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Summary

Background V591 (TMV-083) is a live recombinant measles vector-based vaccine candidate expressing a pre-fusion stabilized SARS-CoV-2 spike protein.

Methods We performed a randomized, placebo-controlled Phase I trial with an unblinded dose escalation and a double-blind treatment phase at 2 sites in France and Belgium to evaluate the safety and immunogenicity of V591. Ninety healthy SARS-CoV-2 sero-negative adults (18-55 years of age) were randomized into 3 cohorts, each comprising 24 vaccinees and 6 placebo recipients. Participants received two intramuscular injections of a low dose vaccine (1×10^5 median Tissue Culture Infectious Dose [$TCID_{50}$]), one or two injections of a high dose vaccine (1×10^6 $TCID_{50}$), or placebo with a 28 day interval. Safety was assessed by solicited and unsolicited adverse events. Immunogenicity was measured by SARS-CoV-2 spike protein-binding antibodies, neutralizing antibodies, spike-specific T cell responses, and anti-measles antibodies. ClinicalTrials.gov, NCT04497298.

Findings Between Aug 10 and Oct 13, 2020, 148 volunteers were screened of whom 90 were randomized. V591 showed a good safety profile at both dose levels. No serious adverse events were reported. At least one treatment-related adverse event was reported by 15 (20.8%) participants receiving V591 vs. 6 (33.3%) of participants receiving placebo. Eighty-one percent of participants receiving two injections of V591 developed spike-binding antibodies after the second injection. However, neutralizing antibodies were detectable on day 56 only in 17% of participants receiving the low dose and 61% receiving the high dose (2 injections). Spike-specific T cell responses were not detected. Pre-existing anti-measles immunity had a statistically significant impact on the immune response to V591, which was in contrast to previous results with the measles vector-based chikungunya vaccine.

Interpretation While V591 was generally well tolerated, the immunogenicity was not sufficient to support further development.

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Key words: SARS-CoV-2; COVID-19; vaccine; measles vector

Research in context

Evidence before this study

We searched the WHO "Novel COVID-19 vaccine tracker" and the "LSTMH VaC tracker" for vaccine candidates from entering clinical development to authorization. As of July 22, 2021, 108 vaccines were under clinical investigation of which 18 had been authorized. Eighty-five of these candidates, including all authorized vaccines, were based on mRNA, non-replicating viral vectors, inactivated virus, or protein subunits. Only 2 vaccine candidates were based on replicating viral vectors, a recombinant live attenuated influenza virus and a recombinant vesiculovirus (rVSV). The measles vaccine is a live attenuated virus that has an excellent safety record, is highly efficacious, and induces humoral and cellular immunity. Using the measles vaccine virus as vector, replicating recombinant measles vaccines can be engineered. A measles-vectored vaccine candidate against chikungunya virus (MV-CHIK) was shown to be well tolerated and highly immunogenic in Phase I and II trials. A large pipeline of measles-vectored pre-clinical candidates has generated the expertise for rapid development of new candidates. Of particular relevance to the development of the V591 against the novel SARS-CoV-2 coronavirus was our experience with the related virus SARS-CoV.

Added value of this study

This is a first-in-human study. The results show that the V591 candidate was well tolerated by intramuscular injection. But immune responses induced by V591 were lower than expected from the previous results with MV-CHIK in Phase I and II trials and from the strong immunogenicity of V591 observed during pre-clinical development. It is interesting to note that pre-existing anti-measles immunity appeared to impact V591 in this study, whereas this was not observed with MV-CHIK, despite leveraging of the same technology.

Implications of all the available evidence

Based on the low immune responses to V591, further development has been abandoned. The results have triggered investigations to identify potential reasons for the poor immunogenicity of V591 in humans and to inform future MV-based vaccine candidates. The previous results with MV-CHIK indicated that the platform technology is able to induce strong immune responses in humans.

