Department for Environment, Food and Rural Affairs

Application for consent to release a GMO – organisms other than higher plants

Part A1: Information required under schedule 2 of the Genetically Modified organisms (Deliberate Release) Regulations 2002

Part I: General information

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

Sanofi Pasteur Inc.
Discovery Drive,
Swiftwater, PA 18370-0187
USA

The qualifications and experience of the scientists are available in a confidential annex 2: 'CVs of the scientists.'
The Principal Investigators at each clinical site are listed in the confidential annex 3: 'list of clinical sites.'

2. The title of the project.

This GMO technical dossier has been developed in the context of the preparation of Phase III, randomized, observer-blind, placebo-controlled, multi-center, multinational study to evaluate the efficacy, immunogenicity, and safety of a Respiratory Syncytial Virus (RSV) vaccine in infants and toddlers.

Part II: Information relating to the organisms
Characteristics of the donor, parental and recipient organisms

3. Scientific name and taxonomy.

The parental strain used to generate live attenuated respiratory syncytial virus is A2.

Name

(i) order and/or higher taxon (for animals) Mononegavirales
(ii) genus - Orthopneumovirus
(iii) species - Respiratory Syncytial Virus (RSV)
(iv) subspecies - Not applicable

(v) strain - A2
(vi) pathovar (biotype, ecotype, race, etc.) - Not applicable
(vii) common name - RSV A2

4. Usual strain, cultivar or other name.

Not applicable

5. Phenotypic and genetic markers.

RSV virus particles are enveloped and pleomorphic, occurring as irregular spherical particles that are 100 to 350 nm in diameter, and as long filamentous fibers that are 60 to 200 nm in diameter and 10 µm in length (1). The virion consists of nine structural proteins and two non- structural proteins. Three proteins are associated with the nucleocapsid and include nucleoprotein (N), phosphoprotein (P), and polymerase or large protein (L). The other five viral proteins are contained within the virus envelope and include non-glycosylated matrix protein (M), M2 (M2-1 and M22), fusion protein (F), glycoprotein (G), and short hydrophobic protein (SH). Two nonstructural proteins are NS1 and NS2. The viral genome consists of a linear, singlestranded, negative-sense, non-segmented RNA (~15.2 kb) and its name is derived from the large syncytium that form when infected cells fuse together.

The vaccine parental strain RSV A2 was first isolated in 1961 from the lower respiratory tract of an infant in Melbourne, Australia (2). Since its initial isolation, RSV A2 has been established as a prototypic RSV strain and has been used extensively as a reverse genetics platform for the development of the majority of liveattenuated vaccine candidates to date (3), but no vaccine is currently available. In addition, the strain is a key system for the study of RSV structure and has played a key role in elucidating immune responses in animal models to RSV infection (4).

6. The degree of relatedness between the donor and recipient or between parental organisms.

Not applicable

7. The description of identification and detection techniques.

RSV can be detected either by the presence of viral nucleic acids, for example genomic detection using quantitative or standard reverse transcriptase polymerase chain reaction (RT-PCR), or by presence of replication competent virus by viral culture, for example by plaque assay. Genome detection is more specific and can distinguish wild-type (wt) A2 from the RSV ΔNS2 vaccine construct, although RTPCR does not distinguish infectious from non-infectious particles.

A genome detection method that allows detection of RSV and differentiation of RSV ΔNS2Δ1313/I1314L from the parental A2 is described in section 30. Viral culture methods for RSV detection and quantification are described in section 30.

8. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

The sensitivity and specificity of the mentioned detection techniques above are described in section 31. In brief, the assay sensitivity at the lower limit of quantitation (LLOQ) is 3.71 log10 genome copies/mL. Dilutional accuracy analysis near the LLOQ showed an absolute difference of at most 0.43 log10 copies/mL between observed and expected values. The intra assay precision standard deviation is 0.2625 and the intermediate precision is 0.2959, log10 genome copies/mL. The assay is thus sensitive, reliable and specific.

9. The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey, parasites and competitors, symbionts and hosts.

RSV was originally recovered and identified as a novel virus in 1956 after an outbreak of common cold infections from a colony of chimpanzees (5). Subsequently, isolated and characterized a virus from adult family members of children who presented with pneumonia and bronchiolitis, which was serologically identical to the virus that affected the respiratory system of captive chimpanzees (6). RSV occurs worldwide and is the most common cause of bronchiolitis and pneumonia among infants and young children. Recently RSV has also been identified as a major pathogen to the elderly.

RSV can spread when an infected person coughs or sneezes, releasing contaminated droplets into the air. Transmission usually occurs when these droplets come into contact with (or inoculate) another person's eyes, nose, or mouth (7).

10. The organisms with which transfer of genetic material is known to occur under natural conditions.

In many organisms, transfer of genetic material could occur by homologous recombination under natural conditions and therefore influence biological evolution at many different levels of the organisms. In most negative sense RNA viruses including RSV, although sporadic authentic examples indicate that homologous recombination can occur, natural recombination seems to be generally rare or even absent (8). To investigate the recombination events of RSV, a co-infection in vitro study by two RSV mutants was conducted.
It turned out that an RSV variant was identified as a recombined RSV in only one of six coinfections (9). The isolation of only one single recombinant RSV under optimized experimental conditions suggests that recombination is rare indeed in

RSV. As a consequence, it is clear that natural recombination is not a concern for vaccine stability and safety.

11. Verification of the genetic stability of the organisms and factors affecting that stability.

Genetic stability could be affected by two mechanisms: homologous recombination and accumulation of mutational changes, which are influenced by selection pressure. The second mechanism that could affect the genetic stability is the error-prone nature of replication of RNA virus genomes. Mutation rates vary between RNA viruses, ranging between 10^{-6} and 10^{-4} per nucleotide site per cell infection, depending on the RNA virus and methods used (10). The point mutations are very rare in RSV A2 strain.

12. The following pathological, ecological and physiological traits:

a. the classification of hazard according to existing Community rules concerning the protection of human health and the environment;

RSV is classified in risk group 2, according to the Directive 2000/54/EC of the European Parliament and of the Council (18 September 2000, Directive on the protection of workers from risks related to exposure to biological agents at work (11)).

b. the generation time in natural ecosystems, and the sexual and asexual reproductive cycle;

RSV has an intracytoplasmic replication cycle and cannot replicate outside a host. RSV infection appears to be limited, as it infects only apical cells in the airway epithelium. People infected with RSV are usually contagious for 3 to 8 days. However, some infants, and people with weakened immune systems, can continue to shed the virus even after they stop showing symptoms, for as long as 4 weeks.

c. information on survivability, including seasonability and the ability to form survival structures, including seeds, spores and sclerotia;

RSV is an enveloped virus and is thus very fragile. RSV from infected individual can survive on fomites (including paper tissues, beds, table tops and toys) for up to 6 h (12). In addition, RSV can survive on contaminated skin (e.g. hands) for up to 25 min (12) (13).
RSV seasonality varies around the world. In Europe, RSV infections exhibit seasonality with an average season starting in the beginning of December, peaking in early February and continuing until early April with wide variation between countries (see Figure 1 and Figure 2). Earlier peaks are observed in southern latitudes while northern latitude experience a longer season (14).
Figure 1: Non-sentinel (n = 14) countries RSV detections by country, season and week of detection, EU/EEA, 2010–2016 (14)

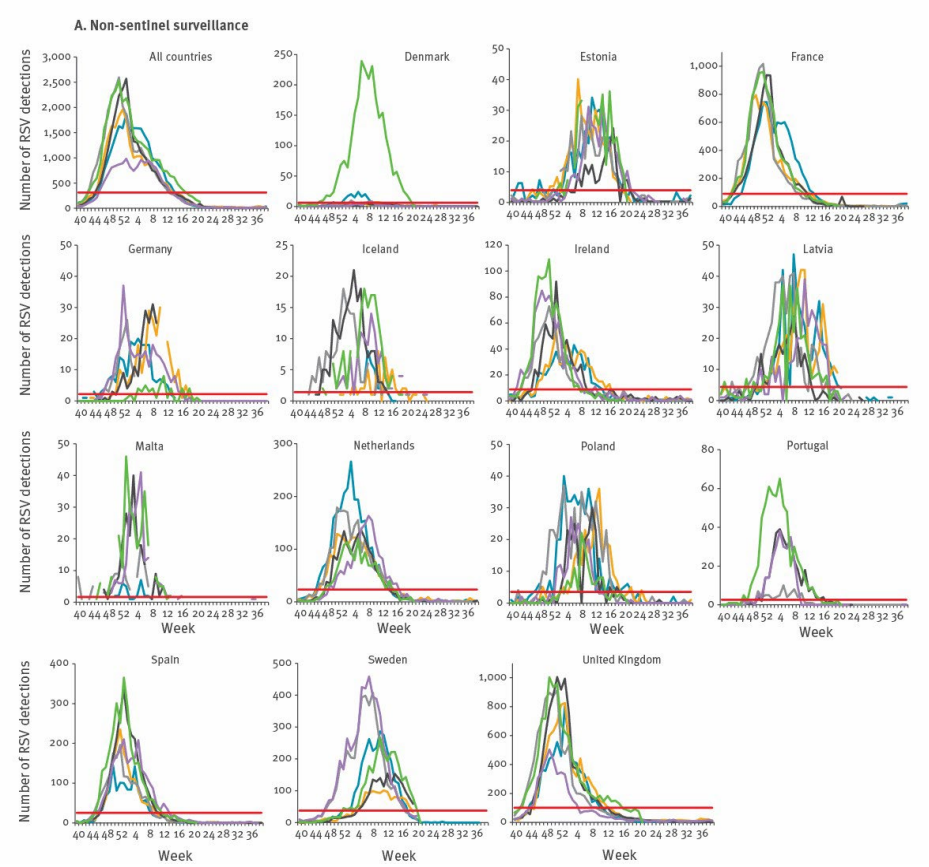
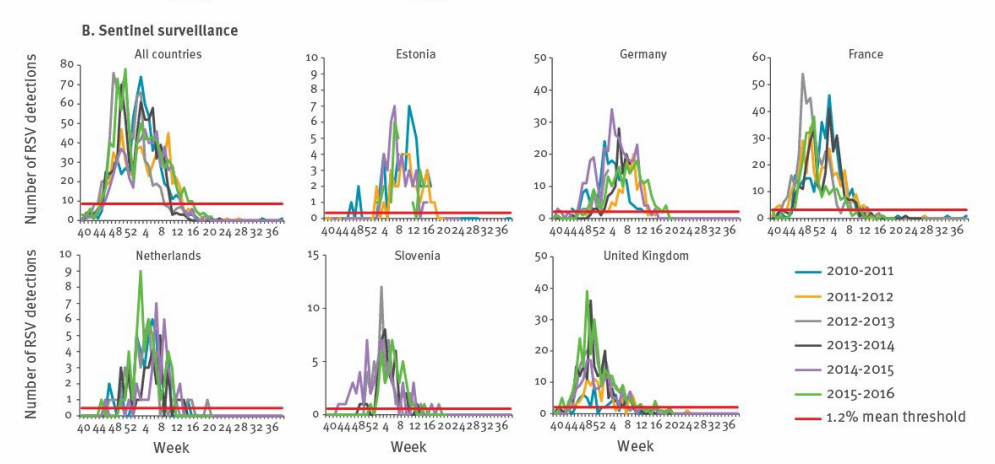


Figure 2: Sentinel (n = 6) countries RSV detections by country, season and week of detection, EU/EEA, 2010–2016 (14)



d. pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms;

RSV only causes disease in humans and Chimpanzees.

In healthy adults RSV infection is often asymptomatic or is limited to the upper respiratory tract (URT) with symptoms similar to the common cold. RSV is the leading viral cause of lower respiratory tract (LRT) infection in infants and young children (15) having caused approximately 33 million cases of low respiratory illness (LRI) and approximately 118000 deaths worldwide in children <5 years of age in 2015 (16).

In Chimpanzees, it is presented as a common cold (5).

There is no carrier vector, infection can only spread by coughs or sneezes releasing contaminated droplets into the air.

e. antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;

Not applicable: RSV virus does not contain any antibiotic resistance genes.

f. involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration.

Not applicable

13. The sequence, frequency of mobilisation and specificity of indigenous vectors and the presence in those vectors of genes which confer resistance to environmental stresses.

Sequence
Not applicable

Frequency of mobilization
Not applicable

Specificity
Not applicable

Presence of genes which confer resistance
Not applicable

14. The history of previous genetic modifications.

The A2 genome backbone was modified by deleting a 112-nucleotide region from the downstream noncoding region of the SH gene and silently modifying the last few codons of the SH open reading frame, to improve the stability of the cDNA during growth in Escherichia coli (17). The mutations were performed by standard cloning techniques.

Characteristics of the vector

The plasmid encoding the ΔNS2/Δ1313/I1314L antigenome was constructed from the parental RSV A2 backbone by standard cloning techniques and the virus generated using a reverse-genetics system (3). The live virus does not contain sequences from the plasmid vector.

15. The nature and source of the vector.

pRSV ΔNS2/Δ1313/I1314L full length cDNA plasmid (18) was generated as described in section 20.a.

To generate live virus from pRSV ΔNS2/Δ1313/I1314L full length cDNA by reverse genetics, 4 supporting plasmids encoding RSV N, P, L and M2-1 proteins and a plasmid encoding T7 polymerase are utilized (see section 19).

16. The sequence of transposons, vectors and other non-coding genetic segments used to construct the genetically modified organisms and to make the introduced vector and insert function in those organisms.

There are no non-native genetic segments in the live attenuated ΔNS2/Δ1313/I1314L.
None of the 5 supplementary plasmids or their products are present in the purified virus.

