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Regulatory aspects of quality and safety for live recombinant viral vaccines against infectious diseases in Japan ☆

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ABSTRACT

Recombinant viral vaccines expressing antigens of pathogenic microbes (e.g., HIV, Ebola virus, and malaria) have been designed to overcome the insufficient immune responses induced by the conventional vaccines. Our knowledge of and clinical experience with the new recombinant viral vaccines are insufficient, and a clear regulatory pathway is needed for the further development and evaluation of recombinant viral vaccines. In 2018, the research group supported by the Ministry of Health, Labour and Welfare, Japan (MHLW) published a concept paper to address the development of recombinant viral vaccines against infectious diseases. Herein we summarize the concept paper—which explains the Japanese regulatory concerns about recombinant viral vaccines—and provide a focus of discussion about the development of recombinant viral vaccines.

1. Introduction

The recent progress in genetic recombination techniques and biotechnology has accelerated the development of new types of prophylactic vaccines for infectious diseases for which conventional vaccines are not available or are insufficiently effective [1]. The new biotechnology and recombination techniques such as the reverse genetics method enable the rapid construction of recombinant viruses and provide an efficient manufacturing system. Vaccines that deliver the gene(s) coding different species antigen (s), such as viral vectors for gene therapy, are also being developed.

These vaccines are produced as a recombinant virus and are intended to prevent infection with a virus or pathogen through the expression of target antigen(s) as the active ingredient (hereinafter referred to as "recombinant viral vaccines"). When such a vaccine is administered, the recombinant virus carrying the gene (s)' coding antigen(s)—rather than injected proteins, virus-like particles (VLPs) or whole virions—may serve as an antigen as in the case of conventional vaccines. This strategy is expected to induce a stronger immune response than the response induced by conventional vaccines because it elicits immunity via a mechanism of action similar to that of viral infection, and thus provides a continuous stimulation of both humoral and cellular immune reactions. Recombinant viral vaccines targeting pathogens that constitute a great public health threat against which no approved effective vaccines are available (e.g., Ebola virus and HIV) are now being developed [2]. Several countries have approved recombinant viral vaccines targeting viruses such as Japanese encephalitis virus and dengue virus [3,4].

☆ The views expressed in this article are those of the authors and do not necessarily reflect the official views of Pharmaceuticals and Medical Devices Agency.

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Table 1
Comparison among recombinant viral vaccines and conventional vaccines.

Vaccine Type	Replication-Competent Viral vaccines	Replication-Incompetent Viral vaccines	Conventional vaccine (live)	Conventional vaccine (inactivated)
Replication	Yes	Partially yes	Yes	No
Immunogenicity				
Antigen	Viral protein expressed by infection	Viral protein expressed by infection	Whole viral particles and, viral protein expressed by infection	Inactivated viral protein
Expected immune response	Humoral and cellular	Humoral and cellular	Humoral and cellular	Humoral
Pathogenesis				
Attenuation/Inactivation	Genetic attenuation	Genetic attenuation	Natural attenuation	Inactivated
Potential risk of revertant	Yes	No	Yes	No
Potential risk of recombination with wild type viruses	High	Moderate	High	No
Potential risk of unexpected infection				
Vertical transmission	High	Moderate	High	No
Horizontal transmission (Shedding and spread to third parties)	High	Moderate	High	No
Others				
Impact to nature	High	Low	Low	No
Experience in clinical use	Poor	Poor	Abundant	Abundant

Although recombinant viral vaccines are expected to have higher efficacy than conventional vaccines, the safety and efficacy of recombinant viral vaccines in medical use are not yet established [5,6]. For example, compared to conventional vaccines the recombinant viral vaccines may have a considerably different safety profile in individuals such as newborns, pregnant women, and immunocompromised patients. Replication-competent recombinant viral vaccines in particular pose a risk for not only immunocompromised individuals but also third parties through the viral shedding from the vaccinated individuals, and additional safety risks due to viral shedding should thus be carefully evaluated [5–7]. In this context, when the development of recombinant viral vaccines is being considered, there is a need for quality, nonclinical, and clinical evaluations and safety measures conducted from a standpoint that differs from that used for conventional vaccines (Table 1).