Introduction

The Coronavirus Disease 19 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic by WHO on March 11, 2020, and has resulted in an unprecedented global public health burden with high socio-economic impact. More than 190 million confirmed cases and 4.0 million deaths were reported as of July 22, 2021.[1] Vaccination is thought to be the most effective long-term measure to control SARS-CoV-2 dissemination and to stop the pandemic. At the time of writing, more than 100 candidates had entered clinical testing and 18 vaccines were in use.[2,3] First population effectiveness studies after vaccine roll-out had provided real-world indications that the vaccines can curb the pandemic.[4,5] The vaccines currently in use are based on four different technologies, 2 mRNA vaccines, 4 non-replicating viral vector vaccines based on chimpanzee adenoviral vector, adenovirus type 5 vector, and/or adenovirus 26 vector, 8 inactivated vaccines, and 4 protein-based subunit vaccines.[3,5]

We aimed to develop a replicating viral vector COVID-19 vaccine based on the measles virus (MV) vector technology.[6] Replicating viral vector vaccines are based on non-pathogenic or attenuated viruses carrying additional heterologous genes to express antigens from target pathogens for vaccination against those pathogens. Based on their replicative nature, they have the potential to elicit long-lived immunity after one or two injections with substantially lower doses than used for non-replicative vectors. [7-10]

The measles vaccine is an attenuated virus that induces humoral and cellular immunity. It has an excellent safety record, is highly efficacious and genetically stable, and likely provides lifelong immunity. Based on these characteristics, the measles vaccine virus provides an attractive vector for generating live recombinant vaccines.[6,11] It has been used for the development of vaccine candidates against a variety of viral diseases, including but not limited to chikungunya, West Nile fever, dengue, HIV infection, SARS, Middle-East respiratory syndrome, Zika, and Lassa fever.[12-18] The most advanced MV-based vaccine candidate, a vaccine against chikungunya virus (MV-CHIK)[12], was shown to be well tolerated and highly immunogenic in Phase I and Phase II trials.[19,20] Additional MV-based candidates currently in clinical development target Zika[17] and Lassa viruses.[18] V591, also called TMV-083, is a recombinant measles vector-based vaccine candidate expressing a

pre-fusion stabilized SARS-CoV-2 spike protein. This candidate elicited strong and sustained spike-binding and SARS-CoV-2 neutralizing antibody responses as well as T helper cell type 1 (Th1) and cytotoxic T cell responses in animal models (preclinical data will be reported separately).

Here we report the results from a placebo-controlled, randomized Phase I study with an unblinded dose-escalation and a double-blind treatment phase to evaluate the safety and immunogenicity of V591.

Methods

Study design and ethics

We performed a randomized, placebo-controlled Phase I trial with an unblinded dose escalation and a double-blind treatment phase at 2 sites, the Clinical Investigation Center (CIC) Cochin-Pasteur (Paris, France) and the Clinical Pharmacology Unit (CPU) of SGS (Antwerp, Belgium). The trial was conducted in compliance with the principles of Good Clinical Practice and the Declaration of Helsinki for biomedical research involving human beings. It was approved by the French National Ethics Committee (Comité de Protection des Personnes, CPP Île de France 3), the UZ Leuven Ethische Commissie, and the Institut Pasteur Institutional Review Board. The trial was registered at ClinicalTrials.gov, NCT04497298. The study protocol is provided as Supplementary Material.

Ninety volunteers were included in 3 cohorts, each cohort comprising 24 vaccinees and 6 placebo recipients. Participants received two upper deltoid intramuscular injections, on day 0 and day 28, of a low dose vaccine ("low dose", 1×10^5 median Tissue Culture Infectious Dose 50 [$TCID_{50}$]), two injections of a high dose vaccine ("high dose [2 inj.]", 1×10^6 $TCID_{50}$), 1 injection of the high dose vaccine and 1 injection of placebo ("high dose [1 inj.]"), or two injections of placebo as outlined in Figure 1. The immunization route and dose levels were selected based on experience with previous MV-based vaccine candidates.[19,20] As a safety precaution, the study began with the enrollment of six sentinel participants (3 receiving low dose and 3 the high dose) in an unblinded manner at the CIC Cochin-Pasteur. A safety phone call was made 24 h after each injection in these sentinel participants. After review of the follow-up safety data up to day 14 of the 6 sentinel participants by the Data Safety Monitoring Board (DSMB) and resulting positive recommendation, 84 participants were randomized into the double-blind study phase. Additionally, safety data after the second injection of the sentinels at day 28 were reviewed by medical monitoring before continuing to the second injection of the double-blind participants.