17. The frequency of mobilisation, genetic transfer capabilities and/or methods of determination of the inserted vector.

There are no insertions in pRSV ΔNS2/Δ1313/I1314L as compared to the parental plasmid D46/6120. All modifications, consisting of two deletions and single amino acid mutation, were checked by DNA sequencing.

18. The degree to which the vector is limited to the DNA required to perform the intended function.

Infectious RNA transcripts produced during rescue of RSV ΔNS2/Δ1313/I1314L by reverse genetics consist of only RSV viral sequences and do not possess any sequence from the molecular vectors used during the construction steps (pRSV ΔNS2/Δ1313/I1314L or supplementary plasmids).

Characteristics of the modified organisms
19. The methods used for the modification.

The RSV sequence on which the RSV ΔNS2/Δ1313/I1314L Seed Virus Drug

Substance is based was derived from wild type RSV strain A2, initially isolated from a child in Melbourne, Australia, in 1961 (2).

The recombinant vaccine strain was generated by a reverse genetics approach at the Laboratory of Infectious Diseases (National Institutes of Health (NIH), Bethesda, MD, USA). The vaccine strain was attenuated by the introduction of three mutations – the deletion of the RSV NS2 gene (nonstructural protein), the deletion of codon 1313 of the L gene (RNA-directed RNA polymerase) and the replacement of isoleucine with leucine at codon 1314L of the L gene. The vaccine strain was derived by electroporating Vero cells with antigenome plasmid ΔNS2/Δ1313/I1314L, and four support plasmids pCITE-N, pCITE-P, pCITE-M2.1, pCITE-L and pT7. The expressed RSV antigenome and support proteins i.e. N, P, M2-1 and L assemble into nucleocapsids that launch a productive infection.

The candidate vaccine virus generated above was used to manufacture clinical trial material at Charles River Laboratories (Malvern, PA, USA). The clinical study is currently underway in the US (IND015465). Sanofi Pasteur has been provided access to this viral modified strain through a collaboration with the NIH (Collaborative Research and Development Agreement [CRADA] agreement June 2016).

The virus vaccine strain from the NIH was re-derived at Sanofi Pasteur (SP) using reverse genetics. This was done to overcome two limitations of the NIH vaccine virus – the use of serum during strain generation, and the lack of plaque purification.

The virus was re-derived using Sanofi Pasteur's serum-free Vero cells and to perform three rounds of viral plaque purification to mitigate the risks associated with Fetal Bovine Serum and to ensure the traceability of the cell substrate and the homogeneity of the virus seed. RSV ΔNS2/Δ1313/I1314L was rescued based on the protocol by NIH involving electroporation of Sanofi Pasteur serum free Vero cells with 6 rescue plasmids followed by three rounds of viral plaque purification. Purified plaques underwent serial amplifications P1, P2, and P3 to generate RSV ΔNS2/Δ1313/I1314L Pre-Master Seed (Pre-MSL).

20. The methods used:

a. to construct inserts and introduce them into the recipient organism;

The pRSV ΔNS2/Δ1313/I1314L full length cDNA plasmid was generated at the NIH (Laboratory of Infectious Diseases, Bethesda, Maryland) by site directed mutagenesis and DNA cloning steps. Plasmid D46/6120 was used as template for site directed mutagenesis (17).

The candidate vaccine full length genome encoded in plasmid D46/6120 differs from the full length wt parent A2 virus by the deletion of 112 nucleotides from the downstream non-translated region of the SH gene, and also contains the introduction of five translationally silent nucleotide changes into the downstream end of the SH open reading frame (17).

This deletion and these silent (at the amino acid level) changes were made to stabilize the cDNA during propagation in bacteria. Importantly, they did not detectably affect the viral phenotype in cell culture or in mice. As the first step to generate pRSV ΔNS2/Δ1313/I1314L, codon 1313 of the L gene was deleted by site directed mutagenesis of plasmid D46/6120, resulting in D46/6120 Δ1313.

To delete the NS2 gene, a 2652 bp EagI/AvrII restriction fragment (containing 1044 bp of the cloning vector and leader, NS1, and part of the N gene) was obtained from the previously described cDNA encoding RSV ΔNS2 (19) and used to replace the EagI/AvrII fragment containing leader, NS1, NS2 and part of the N gene in D46/6120 Δ1313, resulting in D46/6120 ΔNS2 Δ1313. Finally, the I1314L (ATA to CTG) mutation was introduced into D46/6120 ΔNS2 Δ1313 by site directed mutagenesis to increase the genetic stability of this vaccine, resulting in the pRSV ΔNS2/Δ1313/I1314L full length cDNA plasmid.

21. The description of any insert and/or vector construction.

Please refer to section 20.a for a description of the construction of pRSV ΔNS2/Δ1313/I1314L full length cDNA plasmid and of the insert obtained from the cDNA encoding RSV ΔNS2.

22. The purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function.

To verify the purity from any unknown sequence, the full-length genome sequence of RSV ΔNS2/Δ1313/I1314L was obtained by automated sequencing and does not contain any part whose product or function is not known (see section 25).

23. The methods and criteria used for selection

The virus vaccine strain from the NIH was re-derived at Sanofi Pasteur using reverse genetics. The virus was re-derived using Sanofi Pasteur's serum-free Vero cells. The rescued virus was plaque purified 3 times, then underwent serial amplifications to generate RSV ΔNS2/Δ1313/I1314L Pre-Master Seed (Pre-MSL) (see section 19).

The genomic nucleotide sequence of Sanofi Pasteur's Pre-Master Seed Lot (Pre-MSL) was determined and compared to the RSV antigenome plasmid ΔNS2/Δ1313/I1314L, which was obtained from the NIH (see section 25). The PreMSL was used to generate the Viral Master Seed Lot whose genomic nucleotide sequence was like wise determined (see section 27).

24. The sequence, functional identity and location of the altered, inserted or deleted nucleic acid segments in question and, in particular, any known harmful sequence.

The genomic nucleotide sequence of Sanofi Pasteur's Pre-MSL was determined and compared to the RSV antigenome plasmid pRSV ΔNS2/Δ1313/I1314L, which was obtained from the NIH (see Table 1).

The key alterations from the parental RSV A2 are deletions of the NS2 gene and codon 1313 and replacement of isoleucine (I) with leucine (L) at codon 1314 of the L gene performed to attenuate the virus. See section 25 for details.

No plasmid or unknown sequences were transferred into ΔNS2/Δ1313/I1314L.

Characteristics of the genetically modified organisms in their final form

25. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed.

Genetic Traits

Compared to the sequence of wt RSV (GenBank accession number M74568), RSV ΔNS2/Δ1313/I1314L (NIH candidate) has three deletions (the 112 nt phenotypically silent deletion in the SH noncoding sequence (17), the attenuating 523 nt deletion of the NS2 gene (19) (20) (21), and the attenuating 3 nt deletion of codon 1313 of the L gene (2)), and 27 nucleotide differences: 9 silent mutations in noncoding regions, 9 silent mutations in coding regions, and 9 coding changes that are phenotypically inconsequential (21).

The genome structure of the RSV ΔNS2/Δ1313/I1314L is illustrated in Figure 3. The genome sequence of the plasmid pRSV ΔNS2/Δ1313/I1314L which was obtained from the Laboratory of Infectious Diseases at NIAID (NIH) is presented in Appendix 1. Table 1 provides a comparison between the genomic nucleotide sequence of RSV antigenome plasmid ΔNS2/Δ1313/I1314L, which was obtained from the Laboratory of Infectious Diseases at NIAID (National Institute of Allergy and Infectious Diseases) (NIH), and Sanofi Pasteur's Pre-Master Seed Lot (Pre-MSL).

Figure 3: Genome Structure of RSV ΔNS2/Δ1313/I1314L

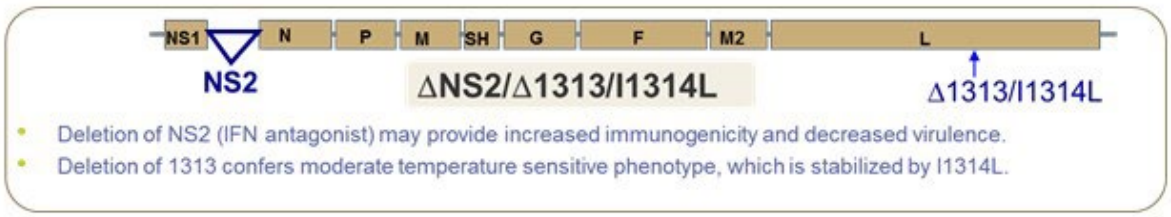


Table 1: Comparison of Genomic Nucleotide Sequence of RSV (NIH) Antigenome Plasmid ΔNS2/Δ1313/I1314L with (Sanofi Pasteur) Pre-Master Seed Lot RSV 2018-053

Region in NIH Antigenome Plasmid ΔNS2/Δ1313/I1314L	RSV ΔNS2/Δ1313/I1314L Sanofi Pasteur Pre-Master Seed Lot *
Non-coding sequence at 3' leader	Identical
NS1	Identical
ΔNS2	Gene deleted as per NIAID antigenome plasmid
Region in NIH Antigenome Plasmid ΔNS2/Δ1313/I1314L	**RSV ΔNS2/Δ1313/I1314L Sanofi Pasteur Pre-Master Seed Lot ***
N	Identical
P	Identical
M	Identical
SH	Identical
G	Identical
F	Identical
M2-1	Identical
M2-2	Identical
L	Identical
Δ1313 at L	Codon deleted as per NIAID antigenome plasmid
I1314L at L	Codon mutated as per NIAID antigenome plasmid

** 99.7% of sequence coverage for RSVΔNS2 Pre-MSL RSV2018-053 by highthroughput sequencing*

Phenotypic traits

RSV ΔNS2/Δ1313/I1314L is a live attenuated RSV with deletion of the NS2 gene (nt 577 –1098) and deletion of codon 1313 and replacement of isoleucine (I) with leucine (L) at codon 1314 of the L gene. The RSV nonstructural protein, NS2, suppresses the production of interferon α/β and also suppresses the cell's ability to establish an antiviral state (22).

Deletion of the NS2 gene attenuates RSV and also potentially provides increased immunogenicity (23). The NS2 protein was recently implicated in pathogenic effects resulting in distal airway obstruction and its deletion may increase vaccine tolerability. Other attenuating mutations in this vaccine candidate were designed by reverse genetics for increased stability of temperature sensitive phenotypes (e.g. Δ1313/I1314L). Deletion of codon 1313 (nt 12,434 to 12,436) in the L gene (Δ1313) yielded a temperature sensitive attenuating mutation (shutoff temperature of 37°C).

Replication of RSV with Δ1313 was reduced about 50-fold in nasal turbinates, and 200-fold in lungs when compared to wild type RSV (24). This mutation was susceptible to a second-site compensatory mutation at codon 1314, but this was stabilized by an I1314L mutation (codon ATA to CTG, nt 12,437 to 12,439) (18). Deletion of NS2 alone reduced RSV replication in cotton rats 100-fold (20). When ΔNS2 and Δ1313 were combined, replication in mice was reduced below the level of detection (24), indicative of an additive effect.

26. The structure and amount of any vector or donor nucleic acid remaining in the final construction of the modified organisms.

Not applicable: no plasmid or unknown sequences were detected in the genomic nucleotide sequence of ΔNS2/Δ1313/I1314L (see section 25).

27. The stability of the organism in terms of genetic traits.

In vitro studies

Genetic Stability

The genetic stability of RSV ΔNS2/Δ1313/I1314L Pre-Master Seed (P3) was assessed by serial passage on SP serum-free Vero cells up to five times (passage 8) under serum-free conditions. For each passage, SP serum-free Vero cells in T225 Flask was infected with RSV ΔNS2/Δ1313/I1314L at multiplicity of infection (MOI) of 0.01 and incubated at 32°C for 5 days with a media exchange on day 3. The infected cells together with viruses in the culture medium were harvested and frozen. After thawing, the harvested virus was mixed with HSG buffer, and then aliquoted and frozen. This was used for the next round of passage. Between each passage, the virus titer was measured as an average of five independent vials to ensure a consistent MOI of 0.01 for each passage.

The pre-MSL as well as virus at passage 8 (5 passages beyond Pre-Master Seed) was then sequenced by high-throughput sequencing (HTS) to assess the genetic stability of RSV ΔNS2/Δ1313/I1314L and to examine if subpopulations of viral mutants with compensatory mutations emerged during serial passage at the cut-off of 5%. 99.7% full length coverage was obtained and the sequence of both the preMSL and P8 virus conformed to the expected sequence.

The RSV reference sequence used for genetic stability assessment of the Pre-MSL at passage 3 and at passage 8 is based on the expected engineered plasmid sequence presented in section “Characteristics of the genetically modified organisms in their final form”. The original NIH plasmid containing the RSV sequence is from NIH. This reference sequence includes the engineered deletion Δ1313 and mutation at I1314L.

The results of the genetic stability study are provided below:

Table 2: Serial Passaging of Pre-Master Seed

Serial Passage	Level of Passage	HTS sequencing

Pre-Master Seed (P3)	Pre-master level	Sequence as expected
Pre-Master Seed+1 (P4)	Master seed level	N/A
Pre-Master Seed+2 (P5)	Product level (Phase I)	N/A
Pre-Master Seed+3 (P6)	Product level + 1	N/A
Pre-Master Seed+4 (P7)	Product level + 2	N/A
Pre-Master Seed+5 (P8)	Product level + 3	Sequence as expected

The Pre-MSL genetic stability results presented in Table 2 at passage 3 and at passage 8 are reported as “sequence as expected”. For clarification, the coding region conforms to the expected sequence with the deleted region for NS2 and the engineered deletion Δ1313, mutation at I1314L.

