNA molecule can enhance the spreading ability of the VLVs. The infectivity of VLVs may also evolve further through the acquisition of mutations in the

genome, for example by sequential passaging of the replicon. In a recent study, the insertion of a second Ebola structural gene into a samRNA Ebola vaccine (next to the gene encoding the Ebola virus glycoprotein) significantly increased the formation of infectious VLVs. Further improvement of the infectivity of the VLVs was achieved by multiple infections of the vaccine in cell culture.[14]

It has been observed that the administration of increased doses of RNA is correlated with an increase in injection site reactions and systemic adverse events in human patients.[22] This is attributed to the innate immune response to RNA and reduces the immunogenicity of RNA vaccines. To overcome this, genetic information coding for viral proteins inhibiting the intracellular vaccine degradation has been integrated into samRNA constructs. It has been shown that such constructs exhibit enhanced protein expression and immunogenicity *in vivo* and downregulate the immune system by repressing NF-κB and IRF3 activation.[23] The enhanced lifetime and expression of samRNAs could also enhance the risk and rate of VLV formation and spreading as well as the likelihood of complementation and recombination.

**3. Regulatory considerations on samRNAs and VRPs described in section 2**

In Directive 2001/18/EC, the legal definition of a GMO is 'an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination', while an organism is described as 'any biological entity capable of replication or of transferring genetic material.'[1]

Many viral vectors currently used in gene therapy or as vaccines are GM viruses that have been altered in such a way that they do not replicate and no longer form progeny virus particles or spread between cells (replication-deficiency). Commonly used viral vectors include replication-deficient adeno-associated virus, adenovirus, and gammaretrovirus or lentivirus. While they do not meet the definition of a GMO with respect to propagation, they are clearly capable of transferring genetic material. Consequently, these replication-deficient vectors fall within the scope of the GMO legislation and an environmental risk assessment must be performed during the marketing authorisation procedure according to the common practice of EMA and EC.

VLVs and VRPs can be regarded as genetically modified ('stripped down') viruses. They are biological entities, because the samRNA is enclosed in proteins of biological origin. samRNAs can be regarded as genetic material since they are continuously propagated. There are differing views on whether samRNAs should be considered to be GMOs or not.

VRPs are able to transfer genetic material to cells and thus fulfil the second criterion of the definition of an 'organism', while VLVs fulfil both requirements for 'organisms' as they are capable of propagation as well as transferring genetic material. Clearly, the genetic material of both VRPs and VLVs has been altered in a way not occurring naturally by mating or natural recombination.

Taken together, both VLVs and VRPs meet the criteria for 'organism' and 'genetically modified organism' as laid down in the European GMO directives. Decisions that samRNAs systems leading to VLVs and VRPs are not considered GMOs would contradict the current regulatory practice for 'classical' replication-deficient viral vector systems.

In both the Netherlands and Germany, VRPs and VLVs have thus far been classified under the GMO legislation. The decision of the EC may lead to undesirable discrepancies in the interpretation of what constitutes a GMO in drug authorisation itself (recombinant viral vectors v. samRNAs/VLVs) and between different fields of law enforcement (medicinal product authorisation v. regulation of GMOs in contained use) and different Member States.

**4. Important points to consider in the ERA of samRNA and VRP systems**

To the best of COGEM's and ZKBS' knowledge, placing on the European market of Kostaive was authorised without consulting GMO competent authorities. COGEM and ZKBS note that regardless of whether samRNAs encapsulated in LNPs are classified as GMOs or not, the formation of recombinant organisms after administration of the LNPs and certain risks to humans and the environment need to be evaluated. VRPs need to be classified as GMOs as discussed in section 3.

The objective of Directive 2001/18/EC is to protect human health and the environment when GMOs are deliberately released into the environment.[1] A decision to consider encapsulated samRNAs and similar medicinal products as non-GMOs in general would mean that the environmental risks of these vaccines would not be properly assessed. This seems to be at odds with the objective of the GMO legislation as these vaccines may not be safe in all conditions, despite their rational design (see section 2). Clear guidelines on samRNAs and similar products are therefore necessary to avoid regulatory inconsistencies and legal uncertainties for applicants and companies in different fields. Such guidelines should include recommendations on the circumstances in which samRNA and VRP systems might lead to the formation of VLVs and on the questions which need to be addressed in an ERA on these products. COGEM and ZKBS recommend that such guidelines address at least the following questions:

- *What is the likelihood of recombination with related wild-type viruses in vaccinated individuals, the general population and the environment?*
  After recombination, replication-competent viruses could be formed and released.
- *What is the likelihood of complementation of deleted viral functions by wild-type viruses in vaccinated individuals, the general population, and the environment?*
  After complementation by related wild-type viruses, replication-deficient recombinant viral particles could be formed and released.
- *What types of transgenes could potentially lead to the formation of VLVs?*
  Guidelines on this topic should include at least the envelope proteins of the virus families mentioned in section 2. The list of sequences may be even longer. The possibility of immune-inhibiting proteins influencing the lifetime of samRNAs and the likelihood of VLV formation also need to be assessed.
- *Questions to be answered based on the results of prior preclinical or clinical studies:*
  - *If the transgene to be expressed has the potential to form VLVs, has it been examined whether or not VLVs are formed?*
  - *If VLVs are formed, are they able to spread from cell to cell and possibly to other organs, and can they be shed from vaccinated individuals?*
  - *Does the spread to other cells and organs have negative consequences for the individual or third parties?*
    To the current knowledge of COGEM and ZKBS, no information is available on shedding and spreading of infectious VLVs from humans or animals. Potential

adverse consequences for humans and the environment of samRNAs are still unclear.

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