In 2009, the European Medicines Agency (EMA) issued a "Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines" (hereafter referred to as the '2009 EMA guideline') [8] which emphasizes safety issues such as the characterization of recombinant viruses, the occurrence of virulent revertant or recombined virus with wild-type viruses, clinical follow-up in healthy patients, and the possibility of integration into a chromosome or germline.

In Japan, there has been neither authorized guidelines nor points-to-consider to address recombinant viral vaccines like the EMA guideline. In 2018, the research group for quality and safety issued on recombinant viral vaccines (RVVRG) organized under the auspices of the Ministry of Health, Labour and Welfare, Japan (MHLW) published a concept paper about recombinant viral vaccines [9]. This review focuses on RVVRG's concept paper and discusses the current regulatory requirements in Japan for recombinant viral vaccines.

2. Objectives and scope

The concept paper has been edited aimed to complement existing guidelines notified by MHLW, i.e., the Guideline for nonclinical studies of preventive vaccines for infectious diseases [10] and the Guideline for clinical studies of preventive vaccines for infectious diseases [11]. And besides, the concept paper aimed to provide considerations about quality, non-clinical, and clinical evaluations that are specific to the development of recombinant viral vaccines.

The scope of the concept paper covers live recombinant viral vaccines intended for infectious diseases that are generated using genetic recombination. The scope does not apply to inactivated recombinant viral vaccines, recombinant protein vaccines, or synthetic vaccines such as DNA vaccines or mRNA vaccines. The concept paper does not cover a recombinant virus with a gene constitution equivalent to that seen in natural viruses, although these vaccines are manufactured using the genetic recombination technique. Nevertheless, some of the principles described herein could be applied to such vaccines.

3. Current status of recombinant viral vaccines

Recombinant viral vaccines can be categorized as mainly two types based on their replication ability: replication-incompetent viral vaccines that express the antigen of interest in infected cells (such as gene therapy vectors), and replication-competent viral vaccines that express the antigen (such as chimeric live viral vaccines) (Fig. 1). There are major safety concerns regarding the replication competency of recombinant viruses and the pathogenicity of host viruses, because proliferated recombinant viruses in vaccinee might cause adverse effects. Some recombinant viral vaccines that are under development replicate under specific artificial or limited conditions, and these vaccines are not characterized into the above-mentioned two types of vaccines.

Since recombinant viral vaccines have many variations that depend on their characteristics and replication abilities, the vaccines should be evaluated on a case-by-case basis in order to evaluate their CMC (chemistry, manufacturing and control) and safety.

3.1. Replication-incompetent viral vaccines

Replication-incompetent viral vaccines are constructed from viral vectors which lack essential gene(s) related to viral replication. In general, transformed cell lines expressing the essential gene(s) are used to produce replication-incompetent viral vaccine. The replication-incompetent viral vaccines can deliver a specific recombinant gene to cells *in vivo* and express foreign proteins such as the antigen of pathogens, but the vaccines are unable to replicate themselves in the infected cells. The vaccines can transfect cells and express the target antigen protein *in vivo*, and they may therefore be expected to induce not only humoral immunity but also cellular immunity.