Blood samples for safety and immunogenicity assessment were collected at day 0, day 7 (sentinel participants only), and days 14, 28, 56 and 91. A final safety follow-up was performed on day 210. The study was originally planned to continue until day 390. The protocol was amended to conclude with the day 210 visit based on an intermediate data analysis after day 56 showing insufficient immunogenicity.

Participants

Adults 18-55 years of age in good health, as established by medical history, vital signs, physical examination and laboratory assessments, and with a body mass index of < 30.0 kg/m^2 were eligible. We excluded participants who were actively or previously infected by SARS-CoV-2 as determined by a positive RT-PCR or serology result or had previously received another investigational COVID-19 vaccine. Other exclusion criteria included contacts with confirmed SARS-CoV-2-infected individuals within 2 weeks prior to enrollment; pregnancy; lactation; history of immunodeficiency or immunosuppressive therapy; any condition that is or might be associated with increased risk of severe COVID-19 disease; and living and/or working with severely immunocompromised people, pregnant or lactating women, or infants under 12 months of age. The protocol in the supplementary material provides a complete list of inclusion and exclusion criteria. All participants provided written informed consent.

Randomization and masking

The first six participants were assigned to the unblinded dose escalation phase, 3 each to cohorts A (low dose) and B (high dose [2 inj.]). The 84 participants of the double-blind part were randomly assigned to one of three different cohorts, receiving V591 or placebo at the allocation ratio 7:2 for cohorts A and B and 4:1 for cohort C (high dose [1 inj.]). Randomization was done via computer-generated random treatment assignments. The randomization number was assigned to a participant by allocation of the next available randomization entry in the randomization list which was established before the start of the study. During the double-blind phase, the participants, investigators, and site personnel performing study-related assessments, as well as the sponsor representatives involved in the data monitoring and conduct of the study were blinded. In addition, the lab tests for both safety and immunogenicity outcomes were carried out in a blinded manner by the lab technicians.

Procedures

Based on the experience with a preclinical MV-based SARS-CoV vaccine[14] a human codon-optimized full-length spike protein of SARS-CoV-2, based on the protein sequence of the original Wuhan strain, was synthesized and inserted as an additional transcription unit

