Preclinical efficacy, safety and immunogenicity assessment of a vaccine candidate in a cynomolgus monkey model of SARS-CoV-2 infection

Joachim Confais[1], Marion Toussenot[1], Stéphane Ployart[1], Gregori Bech-Sabat[2], Mercè Roca[2], Andrés Pizzorno[3], Thomas Julien[3], Manuel Rosa-Calatrava[3,4], Ricard March[2], Hugues Contamin[1]

1 Cynbiose, 1 av. Bourgelat, 69280 Marcy-l'Étoile, France
2 HIPRA, Avda. La Selva, 135, 17170 Amer (Girona), Spain
3 CIRI, Centre International de Recherche en Infectiologie, Team VirPath, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007 Lyon, France
4 VirNext, Faculté de Médecine RTH Laennec, Université Claude Bernard Lyon 1, Université de Lyon, 69008 Lyon, France

INTRODUCTION

Cynbiose, in collaboration with the laboratory VirPath, successfully developed a monkey model of SARS-CoV-2 infection, first in the African Green Monkey (2020), then in the Cynomolgus macaque (2021). HIPRA has developed an adjuvanted vaccine candidate, **PHH-1V**, based on a recombinant fusion heterodimer protein consisting of the RBD domain of the beta and alpha SARS-CoV-2 variants.

OBJECTIVES

A challenge study in **cynomolgus macaques** (*Macaca fascicularis*) was conducted in order to assess the efficacy, immunogenicity and safety of a prime-boost immunization scheme with the PHH-1V vaccine.

METHODS

Animals: Twelve cynomolgus macaques (6 females and 6 males, origin Vietnam, naive, 26-30mo, 2.4-2.6kg), social housing, Cynbiose's AAALAC-accredited facility in France. 4-weeks acclimation period prior to prime vaccination.

Vaccination: IM on day 0 and day 21 with 40 µg of PHH-1V vaccine (3M/F) or PBS (control item; 3M/F).

Viral challenge: On day 36, IT+IN challenge with SARS-CoV-2 (strain D614G, $2*10^6$ PFU/animal) using a microsprayer (Penn-Century).

In vivo monitoring: The animals were monitored for six days post-infection. Blood samples and samples from the airways were taken at different points of the study (oropharyngeal and nasopharyngeal swabs, BAL). Frequent weighing, daily clinical assessment, implanted and rectal temperature measurements. On day 42, all animals were euthanized for organ collection.

Viral copies quantification by RT-qPCR: Airways and organs samples were collected in RNA*later* and stored at -20°C. Analysis by RT-qPCR of the gene ORF1b-nsp14 (SARS-CoV-2), with that of the simian housekeeping gene GAPDH for reference. LOQ cutoff determined at >35Ct (i.e.<2.3 log viral copies/sample). Number of viral copies expressed relative to 1000 copies of GAPDH gene copies, excluding data points below LOQ as negatives.

Viral titer detection: Airways and lung samples were stored in PBS and analyzed within a few hours for $TCID_{50}$ determination. Serial 10-fold dilution from 10^{-1} to 10^{-6} on VeroE6 cells, in quadruplicate. TCID50 determined by observation of cytopathic effect after a 4-days incubation period at +37°C with 5% $C0_2$.

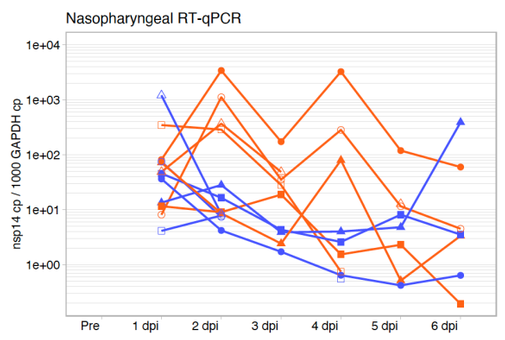
Histopathology: Upper airways and lung samples stored in 4% formalin, FFPE, HE-stained, microscopic inspection by boarded pathologist (Novaxia, France). Compound score for each animal indicates the sum of inflammation scores (0-5) in the 6 lobes inspected.

