Advisory Committee on Releases to the Environment

# Advice on an application for deliberate release of a GMO for research and development purposes

**Applicant:** Sanofi Pasteur Inc.,

**Application:** A Phase III clinical trial of a GM Respiratory Syncytial Virus vaccine in infants and toddlers.

**Ref:** 24/R56/01

**Date:** March 2024

## Advice of the Advisory Committee on Releases to the Environment under section 124 of the Environmental Protection Act 1990 to the Secretary of State for Environment, Food and Rural Affairs and Ministers of the Welsh Assembly Government.

ACRE is satisfied that the information provided by the applicant in accordance with the current regulations on the Deliberate Release of GMOs, demonstrates that the 'release' of this GMO under the conditions of the trial has been fully assessed in relation to the risks of adverse effect on human health or the environment. ACRE therefore sees no reason for the release not to proceed.

## Background

In February 2024 ACRE considered an application from Sanofi Pasteur Inc., for a phase III clinical trial involving the release of a genetically modified respiratory syncytial virus (RSV) vaccine in accordance with the Genetically Modified Organisms (Deliberate Release) Regulations 2002 (as amended). Members assessed the environmental risks (including risks to humans who have not been administered this GM vaccine) associated with the release of this GMO under the conditions of the trial set out in the application.

No public representations were received on this trial.

Wild-type RSV is a fragile, lipid-enveloped virus sensitive to desiccation with an intracytoplasmic replication cycle. It does not replicate outside the host and its infectious potential rapidly decreases in the external environment. There is no vector for RSV transmission, instead it spreads when an infected person coughs or sneezes, thus releasing contaminated droplets into the air.

RSV is only pathogenic to humans and chimpanzees, and in healthy adults is often asymptomatic or limited to the upper respiratory tract. However, RSV is

the leading viral cause of lower respiratory tract infections, such as bronchiolitis and pneumonia, in infants and young children, causing 33 million cases and 118 000 deaths (both approximate figures) worldwide in the under 5 age group in 2015.
This is the first application for deliberate release within the UK of this GMO. The GMO strain RSV ΔNS2/Δ1313/I1314L has undergone six human trials (five Phase I and one Phase II) as an RSV vaccine candidate with no safety concerns identified in any of these trials to date.

**The GMO**
To construct RSV ΔNS2/Δ1313/I1314L standard cloning techniques and a reverse genetics system were employed. The initial modifications included the deletion of a 112-nucleotide region from the downstream, non-coding region of the short hydrophobic (SH) protein gene, along with five translationally silent nucleotide changes to the last few codons of that gene. This was carried out purely to improve the stability of the cDNA when grown in *Escherichia coli*.
Then attenuating alterations were performed on the RSV genome involving introduction of the following three mutations – the deletion of the RSV NS2 gene (non-structural protein), the deletion of codon 1313 of the RNA-directed RNA polymerase or large protein (L) gene and the replacement of isoleucine with leucine at codon 1314L of this L gene. The latter was designed to increase the genetic stability of the recombinant vaccine.
ACRE was content with the extent that the applicant had described the methodology involved in the genetic modifications carried out on the wild-type RSV. To detail this, the RSV non-structural protein, NS2, suppresses the production of interferon α/β and also suppresses the cell's ability to establish an antiviral state. Deletion of the NS2 gene was designed to attenuate RSV and also potentially to provide increased immunogenicity, furthermore its deletion may have increased vaccine tolerability. The other attenuating mutations (e.g. Δ1313/I1314L) in this vaccine were designed for increased stability of temperature sensitive phenotypes.
ACRE noted that the applicant confirmed that no vector DNA remained in the final construction, or in the master seed lot, by way of sequencing the complete genome of RSV ΔNS2/Δ1313/I1314L. Furthermore, The stability of the alterations made to the RSV genome were also comprehensively analysed including sequencing after up to eight serial passages *in vitro*.

*Phenotypic characterisation*
ACRE was content with the applicant's description of the resultant phenotype of the GMO, as far as was required to adequately risk assess the clinical trial with regards to adverse effects on human health and the environment. The NS2 protein, as outlined above, is an IFN antagonist and virulence factor, and the deletion of it from the GM virus is proposed to result in a reduction in replication