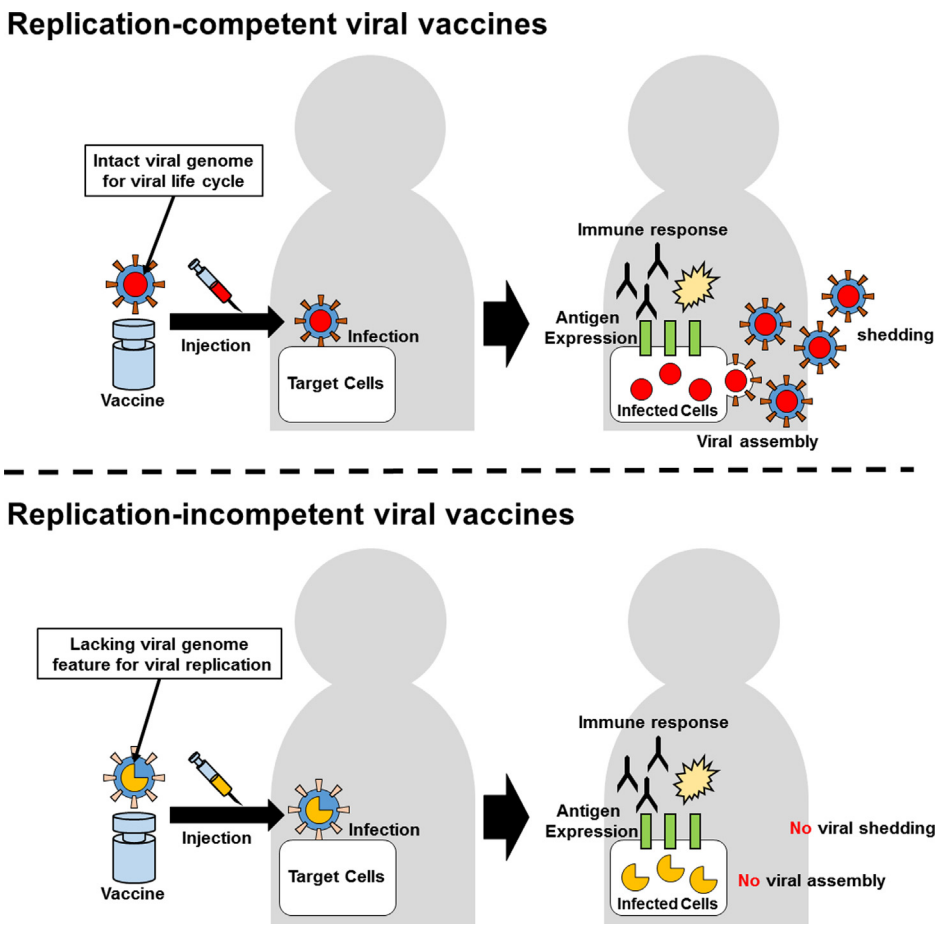

Fig. 1. Comparison between replication-competent and -incompetent recombinant viral vaccines.

There are no approved replication-incompetent viral vaccines available in Japan or other countries at present. Replication-defective adenovirus vectors are one of the candidates for replication-incompetent viral vaccines [12]. Replication-incompetent viral vaccines based on adenoviral vectors have demonstrated immunogenicity and protective immunity in animal models.

3.2. Replication-competent viral vaccines

The design and construction of replication-competent viral vaccines are conducted mainly by two types of methods: the replacement of genes by a reverse genetics system, and the insertion of additional genes to a viral whole genome. A reverse genetics system is used to synthesize molecular clones of live viruses by using cloned cDNA of the viral genome. Vaccines based on a flavivirus backbone are one of the most well-developed chimeric vaccines manufactured by a reverse genetics system [13].

The vaccine Imojev® is constructed from the genes that encode the prM and E proteins of Japanese encephalitis virus and other genes of the live attenuated yellow fever virus (YFV) vaccine [14]. Imojev® is licensed in Australia, Thailand and other Asian countries, but not in Japan, the U.S. or the EU. Dengvaxia®, which is constructed by replacing the genes encoding the prM and E proteins of the attenuated live YFV vaccine with those of four types of dengue viruses, expresses four virus antigens [15]. Dengvaxia® is licensed in the EU and US for adults, young people and children (from 9 to 45 years of age) who had a prior dengue virus infection and who live in endemic areas. Vaccines based on a vesicular stomatitis virus (VSV) backbone are also in advanced clinical trials [16]. rVSV-ZEBOV is a recombinant VSV vaccine containing the gene of a glycoprotein of Zaire strain Ebola virus instead of the gene of G protein of VSV. rVSV-ZEBOV is the only vaccine whose efficacy in preventing Ebola virus disease has been evaluated in clinical trials [17,18], and it is currently used in the Republic of the Congo as a compassionate-use program for outbreaks of Ebola virus (Table 2).

Since poxviruses have large genomes that potentially accept many foreign genes, the vaccinia virus, which belongs to the poxvirus family, is frequently used as the major viral vector for the expression of foreign genes [19]. There are several candidates for vaccinia-based and poxvirus-based chimeric vaccines, but no licensed poxvirus-based chimeric vaccines exist [20].