RSV Genome Sequencing

Sequencing the RSV genome of the RSV ΔNS2/Δ1313/I1314L virus monitors the potential variants that may arise during different stages of vaccine production. RSV genome sequencing by high throughput sequencing (HTS) allows the assessment of the full genome without bias to any existing variants. HTS sequencing is able to generate millions of sequences in parallel and assess each nucleotide position with extensive coverage. An in-house computational tool has been developed specifically to assess HTS data against the expected RSV genome sequence to detect variants above 10% threshold. Any variants are further assessed to verify and confirm the variants.

The results of the genome sequencing for the Master Seed Lot (MSL), Drug Substance (DS) lots are provided in Table 3. The reference sequence used to compare the genome sequences of the MSL and DS lots is based on the sequence obtained from sequencing of the RSV ΔNS2/Δ1313/I1314L Pre-MSL. This reference sequence includes the engineered deletion Δ1313 and mutation at I1314L. The deleted region for NS2 and the engineered deletion Δ1313, mutation at I1314L also conform to the expected sequence.

Table 3: RSV ΔNS2/Δ1313/I1314L Genome Sequencing Results

MSL Final Seed Lot	Drug Substance Development Lot	Drug Substance Clinical (cGMP) Lot (Phase 1/2)
No variants detected	No variants detected	No variants detected

MSL 1.0 was generated to support Phase I/II clinical trial materials. A new MSL (2.0) will be generated to support Phase III and commercial launch. The method of manufacturing for the MSL used to generate the Phase I/II clinical trial materials and the MSL to be generated for Phase III and commercial differs only in one bulk freeze/thaw stage which has been removed for new MSL. There is no impact

expected on virus genetic stability with the removal of the freeze thaw step, as this is not a step that allows for viral replication and so has no potential for genome sequence changes. There are no other differences in the two MSL Manufacturing processes. For process validation/commercial launch the working seed lot (WSL) must be scaled up to meet production demands.

The MSL supporting Phase I/II, MSL supporting Phase III, ISL (if required), WSL and Phase III DS Lot will be tested for the emergence of virus variants by the following methods:

- Genetic testing by HTS sequencing to detect variants present at > 5% of population
- Phenotypic testing by plaque size, Infectivity on Vero and Human Cells (CCID50) and Virus Infectivity at different temperatures. These confirm the attenuated phenotype of virus produced at the MSL and DS stages.

A new genetic stability strategy is under development to support Phase III and commercial manufacturing. This strategy will include at a minimum starting from the MSL and passaging to beyond the crude viral harvest of the DS and testing for the emergence of variants by both genetic (HTS) and phenotypic methods.

In vivo studies

In humans:

The genetic stability of RSV ΔNS2/Δ1313/I1314L was assessed in children by NIH. RT-PCR and partial sequence analysis of nasal wash (NW) isolates obtained at the peak of vaccine shedding from 18 RSV-seronegative vaccinees confirmed the presence of the NS2 deletion and the Δ1313 and I1314L mutations (25). Briefly, to verify the presence and genetic stability of the attenuating elements, viral RNA was obtained from a single passage of NW fluid on Vero cells. The NS2 gene deletion was verified by agarose gel electrophoresis, confirming an 855 base pair RT-PCR amplicon spanning the deletion. The 1313 deletion and the presence of the I1314L mutation was confirmed by sequence analysis of a 758 base pair PCR fragment of the L gene.

28. The rate and level of expression of the new genetic material in the organisms and the method and sensitivity of measurement of that rate and level.

The expression of the new genetic material (RSV ΔNS2/Δ1313/I1314L) can be evaluated by the detection of infectious viral particles. The amount of virus produced is directly related to the expression of the new genetic material.

The assay used at Sanofi Pasteur to quantify infectious RSV ΔNS2/Δ1313/I1314L is titration on Vero cells (see section 30).

The amount of RSV ΔNS2/Δ1313/I1314L is monitored at each passage during the production process as per section 27.

29. The activity of the gene product.

The RSV ΔNS2/Δ1313/I1314L is a live, attenuated, temperature sensitive vaccine. The expressed viral proteins, principally those encoded by the F and G genes (26) (27) contain antigenic determinants for neutralization. Therefore, these proteins act as immunogens and their expression via the RSV ΔNS2/Δ1313/I1314L virus induces a protective immune response against wt RSV.

30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector.

The RSV ΔNS2/Δ1313/I1314L vaccine candidate can be identified by the presence of viral nucleic acids, for example genomic detection using quantitative PCR, and identified by an in vitro test for the attenuated phenotype. It can also be detected by presence of replication competent virus by viral culture, for example by virus infectivity at different temperatures (plaque assay). The PCR method is more specific since it confirms the deletion of the 1313 codon and the Isoleucine (ATA) to leucine (CTG) mutation at codon 1314 in the RSV ΔNS2/Δ1313/I1314L vaccine candidate. The in vitro test for the attenuated phenotype and test for virus infectivity at different temperatures (plaque assay) can differentiate the RSV ΔNS2/Δ1313/I1314L vaccine candidate from wt RSV.

The tests for identification by PCR*, in vitro* test for the attenuate phenotype and test for virus infectivity at different temperatures (plaque assay) are described below.

Identity (qRT-PCR)

To quantify viral shedding (of the RSV ΔNS2/Δ1313/I1314L candidate), a qRT-PCR assay was developed that would specifically detect and quantify RSV ΔNS2/Δ1313/I1314L in nasal swab samples. Beyond the deletion of the NS2 gene, there are few sequence differences between RSV ΔNS2/Δ1313/I1314L and wt RSV A2. Hence the RSV ΔNS2/Δ1313/I1314L qRT-PCR assay was designed using the Light Cycler Probe system from Sigma. This system consists of two hybridization probes that are designed so that they bind the target 1-5 nucleotides apart. Probe 1 (donor) is labeled on the 3' end with a donor reporter. Probe 2 (acceptor) is labeled at the 5' end with an acceptor reporter. During the annealing step, the PCR primers and the LightCycler Probes hybridize to their specific target regions, bringing the probes in close proximity. When this happens, the donor dye is excited by the LightCycler and energy is transferred from the donor to the acceptor dye. The acceptor reporter's emission is detected by the Light Cycler at 640 nm. If the probes bind, but are not in close proximity, no signal is produced. The Light Cycler probes for the RSV ΔNS2/Δ1313/I1314L qRT-PCR assay target the deletion site of the NS2 gene in RSV ΔNS2. Probe 1 binds before the deletion and Probe 2 binds across the

deletion site. While the primers and probes may bind wt RSV A due to high sequence similarity, the two probes would not bind in close enough proximity to create signal as the NS2 gene is over 500 nucleotides long, making this method highly specific. If the probes are not in close proximity, as is the case with wild type RSV, no signal is produced.

In Vitro Test for Attenuated Phenotype

This test can confirm the attenuation phenotype of the RSV ΔNS2/Δ1313/I1314L candidate. Two flasks with confluent MRC-5 cells are inoculated with 25 mL test article/flask, and after an adsorption period, the inoculum is removed, and replaced with media. The next day cells are detached using TrypLE Select and then inoculated onto fresh, confluent MRC-5 monolayers. Flasks are incubated for 14 days, with observations and media exchange performed after 6 to 7 days.
After 14 days, culture fluids are inoculated onto fresh confluent MRC-5 cells, flasks are incubated for a further 14 days, with observations and media exchange performed after 6 to 7 days.

Final observations are performed on day 14 of the second passage (day 29 of test). All incubations are performed at 36°C ± 1°C, 5% ± 2% CO2. Flasks are visually inspected under a light microscope for the presence or absence of cytopathic effects (CPE) in the cell monolayer. At the end of the test, test article flasks should display no signs of viral CPE. A cell control flask, a positive control flask (culture media spiked with 3x limit of detection (LOD) of wt RSV) and matrix control (test article spiked with 3x LOD of wt RSV) are included in each assay. The reportable value for the test is attenuate phenotype confirmed.

Virus infectivity at different temperatures (Plaque Assay)

The RSV ΔNS2/Δ1313/I1314L candidate shows moderate temperature sensitivity at elevated temperatures. This test assesses viral replication at different temperatures (i.e. 34°C, 36°C and 38°C) by plaque assay to characterize the temperature sensitive phenotype of the virus. The RSV vaccine is titrated in 24-well plates on Vero cells infected with different virus dilutions. After a one-hour virus adsorption, culture media containing 1% methylcellulose is added to the wells. After a four-day incubation of the plates at 34°C, 36°C and 38°C, Vero cells are fixed and immunostained with an anti-RSV (F glycoprotein) purified monoclonal antibody conjugated with horseradish peroxidase. Plaques are visualized with a commercially available 3,3',5,5'Tetramethylbenzidine (TMB)-blotting solution and automated plaque counting is performed on a commercially available imaging system. The infectious titer is calculated and expressed as log10 PFU/mL for MSL and DS, and log10 PFU/dose for Drug Product.

An RSV internal control (RSV ΔNS2/Δ1313/I1314L) is prepared in-house, stored at ≤ -60°C, and is included each time the plaque assay is performed. The internal control was qualified by 30 independent titrations, and a mean titer was determined. The internal control titer must be within ± 0.5 log10 PFU/mL of its established titer.

The test is valid if:

- The negative control wells are satisfactory

- The titer of the validity control sample is within ± 0.5 $\log^{10}$ PFU/mL of its established titer
- Reportable values from at least two dilutions can be determined

31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

Identity (qRT-PCR)

The RT-qPCR test was developed to quantify RSV ΔNS2/Δ1313/I1314L genome copies. It is specific to RSV ΔNS2/Δ1313/I1314L and the accuracy is not affected by the presence of other respiratory pathogens including wildtype RSV A2, enabling discrimination between wt RSV and ΔNS2/Δ1313/I1314L. The assay sensitivity at the lower limit of quantitation (LLOQ) is 3.71 $\log^{10}$ genome copies/mL. Dilutional accuracy analysis near the LLOQ showed an absolute difference of at most 0.43 log10 copies/mL between observed and expected values.

The intra assay precision standard deviation is 0.2625 and the intermediate precision is 0.2959, $\log^{10}$ genome copies/mL. The assay is thus sensitive, reliable and specific.

In Vitro Test for Attenuated Phenotype

The test was validated, scaling the test down from two flasks per test condition to one flask per test condition. The limit of detection was determined as the virus spike in which 6/6 independent replicates tested positive. A limit of detection of 200 PFU wild type RSV strain A2 spiked in 25 mL of crude harvest was determined.

Virus infectivity at different temperatures (Plaque Assay)

For the test for infectivity at different temperatures, the plaque assay plates are incubated at 34ºC and two additional temperatures, 36ºC and 38ºC. Initially the plaque assay at 34ºC was qualified. Assessment of linearity revealed R2 of 0.9950 when tested at multiple concentrations. Accuracy, as measured through % recovery across multiple concentrations was calculated as a range of 96.08% to 101.49% for each run. Intermediate precision demonstrated %CV values ranging from 3.99 to 29.20 when calculated across multiple concentrations of formulation. For the two additional temperatures, 36ºC and 38ºC, the qualification of the plaque assay at 34ºC was leveraged. To show the ability to distinguish between wt and the vaccine strain, titrations of the plaque assay vaccine strain control and a wt RSV A2 control were performed at 34ºC, 36ºC and 38ºC showing comparable titers for wt RSV A2 at 34ºC and 36ºC, with a potential slight increase in titer at 38ºC.

In contrast, for the vaccine strain control, slightly reduced titers were observed at 36ºC compared to 34ºC, and a >1 log10 PFU/mL reduction in titer observed at 38ºC compared to 34ºC and 36ºC. Therefore, the assay is considered sensitive, reliable and specific for its intended use.

32. The history of previous releases or uses of the organisms.

To date RSV ΔNS2/Δ1313/I1314L has been assessed in three Phase I clinical trials by the NIH in which over 100 infants and toddlers received ΔNS2/Δ1313/I1314L ((25) (28), and three Sanofi trials. In the recently completed Phase I/II (VAD00001) study (29) a total of 155 infants and toddlers received ΔNS2/Δ1313/I1314L). The Sanofi candidate is also being assessed in an ongoing Phase I study in Japan in which 12 participants received RSV ΔNS2/Δ1313/I1314L and a Ph II transmissibility study in Puerto Rico and USA in which approximately 33 participants have received ΔNS2/Δ1313/I1314L. No safety concerns have been identified in any of these trials to date.

[Confidential information – please refer to Annex 4]

33. In relation to human health, animal health and plant health

To date RSV ΔNS2/Δ1313/I1314L was assessed in three Phase I clinical trials by the NIH and two Phase I trials by Sanofi Pasteur (25) (28) (29) and 1 Phase II trial.
[Confidential information – please refer to Annex 4]
While replicating virus was detected in NW of 90% of vaccinees, rates of lower respiratory tract infection, fever, cough, and otitis media were comparable to placebo recipients. Rhinorrhea occurred more often in vaccinees than in placebo recipients, but the differences were not statistically significant.

b. the toxic or allergenic effects of the non-viable organisms and/or their metabolic products,

The wild type RSV has remarkable tropism for the respiratory tract with no intrinsic propensity for its extra-pulmonary replication (3). Besides, the RSV vaccine ΔNS2/Δ1313/I1314L was constructed with rationally designed gene deletion and mutations well studied for its attenuation and immunogenicity. The nonclinical safety study assessment with RSV ΔNS2/Δ1313/I1314L is thus focused on the respiratory tract besides systemic toxicity and viscerotropism as discussed below. The results from the in vivo attenuation studies are detailed in section 33.