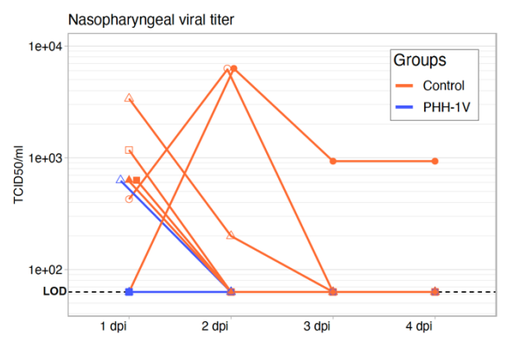
Seroneutralization assays: Serum samples collected on 4 time points. Dilution from 1/20th to 1/10 240^{th} by two-fold steps. Added to Vero E6 cells, infected with a theoretical titer of $100TCID_{50}$/µL of one of four different strains of SARS-COV-2. Used strains were D614G (same used to infect animals), Alpha B.1.1.7 (UK), Gamma P1 (Brazil), Delta B.1.617.2 (India). Absence of cytopathic effect at the highest dilution factor was used to determined the neutralizing antibody titer.

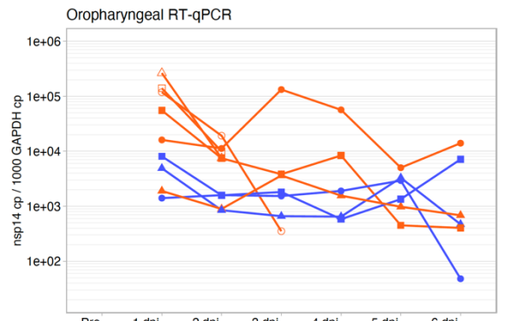
RESULTS

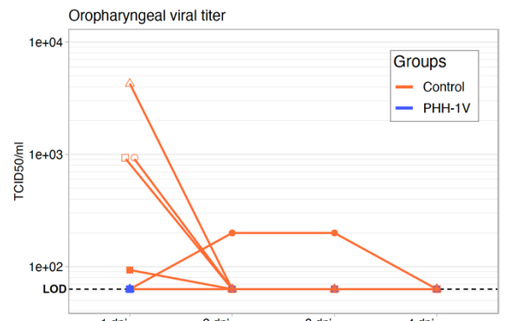
Safety: PHH-1V vaccine was shown to be safe and well tolerated: no adverse reaction. No change in body temperatures or body weight, no change in clinical pathology different from the group.

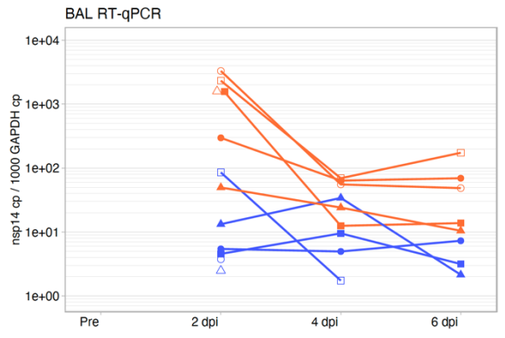
Protection against viral infections (airways): Clear decrease of viral copies in the upper and lower airways in vaccinated animals. Presence of infectious virus in most control animals, but in virtually no vaccinated ones.