There are some licensed replication-competent viral vaccines that are thought to present a potential risk to immunocompromised vaccinee and third parties. The use of these vaccines is limited to regions under the threat of highly pathogenic viruses.

4. Quality aspects of recombinant viral vaccines and their characterization

RVVRG's concept paper requires that quality evaluations of each recombinant viral vaccine include the following three points; (1) the nature of the original virus used to generate the recombinant virus, (2) the characterization of the recombinant virus, and (3) the manufacturing process. The results of these evaluations are expected to reveal potential risks associated with recombinant viral vaccines, and these risks should be considered in the design of non-clinical/clinical studies. There are no major differences

between RVVRG's concept paper and the EMA guideline regarding the quality evaluation requirements.

4.1. Original viruses used to generate recombinant viruses

The characteristics of the original viruses used for the construction of recombinant viruses provide important information for the evaluation of the recombinant viruses' characteristics as mentioned in Section 4.2. The characterization of recombinant viruses is improved by knowledge of the characteristics of the original virus.

In principle, viruses that have a potential to integrate gene(s) into the recipients' chromosome should not be used as the original virus, because recombinant viral vaccines are generally used for the prevention of infection in healthy individuals.

4.2. Characterization of recombinant viruses

The following characterization of recombinant viruses should be performed. Additional studies may be required depending on the characteristics of the recombinant virus.

- Gene sequence analysis.
- Determination of species-specificity, cellular/tissue tropism, replication characteristics, and cytotoxicity.
- Determination of the level, efficiency, and persistence of antigen expression in infected cells.
- Investigation of the risk of recombination/reassortment with wild-type viruses [6].
- Evaluation of the risk of integration into chromosomes.
- Evaluation of process-related impurities specific to recombinant viruses; i.e., unexpected recombinant virus(es), residual plasmids, or helper virus(es).

4.3. Evaluation during the manufacturing process

Drug products should be evaluated for its infectivity titer per virion (or the amount of protein or the viral genome copy), which is a useful parameter for evaluations of the consistency of the production of the recombinant virus. The genetic stability of the recombinant virus (mutation, reversion to virulence, and alteration of replication-competence) during the manufacturing process should also be assessed. The genetic heterogeneity of the recombinant virus in the drug product should be determined, as it serves as a measure for ensuring the consistency of the manufacturing process.

5. Non-clinical studies of recombinant viral vaccines

Non-clinical evaluations of recombinant viral vaccines should follow the Guideline for non-clinical studies of preventive vaccines for infectious diseases notified by MHLW [10]. A biodistribution study and a shedding study should be performed, and their results should be taken into account to the recombinant viral vaccine's specific characteristics. The EMA guideline also requires these additional studies.

5.1. Selection of animal species/models

Animal models capable of mimicking human infectious diseases should be used to prove the potency of the recombinant viral vaccine to prevent symptomatic disease prior to tests involving humans. If no relevant animal models are available, animal models in which the recombinant virus expresses its antigens can be selected.

Toxicity studies are expected to be performed in compliance with Good Laboratory Practice (GLP). However, since recombinant viruses should be handled in a P2-level containment laboratory, some studies employing specialized test systems may not be able to comply fully with GLP. Areas of non-compliance should be identified and their significance should be evaluated relative to the overall toxicity assessment.

5.2. Biodistribution study

To obtain basic data about the characteristics of a recombinant virus and its safety and efficacy, the biodistribution of the virus should be determined before a phase I trial is initiated, in principle. A biodistribution analysis can establish the distribution not only in the expected tissues but also in non-expected tissues and germ cells. This leads to the identification of tissues and cells that should be focused on in the safety assessment and as part of the unintended integration risk in humans, and biodistribution results may thus be useful when considering the toxicological significance of tissue-specific abnormal findings detected in toxicity studies. If a concern arises in a biodistribution study, additional non-clinical studies should therefore be considered. In addition, since an investigation of persistence (including the distribution and elimination) of a recombinant virus yields information that is useful in the determination of the appropriate duration of clinical studies in humans, the obtained biodistribution information should be reflected in the clinical study design.

5.2.1. Evaluations of the risk of germline integration

If a biodistribution study reveals that a recombinant virus is present in gonadal tissues, evaluations should then be conducted referencing the "General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors" issued by ICH [21].