General toxicity, local tolerance (histopathologic assessment of upper respiratory tract) and viscerotropism of the RSV ΔNS2/Δ1313/I1314L following intranasal administration using the intranasal device used for administration was assessed in a pivotal GLP compliant repeated dose toxicity study.

[Confidential information – please refer to Annex 4] The toxicological assessment included following read-outs: general clinical signs, local reactions in nasal tract, clinical pathology and histopathology examination of respiratory tract (including nasal tract and turbinates), lymph nodes and other major organs. The study also aimed at demonstrating the respiratory tropism through viral RNA detection in nasal swabs post administration, as well as the viral RNA detection in serum samples.

There were no mortality or morbid clinical signs, no local reactions in the administration site including untoward respiratory signs or unexpected

ophthalmological observations in any of the animals of the treated group (and control group) throughout the study period. There were no treatment related changes in the body weight, rectal temperature, hematology, coagulation, serum chemistry parameters including CRP in any of the treated animals during the study. There were no changes in organ weights and no vaccine-related macroscopic or microscopic adverse changes in any of the organs examined including the respiratory tract. Lymphoid hyperplasia in the regional lymph nodes and increased cellularity in spleen were consistent findings with the expected systemic stimulation of immune system following administration of a vaccine.

Viremia is regarded as a biomarker of viscerotropism and the serum samples analyzed at multiple timepoints post intranasal administration of the RSV vaccine did not reveal any detectable levels of RSV RNA assuring the absence of systemic distribution. Further, the absence of any excursions in clinical pathology safety markers after vaccine administration together with the absence of viremia and absence of vaccine related changes in the systemic organs underpins the safety profile of the vaccine. RSV RNA in nasal swabs were quantified above the level of quantification by qRT-PCR assay only at 2 days after each intranasal administration demonstrating the respiratory tropism of RSV and progressively reduced to below the limit of detection before the subsequent intra-nasal administration assuring the attenuated nature of the vaccine candidate.

[Confidential information – please refer to Annex 4]

b. the comparison of the organisms to the donor, recipient or (where appropriate) parental organism regarding pathogenicity,

Attenuated phenotype

In vitro evaluation
NS2 is an IFN antagonist and virulence factor, and the mechanism of attenuation is proposed to be a reduction in replication on cells with intact interferon signaling. Therefore, a test was developed on MRC-5 cell, a human lung fibroblast line with intact interferon signaling pathway. Refer to section 30 above.
RSVΔNS2/Δ1313/I1314L strain has been demonstrated to be strongly attenuated on this human lung fibroblast cell line compared to wt RSV.

The RSV ΔNS2/Δ1313/I1314L candidate also shows moderate temperature sensitivity at elevated temperatures. Refer to section 30 above.

In vivo evaluation
The level of attenuation is a critical parameter for a vaccine, as RSV disease and reactogenicity are related to the level of viral replication. Among the experimental animals evaluated to date, the juvenile chimpanzee is the most permissive for RSV replication and has the same body temperature as humans.

RSV ΔNS2/Δ1313/I1314L vaccine shedding was assessed by the NIH (18). *[Confidential information – please refer to Annex 4]*. Animals were monitored twice daily for clinical symptoms. Nasal washes were performed daily for 12 days

postinoculation. On days 2, 4, 6, and 8, bronchoalveolar lavages (BAL) and on days 10 and 12, tracheal lavages (TL) were performed. Virus shedding in the respiratory tract was evaluated by performing nasal washes, BAL and TL. Virus titers in nasal wash, BAL fluid, and TL aspirates were determined by plaque assay on Vero cells at 32°C.

[Confidential information – please refer to Annex 4]

For ethical considerations, the Institute of Medicine has recommended that the use of chimpanzees for RSV research be discontinued. Cynomolgus macaques are an established toxicology (32) and RSV immunogenicity model (33). Therefore, to support further nonclinical safety studies the attenuation of RSV ΔNS2/Δ1313/I1314L was further assessed in cynomolgus macaques by SP.

A total of 24 animals (~50% male:female) free of obvious abnormalities indicative of health problems, tested seronegative for binding antibodies to the RSV fusion protein, and with no baseline RSV neutralizing titers (complement-dependent PRNT) were selected for study inclusion. *[Confidential information – please refer to Annex 4]*. No adverse events were recorded. The vaccine virus was detected by qRT-PCR in nasal swab samples and at much lower levels in tracheal lavage samples indicating that replication of RSV ΔNS2/Δ1313/I1314L was more attenuated in the lower respiratory tract than in the upper respiratory tract. Virus was not detected by plaque titration in either NS or TL samples in any animal at any timepoint.

iii. the capacity of the organisms for colonization, and

By 24 months of age, almost all children are infected by RSV and reinfection occurs throughout life. RSV ΔNS2/Δ1313/I1314L is a temperature sensitive, attenuated vaccine candidate that, when tested by NIH in RSV-seropositive participants did not cause disease and was not shed. While low levels of RSV ΔNS2/Δ1313/I1314L were shed by RSV-seronegative participants, none exhibited LRT disease indicating that RSV ΔNS2/Δ1313/I1314L is unable to replicate outside the URT and is rapidly cleared from the host.

D. if the organisms are pathogenic to humans who are immunocompetent –

i. diseases caused and mechanisms of pathogenicity including invasiveness and virulence,

In the Phase I clinical trial by NIH, rhinorrhea occurred more frequently in RSVseronegative vaccinees than placebo recipients, but these differences were not statistically significant. No other disease was attributable to ΔNS2/Δ1313/I1314L.The same trend was noticed in Sanofi's VAD00001 trial which included both RSV-naïve and experienced participants.

ii. communicability,

Vaccinees shed RSV ΔNS2/Δ1313/I1314L at low levels as compared to wild type virus, making transmission unlikely.

iii. infective dose,

[Confidential information – please refer to Annex 4]

iv. host range and possibility of alteration,

The host range is limited to humans and chimpanzees see sections 9 and 12.

v. possibility of survival outside of human host,

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 (see section 12).

vi. presence of vectors or means of dissemination,

There is no vector for RSV transmission. RSV can spread when an infected person coughs or sneezes, releasing contaminated droplets into the air (see section 9).

vii. biological stability,

The stability of ΔNS2/Δ1313/I1314L in terms of genetic traits has been described in section 27. GMP lots of RSV were sequenced after 5 passages from the Pre-Master Seed Lot with no mutations detected and no compromise of the attenuated phenotype.
The genetic stability of ΔNS2/Δ1313/I1314L was also evaluated in vivo, as described in section 27 and the attenuating mutations were shown to be genetically stable.
According to results obtained in vitro and in vivo, RSV ΔNS2/Δ1313/I1314L is considered to be biologically stable.

viii. antibiotic-resistance patterns,

ΔNS2/Δ1313/I1314L does not contain any antibiotic resistance genes.

ix. allergenicity

While the risk of allergic reaction is possible as with any vaccine, the risk is considered to be low based pre-clinical studies described in section 33.a.

x. availability of appropriate therapies

Not applicable

e. Other product hazards

Not applicable

Part III: Information relating to the conditions of release
The release

34. The description of the proposed deliberate release, including the initial purpose or purposes of the release and any intention to use the genetically modified organisms as or in a product in the future.

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 RSV serostatus. The RSV ΔNS2/Δ1313/I1314L vaccine is intended to be used as a prophylactic vaccine against respiratory tract disease caused by RSV. Clinical trials in infants and toddlers are required to support further clinical development towards a prophylactic vaccine to protect infants and young children against respiratory tract disease caused by RSV. Vaccination against RSV should reduce the number of medically attended acute lower respiratory tract infections, severe RSV, RSV related hospitalizations and deaths.

35. The intended dates of the release and time planning of the experiment including frequency and duration of releases. It is anticipated that enrollment into the planned Phase III efficacy clinical study will begin in UK and EU in May 2024 and will be completed by November 2028.

[Confidential information – please refer to Annex 4]

36. The preparation of the site before the release.

In current clinical trials a freezer is expected to be dedicated to either product or sample storage at the study site if possible and should be identified only for this purpose. If this is not possible, adequate segregation between samples and product must be maintained. A freezer unit should be selected with a lock or installing a latch on the refrigerator door or placing the freezer in a secured room to ensure:

- To prevent unauthorized access to medical freezers access must be limited to site staff
- To ensure that the door closes properly each time and is not inadvertently left ajar
- If it is impossible to lock the refrigerator, a lock box to secure the vaccines inside of the refrigerator should be used.

Transfer of product and specimens between different locations within the same location must be approved by Sanofi Pasteur’s Clinical Logistics. Transfer of product between different sites (including satellite sites) is not recommended and requires the approval of Sanofi Pasteur’s Clinical Logistics and Quality Assurance departments.

When frozen specimens are being shipped to another location for storage, processing or testing, they must be packaged by any authorized designee of the

investigational center who has been trained. Sera shipment will be commonly shipped following the IATA guideline for Category B or Except Human Specimens (34).

Qualified staff at each clinical trial site will be trained how to administer the product as detailed in section 38. Staff dispensing or administering ΔNS2/Δ1313/I1314L will be trained in good clinical practice, expected to follow operational procedure manual, applicable local GMO regulations, and WHO Universal Precautions. Staff working in clinical trial sites are expected to be adults and follow instructions for unused product return or destruction stipulated in the operational procedure manual. In the event of a broken vaccine/ study intervention vial or spillage, study personnel are expected to follow decontamination procedures stipulated in the operational procedure manual.

37. The size of the site.

4 clinical sites are selected in Great-Britain. No specific size for the release, immunizations are going to be performed in separate examination rooms at each proposed study site.

38. The method or methods to be used for the release.

The vaccine is intended to be prepared and administered in clinical trials by trained and qualified unblinded third party investigators. The vaccine will be stored at clinical trial sites in designated -70°C freezers in rooms with restricted access. The frozen vial containing the vaccine must be thawed at room temperature for at least 10 minutes before preparation for administration. A single use intranasal device used for administration in an individually sealed clear plastic bag is supplied for each vaccine/placebo administration.

The individually sealed bag contains (i) a 1-mL standard luer-lock syringe attached with a blue color plastic cannula protected by a clear plastic cover, (ii) 1-piece intranasal device used for administration (atomizer) with a white foam cone. The intranasal device use instructions are as follows:

- Step 1: Remove the clear protective cap from the blue vial access cannula.
- Step 2: Manually invert 5 times the vial that contains the thawed vaccine/placebo. Pierce the rubber stopper of the vial with the blue plastic cannula. Withdraw vaccine candidate formulation by vertically submerging the vial-access cannula.
- Step 3: Invert the vial to draw the required dose volume. De-bubble and aspirate with the syringe.
- Step 4: Disconnect (twist off) the vial-access cannula from the syringe. Discard the cannula into a sharp container or per protocol instructed. Connect the intranasal device used for administration to the syringe via the luer lock connector.
- Step 5: Prime the intranasal device used for administration by gently pushing the plunger and slowly filling up the empty space of the intranasal device used

for administration. Stop priming when the liquid level has reached the target graduation mark of the syringe.
- Step 6: Using the free hand to hold the head stable and at an angle of approximately 45 degrees position. Place the tip of the intranasal device used for administration just inside the nostril up to the foam cone. Spray the vaccine into the subject's nostrils with a single motion. Depress the plunger as rapidly as possible.

After administration of the vaccine the instructions for the disposal of the intranasal device used for administration are as follows:
- The intranasal device used for administration was designed for single-use. Do not re-use the device system.
- Discard the prepared device if it would not be used within 6 hours after the preparation.
- Do not freeze and re-use the prepared device.

All used syringes and needles will be destroyed on site, in specific containers, at the end of each vaccination.
Partially used empty boxes will be monitored by the Clinical Research Associate (CRA). In that case, they will be kept on site in a secure place and well identified and will be destroyed on site after monitoring by the CRA at the end of each cohort. The destruction will be documented on site.

If destruction on site is not possible, the vials will be returned for destruction to the Sponsor, or to a third-party vendor where applicable at room temperature along with the applicable form provided by CRA.

Prior to the return of unused and unusable products (expired, break in the cold chain) the responsible site personnel will account for all study vaccine and the CRA will monitor product accountability on the Investigational Product Dispensing and Reconciliation Form.

39. The quantity of organisms to be released.

In the notified country RSV ΔNS2/Δ1313/I1314L will be administered to subjects at no more than 7.2 log10 PFU per dose.

40. The disturbance of the site, including the type and method of cultivation, mining, irrigation, or other activities.

Not applicable

41. The worker protection measures taken during the release.

The qualified person that will be involved in the management, administration and disposal of the vaccine will follow the Sanofi Pasteur Operating guidelines and

applicable local GMO regulations, and WHO Universal Precautions/ appropriate local guidelines.

42. The post-release treatment of the site.

The rooms in the medical facility used to prepare and administer the vaccine will be cleaned before and after manipulation with a standard disinfectant active against RSV. Surfaces will be decontaminated and cleaned with standard disinfectant active against RSV. After administration of the vaccine the intranasal device will be disposed of and all used materials will be destroyed on site, in specific containers, at the end of each vaccination session.

43. The techniques foreseen for elimination or inactivation of the organisms at the end of the experiment or other purposes of the release.

See section 38.

44. Information on, and the results of, previous releases of the organisms and in particular, releases on a different scale or into different ecosystems.

To date a total of approximately 114 subjects aged 6 to 59 months (10 RSV seropositive subjects aged 12 to 59 months, 35 RSV seronegative subjects aged 6 to 24 months, 43 RSV seronegative and seropositive subjects aged 6 to 18 months) have received at least one injection of the RSV ΔNS2/Δ1313/I1314L vaccine, whatever the formulation, in completed or ongoing Phase I to Phase II clinical studies. Among these subjects, 104 subjects were in the claimed age indication (6 to 24 months of age).
A list of all completed and ongoing trials is presented in Table 4.