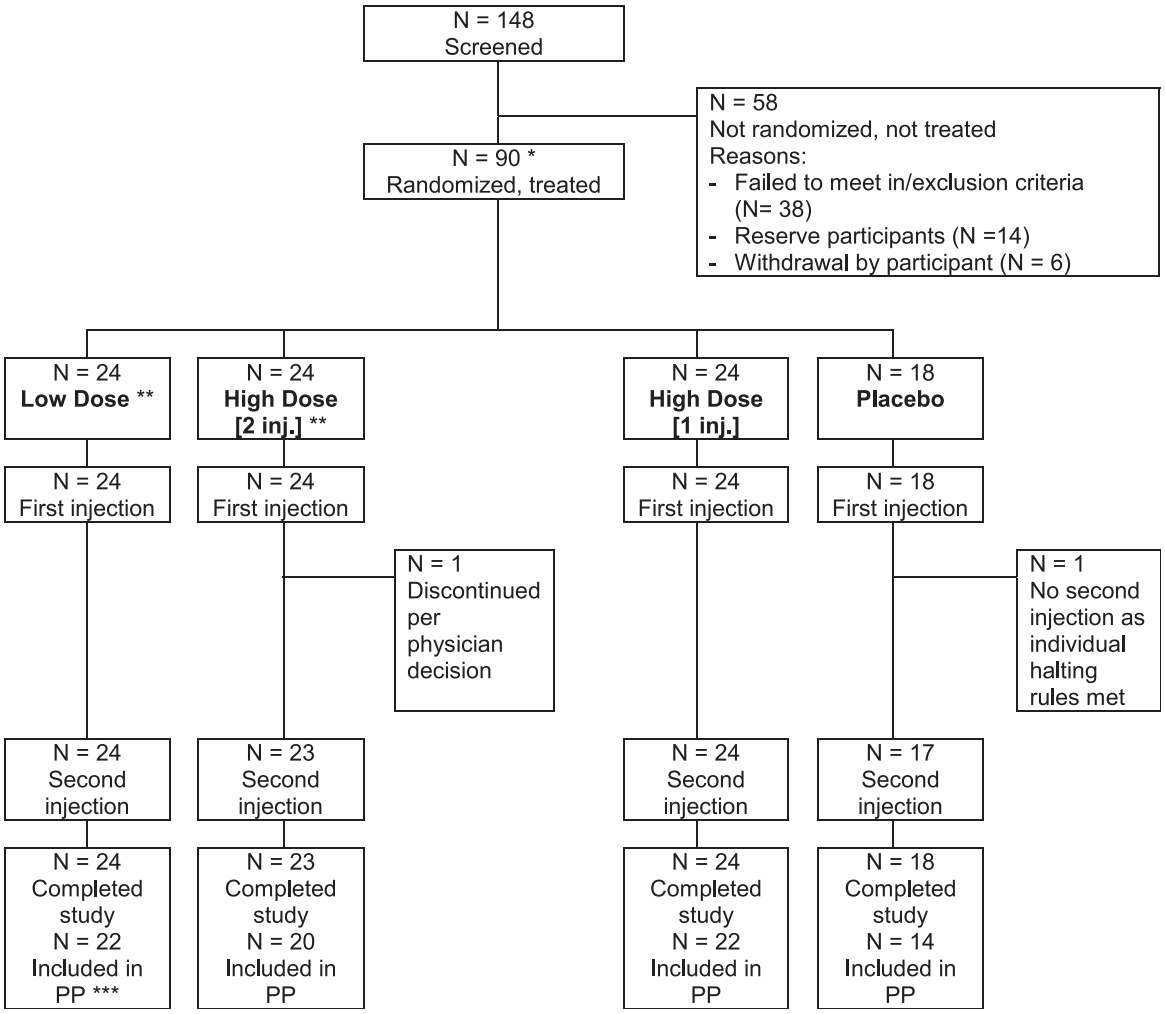

Figure 1. Trial Profile.

Participants received 2 intramuscular injections on day 0 and day 28 of a low dose vaccine (**low dose**), 2 injections of a high dose vaccine (**high dose [2 inj.]**), 1 injection of the high dose vaccine and 1 injection of placebo (**high dose [1 inj.]**), or 2 injections of placebo (**placebo**). * all 90 participants were included in the safety analysis set and in the mITT analysis set, ** The first 3 participants in these groups were assigned as sentinel participants to the unblinded dose-escalation phase, ***12 participants were excluded from the PP analysis set: i) 10 participants due to major protocol deviations, ii) 1 participant did not receive the second injection, iii) 1 participant was discontinued further to investigator decision.

into the Schwarz strain measles vector.[6] Modifications were introduced into the sequence to lock the protein in the pre-fusion conformation (K986P+V987P[21]), remove the loop encompassing the furin cleavage site (675-QTQTNSPRRAR-685) and potentially enhancing cell surface expression by mutation of the endoplasmic reticulum retrieval signal (K1269A+H1271A[22]). The construct was initially named TMV-083 (name used in the clinical protocol) before receiving its final name, V591.

The V591 vaccine was manufactured in Vero 10-87 cells and filled by ABL Europe S.A.S. (France) according to Good Manufacturing Practices. Placebo (0.9% sodium chloride, USP or BP sterile saline) was sourced locally. Vials containing V591 product were stored frozen (≤-65°C) before preparation and vaccination. The vaccine was thawed and administered within 60 minutes after removal from the freezer.