5.3. Evaluations of the shedding of a recombinant virus

In principle, the shedding of a recombinant virus should be evaluated. Shedding of a recombinant virus can be evaluated in a biodistribution study or other toxicity studies, referring to "ICH Considerations: General Principles to Address Virus and Vector Shedding" [22] for an evaluation approach.

5.4. Genotoxicity and carcinogenicity studies

Although genotoxicity and carcinogenicity studies are generally not required for vaccines, if there is a concern about the characteristics of the original virus or the recombinant virus, an evaluation of the concerns should be performed via genotoxicity and carcinogenicity studies using a feasible and appropriate approach.

5.5. Evaluations of immunogenicity

An immunogenicity study should assess not only the immune response specific to the antigen(s) but also the response specific to other viral proteins contained in the recombinant virus, because pre-existing immunity against the viral vectors may hamper a vaccine's clinical use [12,20].

If a recombinant viral vaccine is based on an approved live attenuated vaccine, the two vaccines' mutual interference in immunogenicity should be evaluated by conducting non-clinical studies in which the recombinant viral vaccine is administered after the administration of the approved live vaccine, or vice versa.

5.6. Vaccination studies in immunocompromised animals

Although a replication-competent recombinant viral vaccine may not have any pathogenicity in healthy individuals, it could

induce serious events in individuals such as newborns, pregnant women, and immunocompromised patients. To test this possibility, the necessity of conducting a vaccination study in immunocompromised animals should be considered.

6. Viral shedding study and transmission to third parties

Viral shedding and the potential transmission of the recombinant viruses to third parties should be assessed in at least a phase I clinical trial. RVVRG's concept paper addresses more details of the evaluation of viral shedding and the potential transmission, referring to the 2009 EMA guideline.

6.1. Principles of the evaluation of shedding and transmission to third parties

Recombinant viral vaccines, which not only retain the ability to express the transgene(s) and present antigens in the human body, but also the ability to be shed from the vaccine recipient, may be transmitted to persons in close contact with the recipient and induce undesirable events in these individuals. Replication-competent recombinant viral vaccines in particular present a higher risk by transmission to particular populations such as newborns, pregnant women, and immunocompromised patients. These vaccines should therefore be carefully evaluated for viral shedding in clinical studies.

In a phase I trial, the amount of the recombinant virus at the injection site and in blood, and body fluids where viral shedding is expected should be measured over time using evaluable samples to accurately determine the persistence of the recombinant virus in the human body and the duration of viral shedding. Information on the persistence of a recombinant virus in the human body and its shedding duration will contribute to the design of rational measures to avoid transmission from recipients to third parties that can be applied to subsequent clinical studies. If persistent shedding of a replication-competent recombinant viral vaccine is observed, continuous testing will be required to rule out infection due to close contact with recipients.

6.2. Principles of the duration of contraception

The selection of the appropriate duration of male contraception in clinical studies should take into account the results of a biodistribution study and a shedding study of the recombinant virus. The duration of contraception for women of childbearing potential should be set taking into account the non-clinical study data in addition to data on the persistence of the recombinant virus in human blood and the duration of shedding in body fluids.

6.3. Principles of safety evaluations

The safety of recombinant viral vaccines should be determined with a focus on the following risk factors, which should be carefully investigated in early clinical studies. If necessary, measures to mitigate the risk should be conducted.

- The potential for an unexpected replication of the recombinant virus in the recipient's body (for replication-incompetent recombinant viral vaccines).
- The risk of accidental recombination with other pathogenic viruses in the recipient's body, and the possibility of adverse events caused by recombination variants.
- The relationship between tissue distribution and tissue/organ-specific adverse events (when the recombinant virus is found to localize in certain tissues/organs in a biodistribution study).

7. Efficacy of recombinant viral vaccines

An efficacy evaluation of a recombinant viral vaccine should follow the Guideline for clinical studies of preventive vaccines for infectious diseases notified by MHLW [11].

7.1. Principles of efficacy evaluations

The adequate efficacy of a recombinant viral vaccine is tested in one or more clinical studies that evaluate the disease-protective effect or anti-infective effect as the efficacy endpoint. Efficacy evaluations also serve to identify the potential risks of the adverse events associated with recombinant viral vaccines not only in recipients but also in third parties.