Table 1: Completed or Ongoing Phase I and II Studies

Sponsor (Study #)	**Phase (Country)**	**Age group**	**Objective (population)**	**RSV vaccine Recipients (Total in study)**	**FPFV - LPLV**
NIAID (CIR 288)	I (USA)	4-59 months	Safety and Immunogenicity (S+ or S-)	15 (22) 30 (45)	Jun 2013 – *Apr 2023 (est)*
NIAID (IMPAACT 2018 / 38405	I (USA)	6-24 months	Safety, Immunogenicity and Infectivity (S-)	25 (62)	Sept 2017 – Oct 2020
NIAID (CIR 321*)	See IMPAACT 2018 / 38405				
NIAID (IMPAACT 2021 / 38530)	I/II (USA)	6-25 months	Safety and Immunogenicity (S-)	40 (160)	May 2019 – *Apr 2023 (est)*
Sanofi (VAD00001)	I/II (USA / Chile / Honduras)	6-18 months	Safety, Immunogenicity, Infectivity, Dose-Finding (R+ or R-)	155** (259)	Sept 2020 - *April 2023*
Sanofi (VAD00014)	Puerto Rico	6-24 months	Safety, immunogenicity, transmissibility (R+ or R-)	50 (100)	Feb 2023 – Dec 2023 (est)
Sanofi (VAD00012)	Japan	6-24 months	Safety and immunogenicity	12 (18)	May 2023 – Dec 2023

*Legend: S+ (RSV seropositive); S- (RSV seronegative)[2]; R+ (RSV experienced) R- (RSV naïve) *the portion of IMPAACT 2018, executed at Johns Hopkins University Center for Immunization Research (CIR); **This is the anticipated number, based on the VAD00001 study design; as full unblinding has not taken place, the final number of IP recipients is unknown. FPFV = first participant first visit, LPLV = last participant, last visit.*

The environment (both on the site and in the wider environment)

Based on the fact that the vaccine is intended to be administered as droplets with an intranasal device, the following receiving environments are envisaged:

1. The intended primary receiving environment would be the nose, nasal turbinates and nasopharynx of trial participants, to be delivered via a nasal device used for administration as droplets.
2. The secondary receiving environment would be the room and the clinical trial site where ΔNS2/Δ1313/I1314L is dispensed, administered and disposed of as waste. The rooms in the medical facility used to prepare and administer the vaccine will be cleaned before and after manipulation with a standard desinfectant active against RSV. Surfaces will be decontaminated and cleaned after use with standard desinfectant active against RSV. After administration of the vaccine the intranasal device will be disposed and all used materials will be destroyed on site, in specific containers, at the end of each vaccination.
3. The principal route by which ΔNS2/Δ1313/I1314L may enter the wider environment is by shedding from vaccinated trial participants once they leave the clinical trial site and return home. The tertiary receiving environment thus includes the trial participants' homes and any places they visit during the period when ΔNS2/Δ1313/I1314L is replicating and shedding.

45. The geographical location and national grid reference of the site or sites onto which the release

The proposed release will be conducted at the following locations:

West Suffolk Hospital: Hardwick Lane, Bury St. Edmunds, Suffolk IP33 2QZ, United Kingdom Grid Reference: TL851627	**Halton General Hospital,** Hospital Way, Runcorn, Halton, WA7 2DA, United Kingdom Grid Reference: SJ538809
Connor Downs Surgery Turnpike Road, Hayle, Cornwall, TR27 5DT, United Kingdom Grid Reference: SW594392	**Bristol Royal Hospital for Children** Upper Maudlin Street, Bristol, BS2 8BJ, United Kingdom Grid Reference: ST586734
Royal Devon and Exeter Hospital Barrack Road, Exeter, EX2 5DW, United Kingdom Grid Reference: SX937918	

46. The physical or biological proximity of the site to humans and other significant biota.

Humans and chimpanzee are the only species naturally infectable with RSV. While close biological proximity to other humans by vaccinees will occur once they leave the clinical trial site and return home, the probability of transmission of ΔNS2/Δ1313/I1314L is considered as negligible (see sections of Part IV).

47. The proximity to significant biotopes, protected areas or drinking water supplies.

Not applicable

48. The climatic characteristics of the region or regions likely to be affected.

Not applicable

49. The geographical, geological and pedological characteristics.

Not applicable

50. The flora and fauna, including crops, livestock and migratory species.

Not applicable

51. The description of target and non-target ecosystems likely to be affected.

Not applicable

52. The comparison of the natural habitat of the recipient organisms with the proposed site or sites of release.

Not applicable

53. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

Not applicable

Part IV: Information relating to the interactions between the organisms and the environment
Characteristics affecting survival, multiplication and dissemination

54. The biological features which affect survival, multiplication and dispersal.

Survival:

RSV ΔNS2/Δ1313/I1314L does not replicate outside its hosts and is very fragile (see sections 12 and 33).

Multiplication and Dispersal:
In the vaccinated chimpanzee, the RSV ΔNS2/Δ1313/I1314L was attenuated (18). The details are described in section 33.b.

Furthermore, virus shedding was explored in one Phase I study by NIH (25). In this study nasal wash (NW) samples were obtained from all subjects following vaccination and RSV ΔNS2/Δ1313/I1314L levels were quantified by an immunoplaque assay using a mix of 3 monoclonal antibodies to RSV F and by qRTPCR. When illnesses occurred, nasal wash samples were tested for other viruses and mycoplasma by means of RT-PCR.

In RSV-seropositive participants, upper respiratory illness (URI) was observed in 2 and cough was observed in 1 of 10 vaccinees during the 28-day postimmunization reporting period; in each case, rhinovirus was detected in NW samples at the time of illness. None of these vaccinees shed RSV ΔNS2/Δ1313/I1314L.

In RSV-seronegative participants, URI, cough, and febrile illnesses occurred at similar frequently in both vaccinees and placebo controls (rhinorrhoea occurred more frequently in vaccinees than placebo recipients, but the difference was not statistically significant). Other viruses were detected in 10 of 22 and 7 of 12 symptomatic RSV-seronegative vaccine and placebo recipients, respectively, including rhinovirus, enterovirus, adenovirus, coronavirus, bocavirus, and parainfluenza virus type 3. The NW samples obtained within 3 days after illness onset from the 22 symptomatic vaccinees revealed vaccine virus alone in only 5 children).

[Confidential information – please refer to Annex 4]

55. The known or predicted environmental conditions which may affect survival, multiplication and dissemination, including wind, water, soil, temperature and pH.

RSV ΔNS2/Δ1313/I1314L does not replicate in the environment. It only replicates in the vaccinated host and the shedding in the environment by the vaccinated host is low. No environmental conditions could thus significantly enhance their multiplication and dispersal.

56. The sensitivity to specific agents.

RSV ΔNS2/Δ1313/I1314L has the same structure and physical properties as parental RSV which is a fragile, lipid-enveloped virus sensitive to desiccation with an intracytoplasmic replication cycle. As all enveloped viruses, RSV is sensitive to

detergents and solvents. Like all viruses, RSV does not replicate or survive outside the host cell and is sensitive to heat and ultraviolet radiation. RSV ΔNS2/Δ1313/I1314L, like parental RSV, is susceptible to common disinfectants such as 70% ethanol, various detergents including 0.1% sodium deoxycholate, sodium dodecyl sulphate, and Triton X-100, and to 1% sodium hypochlorite, formaldehyde (5% formalin), 2% glutaraldehyde, 1% iodine and is inactivated by heat (35).

Interactions with the environment

57. The predicted habitat of the organism.

The quantity of RSV ΔNS2/Δ1313/I1314L virus shed in nasal secretions of vaccinees is low and transient (generally <12 days). Besides the human vaccinated hosts, there are no other vectors for transmission of RSV ΔNS2/Δ1313/I1314L. There is no persistence in humans, and therefore, there is no predicted habitat for the vaccine.

58. The studies of the behaviour and characteristics of the organisms and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses.

Not applicable. Due to their biological features, RSV ΔNS2/Δ1313/I1314L is existing low and transient in the natural environment (see sections 54 and 55). Therefore, no stimulated natural environment can be used to study the behavior and characteristics of the vaccine.

59. The capability of post-release transfer of genetic material-

a. from the genetically modified organisms into organisms in affected ecosystems,

In the natural environment, wt RSV transmission is between humans via infected droplets. Humans and chimpanzee are the only organism that could be concerned by post-release transfer of genetic material from the vaccine.

The RSV ΔNS2/Δ1313/I1314L virus will be released in humans for vaccination against RSV.

Some requirements must be fulfilled for incorporation of genetic material of the vaccine organism in whole, or in part, into the human host genome:

- ΔNS2/Δ1313/I1314L genetic material must be in the same cell compartment than the human or animal genetic material, that is to say, in the nucleus. Transfer of genetic material indeed requires interaction between the genomes.
- presence of reverse transcriptase activity to transcribe the chimeric RNA genome into DNA, before insertion into the host genome.

However, ΔNS2/Δ1313/I1314L is a Orthopneumovirus and:

- Like all the Orthopneumovirus, replication cycles of the RSV ΔNS2/Δ1313/I1314L virus are carried out in the cytoplasm of host cells, and not in the nucleus. Therefore, there is no possibility of interaction with the human genome.
- RSV RNA genome does not code for reverse transcriptase.

Therefore, the potential for the genetic material of the vaccine organism to become incorporated in whole, or in part, into the genome of any cells of humans is considered to be null.

b. from indigenous organisms to the genetically modified organisms.

The organisms with which transfer of genetic material could occur by homologous recombination under natural conditions are viruses belonging to the same genus: Orthopneumovirus. The human vaccinated hosts could be infected with another RSV strain around the time of inoculation of the RSV ΔNS2/Δ1313/I1314L vaccine. However, as noted in section 10, homologous recombination of RSV is a very rare event even under optimized experimental conditions. Therefore, the potential risk of genetic transfer between wildtype RSV and ΔNS2/Δ1313/I1314L due to the very low or non-existent levels of natural homologous recombination is negligible.

60. The likelihood of post-release selection leading to the expression of unexpected or undesirable traits in the genetically modified organisms.

According to safety results obtained in monkeys and humans (see section 33.b), the risk of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organisms is considered to be negligible. However, such an event might first assume the occurrence of mutations or genetic transfers that could enhance the pathogenicity of RSV ΔNS2/Δ1313/I1314L.

The genetic stability of the RSV ΔNS2/Δ1313/I1314L has already been evaluated in vivo in vaccinees, verifying the deletion mutations of NS2 and the codon 1313 as well as the codon I1314L mutation (see section 27).
Moreover, in vitro studies sustained the vaccine viruses' genetic stability, showing only as single nucleotide change in a non-coding region after 5 passages (see section 27).

The potential risk of genetic transfer and exchange between wildtype RSV and ΔNS2/Δ1313/I1314L by homologous recombination is negligible, as discussed in section 59.

As the risk of mutation and recombination events that could enhance the pathogenicity of RSV ΔNS2/Δ1313/I1314L is considered to be negligible, the likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism is considered to be negligible.

61. The measures employed to ensure and to verify genetic stability, the description of genetic traits which may prevent or minimise dispersal of genetic material and methods to verify genetic stability.

Measures employed to verify genetic stability
Genetic stability could be affected by two mechanisms: homologous recombination and accumulation of mutational changes due to selection pressure.
The risk of transfer of genetic material by recombination has been described as negligible in section 59.b.

The second mechanism that could affect the genetic stability is the error-prone nature of replication of RNA virus genomes. Mutation rates vary between RNA viruses, ranging between 10^{-6} and 10^{-4} per nucleotide site per cell infection, depending on the RNA virus and methods used (10).

In vitro studies on Vero cells were performed to evaluate the genetic stability of RSV ΔNS2/Δ1313/I1314L. These studies, described in section 27 and utilizing high throughput sequencing to detect potential subpopulations of viral mutants at the 5% threshold, sustain the genetic stability of ΔNS2/Δ1313/I1314L.

In vivo genetic stability of ΔNS2/Δ1313/I1314L has been assessed by confirmation of presence of attenuating mutations in the virus isolated from nasal washes of vaccinees (see section 27). As the RSV backbone is not expected to be unstable (see section 11), this has been done by focusing on the attenuating mutations.

Risk of dispersal of genetic material
Due to its biological features, RSV ΔNS2/Δ1313/I1314L is unstable and unable to replicate outside the host.

Its multiplication in the host is low and transient, and thus unlikely to lead to an effective dissemination in the environment (see section 33.c).

62. The routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact and burrowing.

The RSV ΔNS2/Δ1313/I1314L vaccine does not replicate outside the host. In addition, its survival outside the host is very short as it is affected by many environmental parameters and is sensitive to common disinfecting/cleaning agents (see sections 54 to 56).

The evaluation of RSV ΔNS2/Δ1313/I1314L virus shedding has been performed and is low and transient (see section 54).

In the natural environment, wt RSV is transmitted between humans via nasal or oral secretions, either directly via large droplets (and probably droplets) or indirectly

through contact with contaminated hands and environmental surfaces (e.g. cribs, toys, doorknobs, tabletops).

For the release, the RSV ΔNS2/Δ1313/I1314L will be administered via the intranasal device used for administration which has a soft, conical plug on the tip that forms a seal with the nostril, preventing expulsion of fluid. The release will be handled by qualified persons and the generated waste will be treated to avoid such accidental transmission (see sections “Control of the release” and “Waste Treatment”).

63. The description of ecosystems to which the organisms could be disseminated.

The purpose for release of RSV ΔNS2/Δ1313/I1314L is vaccination to protect human children against RSV disease throughout the world.

