Part B: Information about the release application to be included on the public register

B1 The name and address of the applicant

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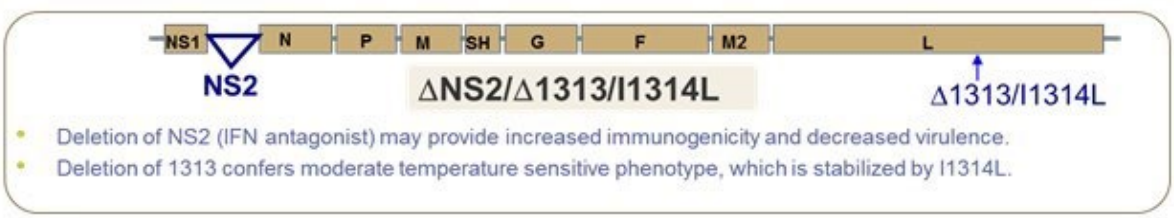
USA

B2 A general description of the genetically modified organisms in relation to which the application is being made.

The RSV ΔNS2/Δ1313/I1314L vaccine candidate is a live attenuated RSV virus constructed using reverse genetics. RSV ΔNS2/Δ1313/I1314L does not express any novel or unnatural genes. Furthermore, each modification in RSV ΔNS2/Δ1313/I1314L can occur naturally, at least in isolation. RSV ΔNS2/Δ1313/I1314L was derived via recombinant Deoxyribonucleic Acid (DNA) technology by deleting the NS2 gene and the 1313 codon and replacing isoleucine at 1314 with leucine in wild type (wt) RSV A2 strain genome (see figure 1). In addition, the A2 genome backbone has been modified by deleting a 112-nucleotide (nt) region from the downstream noncoding region of the SH gene and silently modifying the last few codons of the SH open reading frame. The goal of these modifications was to rationally attenuate RSV to make it a safe and effective live attenuated vaccine. Furthermore, each modification in RSV ΔNS2/Δ1313/I1314L can occur naturally, at least in isolation.

Figure 1: Genome Structure of RSV ΔNS2/Δ1313/I1314L



B3 The location at which the genetically modified organisms are proposed to be released.

The clinical trial will be conducted at the following UK clinical sites:

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

B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

Sanofi is developing a prophylactic RSV ΔNS2/Δ1313/I1314L live attenuated vaccine candidate to prevent RSV. RSV ΔNS2/Δ1313/I1314L will be administered by intranasal route to subjects participating in the Phase III, randomized, observer-blind, placebo-controlled, multi-center, multinational study to evaluate the efficacy, immunogenicity, and safety of a Respiratory Syncytial Virus vaccine in infants and toddlers. Study duration will be between 20 to 21 months for each participant. It is anticipated that around 5334 subjects will be enrolled during this international multicenter study. While the candidate vaccine addresses an urgent need for protecting human health, there is no expected benefit for the environment.

B5 The intended dates of the release.

It is anticipated that enrollment will begin in the UK and EU in May 2024 and will be completed by November 2028.

B6 The environmental risk assessment.

The Environmental risk assessment considers how the genetic modifications to RSV and proposed activities conducted with the RSV ΔNS2/Δ1313/I1314L vaccine candidate might lead to harm to humans or the environment. The risks were characterized in relation to both the seriousness and likelihood of harm, taking into account the current scientific/technical knowledge, information in the application (including proposed limits and controls) and relevant previous approvals. Both the short- and long-term impact were considered.

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Credible pathways of potential harm that were considered include 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 ΔNS2/Δ1313/I1314L vaccine for both humans and the environment is considered as negligible.

The principal reasons for the conclusion of negligible risks are:

- The attenuation phenotype of the ΔNS2 mutation (1) in terms of reduced ability to replicate *in vivo* as demonstrated in nonhuman primates (2) and in RSV seronegative children aged 6 to 24 months of age from NIH studies (3); The attenuating deletion of codon 1313 in the polymerase (L) phenotypically stabilized by substitution of leucine (L) for isoleucine (I) at codon 1314 conferring mild temperature sensitivity (shutoff temperature of 38ºC-39ºC) (4). The temperature sensitive feature of the vaccine candidate will significantly limit its replication capacity in the lower respiratory and therefore ensures safety;
- The absence of shedding of RSV ΔNS2/Δ1313/I1314L in vaccinated seropositive children (3). Furthermore, in seronegative children, the vaccine candidate had excellent low infectivity and was well-tolerated (3) (5);
- There are no added genes to this vaccine candidate other than deletions, i.e., ΔNS2 and Δ1314 as well as the codon replacement of I1314L that can occur naturally, at least in isolation. As a result, no introduction of new genetic materials that can be released into the environment or transferred to others; • The fact that there is no known animal reservoir for RSV (6) (7), and
- The suitability of the controls proposed by Sanofi.

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

B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

Methods for monitoring the GMOs

The intended function of RSV ΔNS2/Δ1313/I1314L is to induce an RSV specific immune response, 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.

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Emergency response plans

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.

Sanofi Pasteur Inc. 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.

References

1. **Wright PF, Karron RA, Madhi SA, et al.** The interferon antagonist NS2 protein of respiratory syncytial virus is an important virulence determinant for humans. J Infect Dis. 2006;193(4):573-81.

2. **Luongo C. 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. . *J Virol. 2012;87(4):1985–96.*

3. **Karron RA, Luongo C, Mateo JS, et al.** Safety and Immunogenicity of the Respiratory Syncytial Virus Vaccine RSV/ΔNS2/Δ1313/I1314L in RSV-Seronegative Children. J Infect Dis. 2020;222(1):82-91.

4. **Liesman RM, Buchholz U, Luongo CL, et al.** RSV-encoded NS2 promotes epithelial cell shedding and distal airway obstruction. J Clin Invest. 2014;124(5):2219-33.

5. **Cunningham CK, Karron RA, Muresan P, et al.** Evaluation of Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV/ΔNS2/Δ1313/I1314L and RSV/276 in RSV-Seronegative Children. J Infect Dis. 2022;226(1.

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6. **Tripp, R.A, Mahy B.W.J, Ter Meulen V.** Pneumovirus and Metapneumovirus: respiratory syncytial virus and human metapneumovirus. Topley & Wilson's Microbiology & Microbial Infections - Virology, 2009: 783-806.

7. **G., Taylor.** Animal models of respiratory syncytial virus infection. Vaccine. 2017;35(3):469-80.