7.2. Principles of immunogenicity evaluations

Humoral and cellular immune responses elicited by recombinant viral vaccines should be investigated at the early stage of clinical development whenever possible. The non-clinical study data and previously available information about the relevant original non-recombinant virus will be helpful in deciding the extent of such investigations.

If the recombinant viral vaccine is based on an approved live attenuated vaccine, the two vaccines' mutual interference in immunogenicity should be evaluated by clinical studies that take into consideration the vaccination schedule used for the approved live vaccine in clinical use.

8. Conclusion

The research into and development of recombinant viral vaccines have been conducted in many laboratories as basic research, but there is as yet no accumulation of clinical experiences with these vaccines. The safety and the efficacy of recombinant viral vaccines are thus poorly understood. A small number of recombinant viral vaccines are licensed only in the limited areas under the threat of high pathogenic viruses (Table.2) [14,15,17]. However, recombinant viral vaccines are expected to induce a stronger immune response than that induced by conventional vaccines, and several recombinant replication-competent and -incompetent viral vaccines have been confirmed to confer protection against target-infectious diseases: rVSV-ZEBOV, Dengvaxia®, and canarypox-based HIV-1 [15,17,20].

Nevertheless, there are several concerns about these new vaccines (Table 3), including their pathogenicity to immunocompromised individuals, potential spread to third parties, recombination with wild-type viruses, and reversion of virulence. RVVRG's concept paper is similar to the 2009 EMA guideline, but the concept paper focuses on the safety of not only vaccine recipients but also those of the people in close contact with the recipients. The reason for this focus is that an unexpected infection with a recombinant virus in persons in close contact with a vaccine recipient can occur because vaccines are usually administered to numerous healthy individuals, as has been observed with the use of live attenuated polio vaccines [23].

In addition, attenuated vaccinia virus, which is one of the major original viruses of recombinant viral vaccines, was reported to present a risk of serious or fatal side effects such as 'progressive vaccinia' [24] and myopericarditis [25] in immunocompromised individuals. A recombinant virus should therefore not be shed from vaccinated individuals; alternatively, recombinant viruses that are shed should be under control. The current knowledge about these vaccines indicates that recombinant viral vaccines could provide insight into the prevention of diseases caused by viruses that can-

Table 2
Licensed recombinant viral vaccines.

Vaccine name	Target pathogen	Replication	Construction of recombinant virus	Approved countries
Dengvaxia®	Dengue virus	Competent	YFV based virus including prM and E genes of dengue virus	EU (restricted areas) US (restricted areas) Philippine
Imojev®	Japanese encephalitis virus	Competent	YFV based virus including prM and E genes of Japanese encephalitis virus	Australia Thailand Malaysia Philippines
rVSV-ZEBOV	Ebola virus	Competent	VSV based virus including G gene of Ebola virus	Congo (as compassionate-use program)

Table 3
Potential benefits and risk of recombinant viral vaccines.

	Replication-competent viral vaccines	Replication-Incompetent viral vaccines
Potential benefits	Immune response stronger and wider vs. conventional inactivated vaccines Suitable for some pathogens against which conventional inactivated and live vaccines are difficalt to produce	Immune response stronger and wider vs. conventional inactivated vaccines Suitable for some pathogens against which inactivated and live vaccines are difficult to produce Lower risk of pathogenicity and reversion to virulence than conventional live vaccines
Potential risks	Poor experience in clinical use Unexpected biodistribution and antigen expression in human bodies Recombination with wild-type viruses Spread to third parties Pathogenicity to immunocompromised individuals Reversion to virulence Effects on animals and nature	Poor experience in clinical use Unexpected biodistribution and antigen expression in human bodies Recombination with wild-type viruses Spread to third parties (partial) Pathogenicity to immunocompromised individuals

not be prevented by conventional vaccines. The risk-benefit balance of each new recombinant viral vaccine must also be determined.

Basic and clinical research groups, vaccine developers, academics, and regulatory bodies should continue discussions of the benefits and risks of recombinant viral vaccines, and the development and regulation of recombinant viral vaccines should be based on a foundation of the latest scientific knowledge.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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