The quantity of RSV ΔNS2/Δ1313/I1314L virus shed in nasal secretions of vaccinees is low and transient (see section 57) and the virus cannot replicate or long survive outside the host. Therefore, no specific ecosystem is concerned by the release.

64. The potential for excessive population increase of the organisms in the environment.

An excessive population increase of ΔNS2/Δ1313/I1314L in the environment would require robust multiplication of the virus in the vaccinated host and a very effective route of transmission from vaccinees to un-immunized humans.

Considering:

- the absence of replication and survival outside the hosts,
- attenuation of RSV ΔNS2/Δ1313/I1314L in RSV seronegative host,
- lack of replication in seropositive host, potential excessive population increase of ΔNS2/Δ1313/I1314L in the environment can be excluded.

65. The competitive advantage of the organisms in relation to the unmodified recipient or parental organism or organisms.

Superinfection with different strains of RSV is possible (36). Due to the attenuation and temperature sensitivity of ΔNS2/Δ1313/I1314L, a competitive advantage in relation to the parental organism is not expected.

66. The identification and description of the target organisms if applicable.

The RSV ΔNS2/Δ1313/I1314L vaccine was designed to protect humans against RSV disease. The target organism is human.

67. The anticipated mechanism and result of interaction between the released organisms and the target organisms if applicable.

RSV ΔNS2/Δ1313/I1314L was designed to vaccinate humans against RSV infection. ΔNS2/Δ1313/I1314L will thus be released in humans to induce an immune response. Following inoculation, RSV ΔNS2/Δ1313/I1314L undergoes replication during which the viral genes are transcribed and translated. This results in presentation of de novo synthesized proteins to the immune system inducing immunity against further RSV infection. A clinical trial showed that RSV ΔNS2/Δ1313/I1314L was immunogenic in human infants with a good safety profile (25).

68. The identification and description of non-target organisms which may be adversely affected by the release of the genetically modified organisms, and the anticipated mechanisms of any identified adverse reaction.

Humans and chimpanzees are the only natural host and the only natural reservoir of RSV. It is highly unlikely that other organisms will be affected by RSV ΔNS2/Δ1313/I1314L. Survival of RSV ΔNS2/Δ1313/I1314L in the environment is short-lived.

The only non-target organisms which may be adversely affected by the release of ΔNS2/Δ1313/I1314L are chimpanzees. RSV was originally recovered and identified as a novel virus in 1956 after an outbreak of common cold infections from a colony of chimpanzees.

However, RSV ΔNS2/Δ1313/I1314L is strongly attenuated in chimpanzees (section 33.b) and presented no serious adverse events. Regardless, as the target organism for release is human infants, accidental transfer to chimpanzees in unlikely.

RSV does not cause disease in any other animal system. Infection would be selflimiting with low viremia and result in the development of antibodies to RSV making further transmission cycles unlikely.

69. The likelihood of post-release shifts in biological interactions or in the host range.

Not applicable

70. The known or predicted interactions with non-target organisms in the environment, including competitors, prey, hosts, symbionts, predators, parasites and pathogens.

RSV is a very fragile enveloped virus that does not replicate nor survive outside its hosts. While transmission of RSV from humans to chimpanzees has been

documented, as the vaccine is targeted to infants, close contact with chimpanzees is highly unlikely.

71. The known or predicted involvement in biogeochemical processes.

Not applicable: RSV ΔNS2/Δ1313/I1314L is not involved in any biogeochemical process.

72. Any other potentially significant interactions with the environment.

None

Part V: Information on monitoring, control, waste treatment and emergency response plans

Monitoring techniques

73. Methods for tracing the organisms and for monitoring their effects.

Peak shedding of the RSV ΔNS2/Δ1313/I1314L virus will be assessed seven days after each administration from a nasal sample. RSV ΔNS2/Δ1313/I1314L can be specifically traced in non-target organisms by a RT-PCR test that distinguishes it from wildtype RSV (see section 30).

74. Specificity (to identify the organisms and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques.

Please refer to sections 7 and 30.

75. Techniques for detecting transfer of the donated genetic material to other organisms.

The risk of transfer of the donated genetic material to other organisms is considered to be negligible. However, if necessary, RSV ΔNS2/Δ1313/I1314L can be specifically traced in non-target organisms by a RT-PCR test that distinguishes it from wildtype RSV (see section 30).

76. Duration and frequency of the monitoring.

The overall risk for both humans and the environment is considered as negligible. Therefore, monitoring is no required.

Control of the release

77. Methods and procedures to avoid and/or minimise the spread of the organisms beyond the site of release or the designated area for use.

The RSV ΔNS2/Δ1313/I1314L vaccine is for human vaccination use only (under prescription and surveillance of a physician).

Moreover, it is submitted to several security levels. The vaccine is stored in vials hermetically sealed, and delivery will be via the intranasal device used for administration to prevent expulsion of fluid. For the release, qualified persons will handle RSV ΔNS2/Δ1313/I1314L and the generated waste will be treated to avoid accidental transmission (see section 82). Spread of ΔNS2/Δ1313/I1314L beyond the site of the release or the designated areas of use is highly unlikely.

78. Methods and procedures to protect the site from intrusion by unauthorised individuals.

Refer to section 36.

79. Methods and procedures to prevent other organisms from entering the site.

Not applicable

Waste treatment

80. Type of waste generated.

Refer to section 38.
The generated waste consists of:

- Vial containing remaining vaccine
- Syringes
- Vial access cannulas
- Intranasal Device used for administration
- Any other consumable material directly in contact with the vaccine

81. Expected amount of waste.

Refer to section 39.

82. Description of treatment envisaged.

As explained in section 56, decontamination of RSV ΔNS2/Δ1313/I1314L can be managed like any other enveloped virus. Decontamination by heat or chemicals (bleach, ethanol/isopropyl alcohol, detergents) can be envisaged. A few minutes at 100°C or with contact chemicals have been shown to achieve full decontamination (see section 56 for more details). Therefore, autoclaving or incinerating, which are

common processes of decontamination, are fully applicable to the RSV ΔNS2/Δ1313/I1314L vaccine.

Emergency response plans

83. Methods and procedures for controlling the organisms in case of unexpected spread.

Refer to section 56.

84. Methods, such as eradication of the organisms, for decontamination of the areas affected.

Refer to section 56.

85. Methods for disposal or sanitation of plants, animals, soils and any other thing exposed during or after the spread.

Not applicable. As release will be performed at a secure location in a clinical site, plants, animals and soil will not be exposed to ΔNS2/Δ1313/I1314L.

86. Methods for the isolation of the areas affected by the spread.

Not applicable

87. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

The RSV ΔNS2/Δ1313/I1314L is a vaccine developed to protect humans from RSV infection.

Data from Phase I clinical trials indicates that RSV ΔNS2/Δ1313/I1314L was well tolerated with rates of upper respiratory symptoms comparable to placebo and no incidence of lower respiratory tract infection in vaccinees (see section33).

Sanofi Pasteur will continue to monitor the safety profile of RSV ΔNS2/Δ1313/I1314L vaccine during future clinical trials and after product launch by conducting:

- Intensive monitoring of the safety profile in vaccinees during clinical trials.
- Routine pharmacovigilance practices allowing a comprehensive, continuous and global overview of post-licensure safety profile.

Part VI: A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this schedule, and the name of the body or bodies responsible for carrying out the studies.

Methods used to construct and introduce the mutations or to delete sequences

The pRSV ΔNS2/Δ1313/I1314L full length cDNA plasmid was generated at the NIH (Laboratory of Infectious Diseases, Bethesda, Maryland) by site directed mutagenesis and DNA cloning steps. Plasmid D46/6120 was used as template for site directed mutagenesis (17). The candidate vaccine full length genome encoded in plasmid D46/6120 differs from the full length recombinant wt parent A2 virus by the deletion of 112 nucleotides from the downstream non-translated region of the SH gene, and also contains the introduction of five translationally silent nucleotide changes into the downstream end of the SH open reading frame (17).

This deletion and these silent (at the amino acid level) changes were made to stabilize the cDNA during propagation in bacteria. Importantly, they did not detectably affect the viral phenotype in cell culture or in mice. As the first step to generate pRSV ΔNS2/Δ1313/I1314L, codon 1313 of the L gene was deleted by site directed mutagenesis of plasmid D46/6120, resulting in D46/6120 Δ1313 (18).

To delete the NS2 gene, a 2652 bp EagI/AvrII restriction fragment (containing 1044 bp of the cloning vector and leader, NS1, and part of the N gene) was obtained from the previously described cDNA encoding RSV ΔNS2 (19) and used to replace the EagI/AvrII fragment containing leader, NS1, NS2 and part of the N gene in D46/6120 Δ1313, resulting in D46/6120 ΔNS2 Δ1313. Finally, the I1314L (ATA to CTG) mutation was introduced into D46/6120 ΔNS2 Δ1313 by site directed mutagenesis to increase the genetic stability of this vaccine, resulting in the pRSV ΔNS2/Δ1313/I1314L full length cDNA plasmid (18).

The live attenuated virus RSV ΔNS2/Δ1313/I1314L was rescued through reverse genetics based on the full-length cDNA clone of RSV ΔNS2/Δ1313/I1314L. In brief, Vero cells were transfected by electroporation with the ΔNS2/Δ1313/I1314L cDNA, and four support plasmids encoding the RSV N, P, M2.1 and L genes, all under the T7 promoter, and a fifth support plasmid encoding T7 polymerase. In transfected cells, full-length RSV RNA is generated, encapsulated, and then serves as a template for transcription and replication mediated by the RSV N, P, M2-1, and L proteins. Only the full-length RSV genome is present in the rescued virus. Table 1 summarizes all modifications relative to the wt parent A2 virus and their purposes.

Table 5: Description of the modification(s) and purposes

Modification(s), with the gene and genome nt sequence position indicated	**Purposes**
Deletion of the entire NS2 gene, nt #577–1098	Genetically introduced attenuating modification

The deletion of 112 nucleotides from the downstream non-translated region of the SH gene, and the introduction of four translationally silent codons into the SH open reading frame • Nucleotide substitutions in the last 4 codons (5 nucleotides) of the SH Open Reading Frame (ORF), including the stop codon: #4489 C→T silent #4492 C→T silent #4495 A→T silent #4497 A→G silent #4498 G→A silent • Deletion of nucleotide positions # 4499 – 4610, which comprise 112 nucleotides from the downstream non-translated region of the SH gene	Genetically introduced deletion with silent mutations. This helps stabilize the antigenome plasmid during propagation in bacteria.
Deletion of codon 1313/change of codon I1314L	Genetically introduced attenuating deletion Δ1313 confers temperature-
Modification(s), with the gene and genome nt sequence position indicated	**Purposes**
• Deletion of codon 1313 in the ORF encoding the L polymerase (Δ1313), nucleotide # 12,435 – 12,437 • Two missense nucleotide substitutions in codon 1314 of the ORF encoding the L polymerase, nt #12,438 A→C #12,440 A→G (I1314L)	sensitivity and attenuation phenotypes, which is stabilized by the I1314L genetically introduced point mutation.

Nucleotide substitution in the noncoding 5' trailer region, nucleotide #15,093 T→A	Spontaneous biological modification occurring during manufacturing

Detailed functionality and stability of the mutations

NS2 is a type I and III interferon antagonist that interferes with interferon induction and signaling. Deletion of the NS2 gene diminished RSV replication in vivo. NS2 also functions as a virulence factor, promoting epithelial cell shedding in vitro and in the hamster model, potentially contributing to small airway obstruction. Thus, deletion of NS2 may be beneficial for vaccine safety by increasing immunogenicity and decreasing reactogenicity.

The 112-nucleotide deletion is noncoding and the nucleotide substitutions in the last 4 codons of the SH Open Reading Frame, including the stop codon are translationally silent. These modifications are not known to impact translationally or phenotypically but instead create stability for the plasmid during propagation in bacteria.

The Δ1313 modification confers mild temperature sensitivity to the virus (shutoff temperature of 38ºC–39ºC), conferring restriction of virus replication that is greater in the warmer lower respiratory tract. As Δ1313 is subject to de-attenuation by a spontaneous I1314T modification; this is blocked by modifying codon 1314 to CTG (I1314L) (18).

The spontaneous nucleotide #15,093 T→A biological modification occurring during manufacturing, had no impact on viral characteristics during characterization of the viral stock. No other mutations were observed during evaluation of the RSV ΔNS2/Δ1313/I1314L genetic stability by 8 rounds of serial passage.

The GM vaccine strain is manufactured to GMP (good manufacturing standards) according to the quality dossier for it and will be subject to approval by the MHRA via a Clinical Trial Application. The GM vaccine is only being handled and administered by authorized staff at each site, and these staff are trained in laboratory safety and experienced in vaccination/controlled infection studies in adult volunteers.