Adverse Events (AEs) observed by the study physician or delegate or reported by the participants were collected throughout the study. All AEs were coded and grouped for System Organ Class (SOC) and Preferred Term (PT) according to MedDRA version 23.0. The severity of clinical and laboratory adverse events was graded according to the FDA "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled

in Preventive Vaccine Clinical Trials". The relatedness of the AE to the treatment was evaluated by the clinical investigator.

Samples to assess potential measles virus shedding were collected from the six sentinel participants. Saliva, nasal swabs, urine, and whole blood were collected at days 0, 7, 14, 28, and 42 and analyzed for the presence of measles virus RNA by quantitative Polymerase Chain Reaction following reverse transcription (RT-qPCR)[13] at Texcell (Evry, France).

Serum and peripheral blood mononuclear cells (PBMC) were prepared from blood samples at all time points and frozen until assayed. SARS-CoV-2 spike protein-binding antibodies were analyzed by enzyme-linked immunosorbent assay (ELISA) using as target antigen a recombinant, purified, trimerized spike protein ectodomain stabilized in the pre-fusion configuration.[23] The presence of serum neutralizing antibodies was assessed using a pseudotyped virus neutralization assay.[24] Both assays were performed at Nexelis (Laval, Canada). Antibodies to measles were measured at Bioaster (Lyon, France) using the Anti-Measles Virus ELISA [IgG] kit (Euroimmun EI 2610-9601G). Antibody responses to the SARS-CoV-2 nucleoprotein (N) were measured as control of exposure of the study participants to SARS-CoV-2 (N protein not included in vaccine) using the Roche-Elecsys® Anti-SARS-CoV-2 electro-chemiluminescent immunoassay at PPD (Zaventem, Belgium). To assess T cell responses, PBMC were stimulated with peptide pools spanning the S1 and S2 domains of the spike protein (15 amino acid peptides with 10 amino acids overlap), stained with α-CD3, α-CD4, α-CD8 and analyzed by intracellular cytokine staining for INF-γ, TNF-α, IL-5, IL-13 and flow cytometry. The T cell analyses were performed at Bioaster.

Outcomes

The primary outcome was safety and tolerability of V591 following one or two injections as evaluated by i) the rate of solicited AEs up to 14 days after each injection, ii) the rate of unsolicited AEs up to 28 days after each injection, and iii) the rate of serious adverse events (SAEs), serious adverse reactions (SARs), suspected unexpected serious adverse reactions (SUSARs), and adverse events of special interest (AESIs) all along the study period.

Secondary outcomes were the induction and persistence of i) SARS-CoV-2 spike protein-binding antibodies and ii) neutralizing antibodies up to study day 91, iii) induction of SARS-CoV-2 spike protein-specific T cells up to day 91 (stopped after day 56 based on results of an intermediate analysis up to day 56 showing insufficient immunogenicity), and iv) occurrence of potential measles virus shedding. Exploratory endpoints were: i) anti-measles antibody levels at days 0, 28, 56, ii) natural exposure of the participants to SARS-CoV-2 during the study measured by anti-N antibody response until day 91, and iii) occurrence of diagnosed COVID-19 cases in study participants during the study.

Statistical analysis

No formal sample size calculation was performed. The sample size of 90 participants was determined based on prior experience with measles-vectored vaccine candidates[19,20] and estimated to detect general differences.

All participants who received at least one injection were included in the safety analysis set and the modified intention to treat (mITT) analysis set. Solicited (local and systemic) and unsolicited AEs were summarized for each cohort and analyzed descriptively. Solicited (local and systemic) and unsolicited AEs were compared pairwise between each active treatment group and the pooled placebo group using Fisher's exact tests.