of cells with intact interferon signalling. This was tested and confirmed *in vitro* using a human lung fibroblast line, with the vaccine strain revealing strong attenuation in this line.
The two mutations (Δ1313/I1314L) yielded a temperature sensitive attenuation, with a shutoff temperature of 37°C, as confirmed by plaque-forming assay where a >log 10 reduction was seen between 36 and 38°C. (see application part A1, Section 31).
ACRE was content that the above mutations meant that RSV ΔNS2/Δ1313/I1314L therefore was a live, attenuated, temperature sensitive vaccine. The expressed viral proteins, principally those encoded by the fusion protein (F) and glycoprotein (G) genes contain antigenic determinants for neutralization. Thus, these proteins act as immunogens and their expression via the RSV ΔNS2/Δ1313/I1314L virus induces a protective immune response against wildtype RSV.
Clinical studies conducted to date, have resulted in 114 infant and toddler subjects receiving at least one dose of RSV ΔNS2/Δ1313/I1314L across a number of both Phase I and II clinical trials, with 104 being within the age indication of 6 to 24 months of age. ACRE noted that by the age of 24 months almost all children will have been exposed to RSV, with subsequent reinfection throughout life.
The RSV ΔNS2/Δ1313/I1314L was detectable as replicating virus in 90% of vaccinees in clinical trials to date, but this was not associated with any increase in lower respiratory tract infection beyond that seen in placebo-treated subjects. However, ACRE noted the observation that, in seronegative subjects virus shedding was detectable albeit at low levels compared to wild type virus. The committee was therefore content with the applicant's conclusion that, based on clinical trial data, the vaccine strain was no more invasive than the wild type virus and in addition, to possess no selective advantage in the environment. This was evidenced in those clinical trials conducted to date, where detectable shedding of virus in nasal secretions was low and transient, generally disappearing within 12 days following vaccination. The applicant notes that superinfection with different strains of RSV is possible. However, the attenuation and temperature sensitivity of ΔNS2/Δ1313/I1314L, would mean that a competitive advantage in relation to the parental organism is not expected.
The application refers to an initial non-clinical study assessment that was carried out to investigate the local tolerance, any systemic toxicity and viscerotropism arising from intranasal administration of the vaccine strain.
ACRE noted that both the above non-clinical, and subsequent clinical studies, served as sufficient confirmation of the lack of any toxic or allergenic effects of ΔNS2/Δ1313/I1314L beyond the expected immunogenic properties of a vaccine against RSV.
ACRE was also aware that, as noted in the risk assessment, that the intramuscular delivery of an inactivated RSV vaccine was the cause of RSV

disease enhancement following subsequent natural RSV infection and resulted in the two child fatalities. Furthermore, the committee accepted that this disease enhancement does not occur following intranasal immunisation with similarly attenuated live RSV vaccines.

**The Clinical Trial**

The GMO, RSV ΔNS2/Δ1313/I1314L is being studied in a large multinational, multi-centre phase III clinical trial in infants and toddlers, as a follow-up to previous Phase I and II studies that involved a total of 192 children (114 of whom received the GMO) aged 6 to 59 months. The purpose of the release is in human clinical trials to investigate the safety, infectivity, immunogenicity and efficacy of RSV ΔNS2/Δ1313/I1314L in infants and toddlers regardless of initial RSV serostatus.

It is anticipated that enrollment into the planned Phase III efficacy clinical study will begin in the UK and EU in May 2024 and will be completed by November 2025. The applicant aims to recruit subjects at five sites within England and will run concurrently with trials in both Spain and Finland.

The subjects in the study will receive a single dose of either RSV ΔNS2/Δ1313/I1314L or placebo. All treatments will be by intranasal administration, which will be controlled to ensure the given dose of GMO will be no more than 7.2 log10 plaque forming units (PFU), (15 848 932 PFU).

ACRE was cognisant of observations that in clinical practice RSV is increasingly recognised as a pathogen in older, immunocompromised patients, including for example those receiving steroid treatment for an asthmatic condition. Therefore, ACRE requested that the applicant provide information on the measures that subjects pot-vaccination were expected to adhere to in terms of reducing or addressing this risk aspect of the clinical trial. This could therefore be one of a risk between such close-contact individuals and the trial participants whilst the latter are shedding, but there may also be other scenarios where immunocompromised individuals are, or could be, exposed to the vaccine virus.

To address the above risk of exposure of the most vulnerable to this attenuated RSV from shed vaccine virus the applicant has included several exclusion criteria for recruitment of subjects in all clinical protocols. These include subjects who are a member of a household that contains an immunocompromised individual, including, but not limited to: a person who is HIV infected; a person who has received chemotherapy within the 12 months prior to study enrollment; a person who has received (within the past 6 months) or is receiving (at the time of enrollment) immunosuppressant agents, and a person living with a solid organ or bone marrow transplant. The applicant also indicated that subjects would be excluded where they had potential close contact with other immunocompromised individual within 30 days after each

vaccination.
ACRE noted the risk of transmission of shed vaccine virus was however considered minimal and data gathered to date in a study designed to demonstrate transmission of the vaccine virus had not revealed any transmission. However, as part of the clinical development the applicant will continue to monitor transmission to healthy individuals so as to assess this potential risk. ACRE therefore was content that such measures were to be put in place, whilst simultaneously gathering more data on this potential risk.

**Comment**

Following a detailed consideration of the application, along with seeking clarification of the exclusion criteria, ACRE was content that the environmental risk assessment provided by the applicant was thorough and included sufficient consideration of the risks to human health and the environment as well as a good description of appropriate measures employed in order to minimise these risks.
Given the nature of the vaccine candidate, its level of attenuation, species (human and chimpanzee) specificity, evidence from previous clinical trials and, very limited propensity for shed and spread, ACRE assessed it unlikely that there will be any significant impact upon the environment. The preliminary risk assessment for this study suggests there is an extremely low risk for potential environmental impact associated with administering RSV ΔNS2/Δ1313/I1314L to study subjects. Furthermore, ACRE was content with the applicant's conclusion that overall, for this study there is a very low potential risk for the study subjects and impact associated with administering RSV ΔNS2/Δ1313/I1314L.

ACRE further concluded that given the evidence and arguments in the application it seemed that any environmental risk would be to immunocompromised contacts of the subjects, and that measures to reduce this risk were in place and appropriate.
ACRE concluded that the trial as proposed was well designed and noted that it included a description of follow up protocols for monitoring peak shedding in vaccinates. ACRE also recognised that the trial was designed to address a real issue, namely the spread of RSV among naive infant and toddler populations, and to this end should provide data of significant benefit in public health planning while presenting a negligible risk to other people, animals or the environment. ACRE therefore sees no reason for the release not to proceed.

April 2024