References

1. **Jeffree CE, Rixon HW, Brown G, et al.** Distribution of the attachment (G) glycoprotein and GM1 within the envelope of mature respiratory syncytial virus filaments revealed using field emission scanning electron microscopy. Virology. 2003 Feb 15; 306(2):254-67.
2. **Lewis FA, Rae ML, Lehmann NI, et al**. A syncytial virus associated with epidemic disease of the lower respiratory tract in infants and young children. Med. J. Aust. 1961;48:932–933.
3. **Collins PL et al**. Production of infectious human RSV from cloned cDNA confirms an essential role for the transcription elongation factor from the 5'

proximal open reading frame of the M2 mRNA in gene expression and provides a capability for vaccine development. Proc. Natl. Acad. Sci. USA. 1995;92:11563–11567.
4. **Woolums AR, Lee S, Moore ML.** Animal Models of Respiratory Syncytial Virus Pathogenesis and Vaccine Development: Opportunities and Future Directions. [(accessed on 5 May 2019)] and 2011., RSV. Available online: https://www.intechopen.com/download/pdf/24392.
5. **Blount RE, Morris JA, Savage RE.** Recovery of cytopathogenic agent from chimpanzees with coryza. Proc. Soc. Exp. Biol. Med. 1956,92: 544–549.
6. **Channock R, Roizman B, Myers R.** Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). Am. J. Hyg. 1957,66: 281–290.
7. **Hall CB, Douglas RG, Schnabel KC, et al.** Infectivity of respiratory syncytial virus by various routes of inoculation. Infect. Immun. 1981,33: 779–783.
8. **Chare ER, Gould EA, Holmes EC.** Phylogenetic analysis reveals a low rate of homologous recombination in negative-sense RNA viruses. J. Gen. Virol. 2003, 84, 2691–2703.
9. **Spann KM, Collins PL, Teng MN.** Genetic recombination during coinfection of two mutants of human respiratory syncytial virus. J. Virol. 2003, 77, 11201–11211.
10. **Peck KM, Lauring AS.** Complexities of Viral Mutation Rates. J Virol. 2018 Jun 29 and 92(14):e01031-17.
11. Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. [Online] September 18, 2000. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:262:0021:0045:EN:PDF.
12. **Hall CB, Douglas RG Jr, Geiman JM**. J Infect Dis. 1980 Jan; 141(1):98-102.
13. **Drysdale SB, Green CA, Sande CJ.** Best practice in the prevention and management of paediatric respiratory syncytial virus infection. Therapeutic Advances in Infectious Disease. 2016 April; 3 (2): 63–71.
14. **Broberg EK, Waris M, Johansen K, et al.** European Influenza Surveillance Network. Seasonality and geographical spread of respiratory syncytial virus epidemics in 15 European countries, 2010 to 2016. Euro Surveill. 2018 Feb;23(5):17-00284.
15. **Hall CB, Weinberg GA, Iwane MK, et al.** The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009; 360:588–98.
16. **Shi T, McAllister DA, O'Brien KL, et al.** RSV Global Epidemiology Network. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017; 390:946-58.
17. **Bukreyev A, Belyakov IM, Berzofsky JA, et al.** Granulocyte-macrophage colony-stimulating factor expressed by recombinant respiratory syncytial virus attenuates viral replication and increases the level of pulmonary antigenpresenting cells. J Virol. 2001 Dec;75(24):12128-40.
18. **Luongo C, Winter C, Collins P, et al**. Respiratory Syncytial Virus Modified by Deletions of the NS2 Gene and Amino Acid S1313 of the L Polymerase Protein Is a Temperature-Sensitive, Live-Attenuated Vaccine Candidate That

Is Phenotypically Stable at Physiological Temperature. Journal of Virology. 2012;87(4):1985-1996.
19. **Teng M, Collins P.** Altered Growth Characteristics of Recombinant Respiratory Syncytial Viruses Which Do Not Produce NS2 Protein. Journal of Virology. 1999;73(1):466-473.
20. **Jin H, Zhou H, Cheng X, et al.** Recombinant Respiratory Syncytial Viruses with Deletions in the NS1, NS2, SH, and M2-2 Genes Are Attenuated in Vitro and in Vivo. Virology. 2000;273(1):210-218.
21. **Whitehead S, Bukreyev A, Teng M, et al.** Recombinant Respiratory Syncytial Virus Bearing a Deletion of either the NS2 or SH Gene Is Attenuated in Chimpanzees. Journal of Virology. 1999;73(4):3438-3442.
22. **Spann K, Tran K, Chi B, et al.** Suppression of the Induction of Alpha, Beta, and Gamma Interferons by the NS1 and NS2 Proteins of Human Respiratory Syncytial Virus in Human Epithelial Cells and Macrophages. Journal of Virology. 2004;78(8):4363-4369.
23. **Wright P, Karron R, Madhi S, et al.** The Interferon Antagonist NS2 Protein of Respiratory Syncytial Virus Is an Important Virulence Determinant for Humans. The Journal of Infectious Diseases. 2006;193(4):573-581.
24. IND015465. General Properties. RCHSB_NIAID_NIH.
25. **Karron RA, Luongo C, Mateo JS, et al.** Safety and Immunogenicity of the Respiratory Syncytial Virus Vaccine RSV/ΔNS2/Δ1313/I1314L in RSVSeronegative Children. J Infect Dis. 2020 Jun 16;222(1):82-91.
26. **Cortjens B et al.** Broadly Reactive Anti-Respiratory Syncytial Virus G Antibodies from Exposed Individuals Effectively Inhibit Infection of Primary Airway Epithelial Cells. Journal of Virology 91, 2017,e02357-02316.
27. **Ngwuta JO et al.** Prefusion F–specific antibodies determine the magnitude of RSV neutralizing activity in human sera. Science Translational Medicine 7, 2015,309ra162.
28. Evaluating the Infectivity, Safety, and Immunogenicity of Recombinant LiveAttenuated RSV Vaccines RSV ΔNS2/Δ1313/I1314L or RSV 276 in RSVSeronegative Infants 6 to 24 Months of Age. ClinicalTrials.gov Identifier: NCT03227029. [Online] https://clinicaltrials.gov/ct2/show/results/NCT03227029?view=results.
29. Study of a Live-Attenuated Respiratory Syncytial Virus Vaccine in Infants and Toddlers (VAD00001). ClinicalTrials.gov Identifier: NCT04491877. [Online] https://clinicaltrials.gov/ct2/show/NCT04491877.
30. **Belshe RB, et al.** Experimental respiratory syncytial virus infection of four species of primates. J Med Virol. 1977;1(3):157-62.
31. **Crowe JE Jr, and al.** A comparison in chimpanzees of the immunogenicity and efficacy of live attenuated respiratory syncytial virus (RSV) temperaturesensitive mutant vaccines and vaccinia virus recombinants that express the surface glycoproteins of RSV. Vaccine. 1993 Nov;11(14):1395-404.
32. **Clark M, Steger-Hartmann T.** A big data approach to the concordance of the toxicity of pharmaceuticals in animals and humans. Regul Toxicol Pharmacol. 2018 Jul;96:94-105.

33. **Grandin C**. Evidence for an intranasal immune response to human respiratory syncytial virus infection in cynomolgus macaques. J Gen Virol. 2015 Apr;96(Pt 4):782-792.
34. (IATA), International Air Transport Association. [Online] https://www.iata.org/en/publications/standards-manuals/.
35. World Health Organization. 1993. Disinfection and Sterilization. Laboratory Biosafety Manual (2nd ed., pp. 60-70).
36. **Gamiño-Arroyo AE et al**. Mexico Emerging Infectious Diseases Clinical Research Network (La Red). Epidemiology and clinical characteristics of respiratory syncytial virus infections among children and adults in Mexico. Influenza Other Respir Viruses. 2017 Jan;11(1):48-56.
37. **Cunningham, C. K., Karron, R. A., Muresan, P., et al**. International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) 2018 Study Team (2022). Evaluation of Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV/ΔNS2/Δ1313/I1314L and RSV/276 in RSVSeronegative Children. The Journal of infectious diseases, 226(12), 2069– 2078.
38. **Acosta PL, Caballero MT, Polack FP.** Brief History and Characterization of Enhanced Respiratory Syncytial Virus Disease. Clin Vaccine Immunol. 2016;23(3):189-95. doi: 10.1128/CVI.00609-15. PMID: 26677198; PMCID: PMC4783420.
39. **Buchholz UJ et al.** Live Respiratory Syncytial Virus (RSV) Vaccine Candidate Containing Stabilized Temperature-Sensitivity Mutations Is Highly Attenuated in RSV-Seronegative Infants and Children. J Infect Dis. 2018 Apr 11; 217(9):1338-1346.
40. **McFarland EJ et al.** Live Respiratory Syncytial Virus Attenuated by M2-2 Deletion and Stabilized Temperature Sensitivity Mutation 1030s Is a Promising Vaccine Candidate in Children. J Infect Dis. 2020 Feb 3; 221(4):534-543.
41. **Wright PF, Shinozaki T, Fleet W, et al.** Evaluation of a live, attenuated respiratory syncytial virus vaccine in infants. J Pediatr. 1976 Jun et 88(6):9316.
42. **Malkin E et al.** Safety and immunogenicity of a live attenuated RSV vaccine in healthy RSV-seronegative children 5 to 24 months of age. PLoS One. 2013 Oct 29; 8(10):e77104.
43. **Pirtle EC, Beran GW.** Virus survival in the environment. Rev Sci Tech. 1991 Sep et 10(3):733-48.
44. **Battles MB, McLellan JS.** Respiratory syncytial virus entry and how to block it. Nat Rev Microbiol. 2019 Apr et 17(4):233-245.

Application for consent to release a GMO

Part A2: data or results from any previous releases of the GMO
Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].

The safety and immunogenicity of the investigational RSV vaccine has been evaluated in 7 clinical trials so far:

- Four studies of the RSV ΔNS2/Δ1313/I1314L vaccine*, conducted by the US National Institutes of Health (NIH)/NIAID, also referred to as non-Sanofi trials
- One study of the Live-attenuated Respiratory Syncytial Virus vaccine candidate *, conducted by Sanofi (VAD00001).
- Two additional Sanofi trials are ongoing (VAD00014 and VAD00012)

** See Table 4 for a description of the NIH RSV ΔNS2/Δ1313/I1314L vaccine and the Sanofi Live-attenuated Respiratory Syncytial Virus vaccine candidate.*

Overall, more than 110 study participants have received the NIH RSV ΔNS2/Δ1313/I1314L vaccine at one or two doses. In the Sanofi VAD00001 study, approximately 155 study participants (infants and toddlers) have received the investigational product, at any dose or dosing regimen. The vaccine candidate was found to be well-tolerated in all studies, showed high infectivity, genetic stability and a robust immune response. The clinical trials completed to date or ongoing are listed in Table 1 and are described in further detail below. Subject participation in another Phase II study in the US (Puerto Rico) (VAD00014), involving approximately 50 Liveattenuated Respiratory Syncytial Virus vaccine candidate recipients was initiated in Q1 of 2023.

The quantities that will be released in the environment by vaccinated individuals will be negligible because of the restricted shedding of the attenuated virus. Due to the attenuation, and because most humans have pre-existing immunity against wt RSV, it is unlikely that the RSV ΔNS2/Δ1313/I1314L vaccine will cause a measurable infection in adults and older children. If infection with RSV ΔNS2/Δ1313/I1314L occurs, it is most likely to be asymptomatic, but symptoms of an upper respiratory tract infection may occur, such as rhinorrhea, pharyngitis, sneezing, and cough. Because of the attenuated phenotype of RSV ΔNS2/Δ1313/I1314L, symptoms caused by infection with RSV ΔNS2/Δ1313/I1314L, if any, are expected to be mild. In addition, the vaccine will induce immunity against natural occurring RSV.

RSV is not a persistent or invasive virus, and it is therefore highly unlikely that the attenuated virus (RSV ΔNS2/Δ1313/I1314L) becomes persistent and invasive. Because of its attenuation, the infective dose of the RSV vaccine, to be determined from the Phase I/II safety, infectivity and immunogenicity (VAD00001) study, is expected to be much higher than the wt RSV. The host range of RSV has not been changed by the attenuation of the virus.
Recombination of the RSV ΔNS2/Δ1313/I1314L virus with wt RSV is highly unlikely. The probability of RSV ΔNS2/Δ1313/I1314L recombining with other virus strains is

unlikely. RSV is a negative stranded non-segmented RNA virus for which recombination is generally rare or even absent. Being a non-segmented virus, RSV cannot recombine through re-assortment like influenza viruses. In this light, it is highly unlikely that RSV ΔNS2/Δ1313/I1314L will regain the complete genetic material for the NS2 gene deletion through recombination. The temperature sensitive attenuating deletion of codon 1313 in the polymerase (L) is stabilized by substitution of leucine (L) for isoleucine (I) at codon 1314.

Part A3: Details of previous applications for release

Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

This is first application submitted to the Secretary of State under the 2002 Regulations for release of RSV ΔNS2/Δ1313/I1314L in the UK.

An application to release RSV ΔNS2/Δ1313/I1314L under the Deliberate Release Directive has been submitted in the following European Member States:

- Spain on 28 August 2023 – Notification number B/ES/23/22

- Finland on 27 October 2023 – Application number (diary number): 001/MA/2023

Part A4: Risk assessment and a statement on risk evaluation

Risk Assessment: environmental impact of the release of the GMOs

When considering how the genetic modification to RSV and proposed activities conducted with RSV ΔNS2/Δ1313/I1314L might lead to harm to humans or the environment, all potential risks were characterized in relation to both the seriousness and likelihood of harm, taking into account the current scientific/technical knowledge and the proposed limits and controls. Both the short- and long-term impact were considered. Credible pathways of potential harm that were considered included exposure of people or animals to RSV ΔNS2/Δ1313/I1314L, potential for persistence of RSV ΔNS2/Δ1313/I1314L and the potential for recombination with other viruses. Potential harms that were considered in relation to these pathways included severe RSV disease and increased disease burden in people.

The overall risk linked to the use of RSV vaccine ΔNS2/Δ1313/I1314L for both humans and the environment is considered **negligible**.

The principal reasons for the conclusion of negligible risks are:

- the attenuation phenotype of the ΔNS2 and Δ1313/I1314L mutations in terms of reduced ability to replicate in vivo as demonstrated in nonhuman primates (18) and in RSV seronegative children aged 6 to 24 months of age (25) (37)

- the absence of shedding of RSV ΔNS2/Δ1313/I1314L in vaccinated seropositive children (25),
- there is no known animal reservoir for RSV,
- the suitability of the proposed limits and controls.