The secondary immunogenicity endpoints and anti-MV antibody results were analyzed within each group and summarized by descriptive statistics. Spike-binding antibody and neutralizing antibody Geometric Mean Titers (GMT) were compared between the four groups by applying a longitudinal model (Mixed Model for Repeated Measures, MMRM) including the fixed effects treatment, timepoint, and the interaction between treatment*timepoint, with and without anti-MV ELISA titer at baseline (10log IU/L) as an additional fixed factor. Aside from main effects of the model, simple effects were shown together with pairwise comparison between treatment groups at different timepoints. Within-participant variability was captured with an unstructured (type=UN) covariance matrix. GMTs and GMT ratios were estimated using log10 transformed data and taking the anti-log of the resulting point estimates. This approach was followed for the least squares means, least squares means differences, and the corresponding two-sided 95% Confidence Intervals.

In addition, SARS-CoV-2 spike-specific IgG Ab levels were analyzed against the anti-MV antibody levels at baseline. The 72 participants having received V591 were stratified into quartiles based on anti-measles IgG Abs titers at day 0. The GMTs in the 4 quartiles were compared using a similar MMRM model as described above with quartile groups as factor.

Statistical analyses and programming were performed with SAS version 9.4. Summary graphs were prepared using GraphPad Prism 8.4.

Role of the funding source

Themis provided the V591 GMP batches, funding for the clinical trial, and contributed to the clinical study design, data interpretation, and review of the report. CEPI supported the pre-clinical activities to develop the V591 vaccine candidate, manufacturing set-up, and clinical trial set-up, and participated in the clinical study

Parameter	Low Dose N = 24	High Dose [2 inj.] N = 24	High Dose [1 inj.] N = 24	Placebo N = 18	All Participants N = 90
Age, years					
Median	37.0	40.0	35.0	39.5	38.0
Range	(19; 54)	(23; 55)	(20; 55)	(21; 55)	(19; 55)
Height, cm					
Median	168.6	170.0	172.2	172.8	170.0
Range	(155; 188)	(154; 193)	(148; 191)	(157; 188)	(148; 193)
Weight, kg					
Median	71.50	66.70	70.00	69.50	68.85
Range	(52.0; 103.0)	(52.5; 108.3)	(48.5; 97.9)	(55.8; 83.8)	(48.5; 108.3)
BMI, kg/m^2					
Median	24.70	24.05	22.25	23.40	23.65
Range	(18.4; 29.7)	(17.8; 29.6)	(19.7; 29.9)	(19.1; 29.8)	(17.8; 29.9)
Gender, n (%)					
Female	16 (66.7)	14 (58.3)	13 (54.2)	9 (50.0)	52 (57.8)
Male	8 (33.3)	10 (41.7)	11 (45.8)	9 (50.0)	38 (42.2)

***Table 1*: Demographic data.**
N = number of participants; n = number of participants with that observation.
Demographic data and baseline characteristics were similar across intervention groups (mITT set). Screening tests (urine drug screen, alcohol breath test, and viral and SARS-CoV-2 serology) were negative for all participants.

design but had no role in data collection, analysis, and interpretation, or writing of the report.

Results

From Aug 10 to Oct 13, 2020, 148 volunteers were screened and 90 participants were randomized to participate in the trial, of whom 89 completed the study (Figure 1). Baseline demographics for all participants were similar between groups as shown in Table 1. Study duration per participant was 210 days (last subject last visit May 12, 2021). All participants were included in the safety analysis set and in the modified intention to treat (mITT) analysis set. Seventy-eight participants were included in the Per Protocol (PP) analysis set. Twelve participants were excluded from the PP: 1 participant did not receive the second injection as individual halting rules were met (COVID-19 infection), 1 participant was discontinued further to investigator decision, and 10 participants were excluded due to major protocol deviations (mainly assessments and visits performed outside the time window and one missing diary card).

V591 was generally well tolerated. No SAEs were reported during the study. At least one AE was reported in 45 (62.5%) participants in the active treatment groups and in 12 (66.7%) participants in the placebo group (Table 2). No significant differences were observed between any of the active treatment groups, i.e. 16 (66.7%) participants in the low dose group, 13 (54.2%) in the high dose [2 inj.] group, 16 (66.7%) in the high dose [1 inj.] group, and the placebo group (12 [66.7%]). At least one unsolicited event was reported in 39 (54.2%) participants receiving V591 and in 9 (50.0%) in the placebo group. Similar to all AEs, no significant differences were found for unsolicited AEs (Table 2) between any of the treatment groups and the placebo group (12 (50.0%), 12 (50.0%), 15 (62.5%) vs. 9 (50.0%).