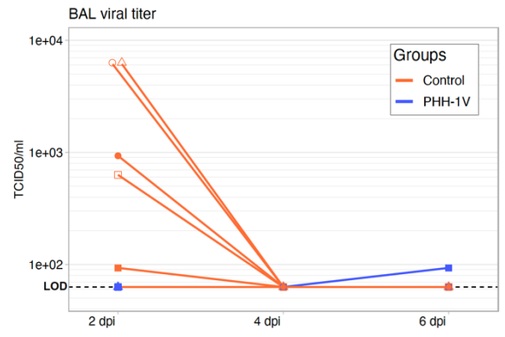




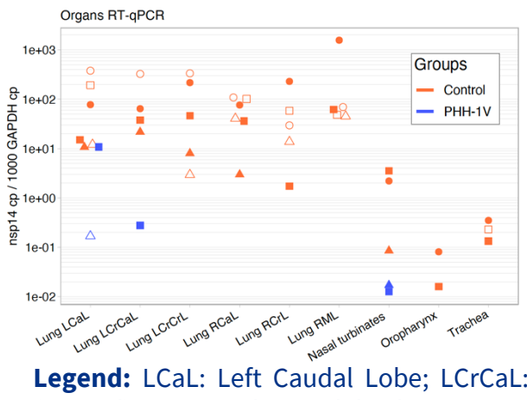


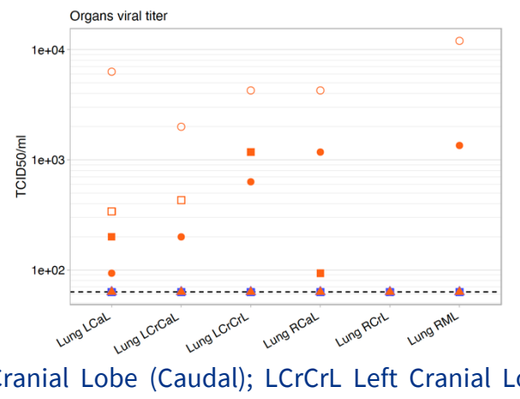






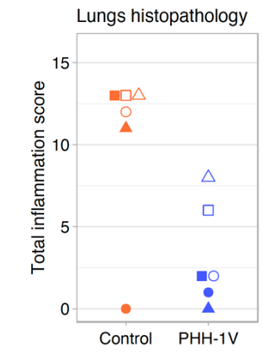
Protection against viral infections (organs, 6 dpi): Clear decrease of viral copies in the lungs and airways in vaccinated animals. Presence of infectious virus in 4/6 control animals, but in no vaccinated ones.

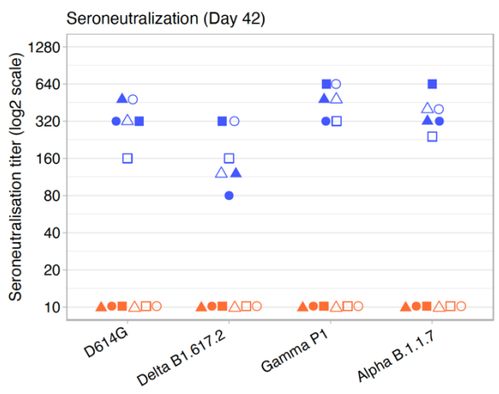




Legend: LCaL: Left Caudal Lobe; LCrCaL: Left Cranial Lobe (Caudal); LCrCrL Left Cranial Lobe (Cranial); RCaL: Right Caudal Lobe; RML: Right Medial Lobe; RCrL: Right Cranial Lobe

Protection against bronchointerstitial inflammation (6 dpi):
All but one control animals showed a higher degree of lung inflammation than vaccinated ones.





Multi-strain seroneutralization:
Vaccinated animals show strong seroneutralization against 4 different strains of SARS-CoV-2.

CONCLUSION

With 6 vaccinated and 6 placebo cynomolgus monkeys, Cynbiose and VirNext's NHP model of SARS-CoV-2 infection showed Hipra's PHH-1V vaccine to be safe, to induce a strong multi-strain neutralizing antibody response, and to be protective against viral replication in the upper and lower airways and against bronchointerstitial inflammation.

CONTACT@CYNBIOSE.COM