Risk assessment: factors affecting dissemination

RSV can spread when an infected person coughs or sneezes, releasing contaminated droplets into the air. Dissemination is affected by the titers of virus shed by the infected person. Dissemination is also affected by the external environmental factors such as temperature and humidity; in Europe, RSV infections exhibit seasonality with an average season starting in the beginning of December, peaking in early February and continuing until early April with wide variation between countries.

Risk assessment: human health impact

Five characteristics of the clinical vector that could pose risks to healthcare professionals and/or close contacts of the clinical trial subjects are:

1. Pathogenicity
2. Enhanced RSV disease
3. Production of biologically active/toxic products
4. Genetic stability; reversion to virulence, or recombination with other pneumoviruses
5. Survival and dissemination

Pathogenicity

While wildtype RSV viruses are pathogenic for humans, live-attenuated intranasal (IN) vaccine candidates have undergone decades of development to ensure safety in the most vulnerable population, RSV seronegative children 6 to 24 months of age. Data from NIH studies confirm the attenuation phenotype of the clinical vector (25) (37), showing it to be sufficiently safe and attenuated in RSV seronegative children. Therefore, the magnitude of the potential consequences of exposure of healthcare professionals and/or close contacts of the clinical trial subjects to the clinical vector in terms of pathogenicity are **negligible**.

Clinical trials demonstrated the minimal to absent infectivity by live-attenuated RSV vaccines in adults and seropositive children, and strong attenuation of the clinical vector in RSV-seronegative children (37) (25). Therefore, the probability of occurrence of disease in healthcare professionals and/or close contacts of the clinical trial subjects due to the clinical vector is **negligible**.

Enhanced RSV disease

Intramuscular delivery of a formalin inactivated RSV vaccine caused RSV disease enhancement following subsequent natural infection with wildtype RSV, resulting in the deaths of two children (38). Therefore, the severity of the potential consequence of enhanced disease in young children who are in close contact with the clinical trial subjects due to the exposure to the clinical vector is **high**.

Enhancement of RSV disease does not occur following IN immunization with live attenuated RSV vaccines (23) nor following a natural exposure to live wt RSV. *[Confidential information – please refer to Annex 4]*. Several studies with various liveattenuated RSV vaccine candidates showed no evidence of enhanced RSV disease (39) (40) (41) (23) (42). Therefore, the probability of the occurrence of enhanced RSV disease in young children who are in close contact with the clinical trial subjects is **negligible**.

Production of biologically active/toxic products

The clinical vector itself is the active substance. It does not produce any unnatural or non-native products or antigens that could be active or toxic. Thus, the magnitude of the hazard to healthcare professionals and/or close contacts of the clinical trial subjects is **negligible.**

As the clinical vector does not produce any unnatural or non-native products or antigens that could be active or toxic, the likelihood of hazard to healthcare professionals and/or close contacts of the clinical trial subjects is **negligible**.

Genetic stability; reversion to virulence, or recombination with other pneumoviruses

While transfer of genetic material from the clinical vector to wildtype viruses of the same genus could theoretically occur by homologous recombination under natural conditions, the clinical vector differs from wt RSV only by the attenuating mutations whose transfer would attenuate the wt virus. Transfer of genetic material from a wildtype virus to the clinical vector could at worst cause a reversion to wt virulence. However, reversion to wt would result in disease no worse than that caused by the wt RSV which is in constant circulation in the environment. Almost all children have been infected with RSV by the age of 2 years and infections recur throughout the lifetime. Consequently, the hazard poised to healthcare professionals and/or close contacts of the clinical trial subjects by the full reversion of the clinical vector to wild type virulence or by the recombination of the clinical vector with other pneumoviruses is **low**.

Genetic stability could be affected by two mechanisms: homologous recombination and accumulation of mutational changes due to selection pressure.

The organisms with which transfer of genetic material could occur by homologous recombination under natural conditions are viruses belonging to the same genus: Orthopneumovirus. In most negative sense RNA viruses including RSV, while sporadic examples indicate that homologous recombination does occur, natural recombination seems to be generally low (8). In an in-vitro co-infection study of two RSV mutants, a recombined variant was detected in only one of six coinfections (9). The isolation of only a single recombinant RSV under optimized experimental conditions suggests that such recombination is rare, and the likelihood of

recombination between the clinical vector and wt RSV or other pneumoviruses is negligible.

The second mechanism that could affect the genetic stability is the error-prone nature of replication of RNA virus genomes. Mutation rates vary between RNA viruses, ranging between 10^{-6} and 10^{-4} per nucleotide site per cell infection, depending on the RNA virus and methods used (10). The occurrence of point mutations is very rare in RSV A2 strain. Therefore, the risk linked to point mutations is negligible.

In a clinical study, the presence of the attenuating mutations was confirmed in all samples obtained at the peak of vaccine shedding from children immunized with the clinical vector (25).

In sum, the likelihood of the reversion to virulence of the clinical vector via mutation or recombination with wild type RSV is **negligible**.

Survival/dissemination

The severity of the potential consequence of the survival and dissemination of the clinical vector is linked to the assessment of its pathogenicity (please see above). The clinical vector is highly attenuated in humans and even in the worst case would result in disease no worse than that caused by wild-type RSV. Thus, the consequences of the survival and dissemination of the clinical vector beyond the clinical trial participants is **low**.

RSV does not survive well outside the human body (12) (43) and is very vulnerable to environmental changes. Given the attenuation of the clinical vector, it is likely to replicate to lower titers than wild-type RSV and be shed in reduced quantities and/or for a shorter period (25). The likelihood of survival and dissemination of the clinical vector beyond the vaccinees is considered **negligible**.

Table 6: Risk assessment for the RSV vaccine for each hazard for human health

Potential adverse effect	**Consequence**	**Likelihood**	**Overall Risk**
Pathogenicity	Negligible	Negligible	**Negligible**
Enhanced RSV disease	High	Negligible	**Negligible**
Production of biologically active/toxic products	Negligible	Negligible	**Negligible**
Genetic stability; reversion to virulence or recombination with other pneumoviruses	Low	Negligible	**Negligible**

Survival/dissemination	Low	Negligible	**Negligible**

Risk assessment: environmental impact

Five characteristics of the clinical vector could possibly result in adverse effects on the environment and organisms other than humans are:

1. Survival and dissemination
2. Recombination
3. Genetic stability; reversion to virulence
4. Pathogenicity to other organisms
5. Change of tropism

Survival and dissemination

Even under laboratory conditions, attenuated RSV strains do not cause disease and replicate only at low levels in animal models such as mice and non-human primates. Thus, it is unlikely that the clinical vector could infect and cause disease in wild mammals. The only significant impact could be on chimpanzees as this is only species besides humans known to experience RSV disease. The attenuation of the clinical vector was initially established in chimpanzees where inoculation resulted in a self-limiting infection with low levels of viral shedding which makes further transmission cycles unlikely (18). Thus, the consequence of potential survival and dissemination of the clinical vector outside the host are **negligible**.

RSV is a fragile, lipid enveloped virus sensitive to desiccation with an intracytoplasmic replication cycle. RSV does not replicate or long survive outside a host. The clinical vector viral shedding was evaluated in pre-clinical and clinical studies. *[Confidential information – please refer to Annex 4]*

The clinical vector will be administered via the intranasal delivery device which has a soft, conical plug on the tip that forms a seal with the nostril, preventing expulsion of fluid. The release will be handled by qualified persons and the generated waste will be treated to avoid accidental transmission. Therefore, the risk of dissemination from vaccinees is **low**.

Recombination

Natural recombination of negative-sense RNA viruses including RSV in the environment appears to be vanishingly rare or altogether absent (8). However, even in the “worst case scenario”, recombination could only result in reversion to wild type RSV which is naturally and constantly circulating in the environment. Therefore, the consequences of the potential recombination are **negligible**.

Natural recombination of negative-sense RNA viruses including RSV in the environment appears to be vanishingly rare or altogether absent (8). Moreover, even under optimized laboratory conditions, recombination of RSV is a very rare event (9). Therefore, the risk of recombination of the clinical vector is **negligible**.

Genetic stability; reversion to virulence

Apart from humans, chimpanzees are the only species known to be naturally susceptible to RSV infection and experience RSV disease. RSV infection in chimpanzees causes them to experience moderate nasal discharge and mild to moderate nasal congestion, similar to the common cold. Beyond these symptoms, there does not seem to be any further progression of the disease in chimpanzees. Therefore, the consequences of reversion to virulence of the clinical vector in the environment due to genetic instability would be **negligible**.

As the clinical vector was generated by the complete deletion of the NS2 gene, reversion to full virulence in the absence of recombination with wild type RSV is negligible. The Δ1313 mutation, which largely limits the virus infection to the upper respiratory tract and is stabilized by the I1314L mutation, was shown to be genetic stable in vivo in both chimpanzees and humans. Thus, the probability of reversion to virulence is considered **negligible**.

Pathogenicity to other organisms

Aside from the natural human hosts, chimpanzees are the only species known to experience RSV disease. While other animal models such as mice, cotton rats and African green monkeys are semi-permissive to wildtype RSV infection without disease symptoms, the ability of the clinical vector to replicate in these species is further suppressed. The clinical vector was initially tested for attenuation in RSV seronegative juvenile chimpanzees (18). Its attenuation profile and lack of pathogenicity in this model permitted progression to clinical evaluation in RSV seronegative human infants for whom safety concerns are paramount. Therefore, the risk of pathogenicity to other organisms is **negligible.**

In the natural environment RSV is only known to be pathogenic to humans and chimpanzees. The clinical vector was shown to be highly attenuated in both these species and to cause only mild upper airway symptoms. Therefore, the probability of the clinical vector being pathogenic to other organisms is considered **low**.

Change of tropism

Wildtype RSV is naturally constantly circulating worldwide. The clinical vector is attenuated by 1) the complete deletion of the NS2 gene, which is involved in the evasion of the innate immune system and 2) by the Δ1313 and I1314L mutations, which greatly decrease its ability to replicate in species with high body temperatures, as it is restricted for replication at 37°C (18). As a result, in all animal models the clinical vector was highly attenuated compared to wild type RSV. Therefore, should the clinical vector change the tropism and gain better infectivity in species other than humans, the severity of infection would be **low**.

RSV tropism is highly restricted to the apical surface of ciliated airway epithelial cells and type I alveolar pneumocytes and the tropism of RSV is most likely defined by its envelope genes F and G (44). Due to the excellent genetic stability demonstrated for the clinical vector and the low recombination rate, the probability of a tropism change is considered negligible.

Table 7: Likelihood of occurrence of adverse effect

Characteristics	Severity	Probability	Risk
Survival and dissemination	Negligible	Low	**Negligible**
Recombination	Negligible	Negligible	**Negligible**
Genetic stability; reversion to virulence	Low	Negligible	**Negligible**
Pathogenicity to other organisms	Negligible	Low	**Negligible**
Change of tropism	Low	Negligible	**Negligible**

Risk assessment: monitoring the GMO

The intended function of RSV ΔNS2/Δ1313/I1314L is to induce an RSV specific immune responses, which will be measured by assessment of immune responses against RSV. In addition, subjects participating in the clinical trial using RSV ΔNS2/Δ1313/I1314L will be monitored for clinical assessment (e.g. physical examinations), and adverse event monitoring.

Risk assessment: emergency response

Emergency response plans for accidental self-administration during handling or administering the clinical vector:

Accidental self-administration is unlikely because this is an intranasally vaccine administered by Healthcare Professionals. Monitoring, reporting and follow-up of adverse events will be conducted in the event accidental self-administration.

Emergency response plans for accidental release into the environment of the clinical vector:

Materials in contact with the RSV ΔNS2/Δ1313/I1314L will be considered as contaminated and all wastes (including vaccine vials, syringes, vial access cannulas, and nasal devices) will be placed in bins suitable for biohazardous waste directly following administration of the vaccine.

Decontamination of RSV ΔNS2/Δ1313/I1314L will be managed like any other enveloped virus. Decontamination by heat or chemicals (bleach, ethanol/isopropyl alcohol, detergents) can be envisaged. A few minutes at 100°C or with contact chemicals have been shown to achieve full decontamination. Therefore, autoclaving or incinerating, which are common processes of decontamination, are fully applicable to the RSV ΔNS2/Δ1313/I1314L vaccine.

Part A5: Assessment of commercial or confidentiality of information contained in this application.

Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

This electronic application containing the documents listed hereafter does not contain any commercially confidential information:

- Part A1-A6 non plant application form
- Part B public register
- Part C SNIF EU

The following confidential information which is not available in the public domain is sent to DEFRA separately as hard copy:

- Annex 1 to Part A1-A6 - Genetic sequence as it constitutes industrial commercial property
- Annex 2 to Part A1-A6 - CVs of the responsible persons as personal data is available
- Annex 3 to Part A1-A6 - List of clinical sites and their associated Principle Investigator personal data
- Annex 4 to Part A1-A6 - Dosage information as it constitutes industrial commercial property
- Part D declaration as personal data is available

Part A6: Statement on whether detailed information on the description of the GMO and the purpose of release has been published.

Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.

This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register

A description of RSV ΔNS2/Δ1313/I1314L and the purpose of release is published in International Publication Number WO 2013/154728 and at least in the following article: Karron RA, Luongo C, Mateo JS, Wanionek K, Collins PL, Buchholz UJ.

Safety and Immunogenicity of the Respiratory Syncytial Virus Vaccine RSV/ΔNS2/Δ1313/I1314L in RSV-Seronegative Children. J Infect Dis. 2020 Jun 16;222(1):82-91.