The majority of AEs were mild or moderate in intensity. Four participants were reported with a severe AE. Three of these were considered unrelated to study treatment: one participant developed acute cystitis 13 days after the second injection that resolved within 3 days, one participant was diagnosed with anemia 15 days after the first injection which was persistent at the end of the study, and one participant developed headaches just before the second injection which resolved within 3 days. One participant in the placebo group reported severe fatigue after the second injection which was considered as treatment-related. As this event occurred in the placebo group, in summary, no severe AE related to V591 treatment was observed during the study.

AEs considered to be at least possibly treatment-related by the investigators (Table 3) were reported by 20.8% of participants with similar distribution between the treatment groups, i.e. 5 (20.8%) participants each in the low dose, high dose [2 inj.], and high dose [1 inj.] groups, and 6 (33.3%) participants in the placebo group. The most frequently reported treatment-related AEs were headache (4 [5.6%] participants receiving V591 and 5 [27.8%] receiving placebo), followed by fatigue (4 [5.6%] receiving V591 and 1 [5.6%] receiving placebo) and musculoskeletal pain (3 [4.2%] receiving V591, none receiving placebo). AEs at the injection site were reported by 3 [4.2%] participants receiving V591. No

Adverse Events	Low Dose N = 24		High Dose [2 inj.] N = 24		High Dose [1 inj.] N = 24		Active Total N = 72		Placebo N = 18	
	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
At least one AE	16 (66.7)	29	13 (54.2)	30	16 (66.7)	42	45 (62.5)	101	12 (66.7)	33
*Comparison to placebo group**	*p=1.0000*		*p=0.5302*		*p=1.0000*					
At least one unsolicited AE	12 (50.0)	24	12 (50.0)	22	15 (62.5)	31	39 (54.2)	77	9 (50.0)	21
*Comparison to placebo group**	*p=1.0000*		*p=1.0000*		*p=0.5328*					
At least one SAE	0	0	0	0	0	0	0	0	0	0
At least one treatment-related AE	5 (20.8)	6	5 (20.8)	6	5 (20.8)	6	15 (20.8)	18	6 (33.3)	6
At least one serious treatment-related AE	0	0	0	0	0	0	0	0	0	0
At least one AE for which the study drug was discontinued	0	0	0	0	0	0	0	0	1 (5.6)**	3
At least one AE for which the study was discontinued	0	0	0	0	0	0	0	0	0	0
At least one AE of interest special (AESI)***	0	0	0	0	1 (4.2)	1	1 (1.4)	1	3 (16.7)	5

***Table 2*: Summary of adverse events.**

N = number of participants with data; n = number of participants with event; m = number of events.

* The frequency of subjects reporting adverse events was compared pairwise between the placebo group and each of the treatment groups, respectively, using Fisher's exact test.

** Participant met individual halting rules: COVID-19 infection with anosmia and dysgeusia (AESIs) before day 28

*** All AESIs in this study were anosmia and dysgeusia linked to COVID-19 infection and not treatment-related.

System Organ Class Adverse event, n (%)	Low Dose N = 24		High Dose [2 inj.] N = 24		High Dose [1 inj.] N = 24		Active Total N = 72		Placebo N = 18	
	n (%)	m	n (%)	m	n (%)	m	n (%)	m	n (%)	m
Any Treatment-related AE	**5 (20.8)**	**6**	**5 (20.8)**	**6**	**5 (20.8)**	**6**	**15 (20.8)**	**18**	**6 (33.3)**	**6**
Gastrointestinal Disorders	**0**	**0**	**1 (4.2)**	**1**	**0**	**0**	**1 (1.4)**	**1**	**0**	**0**
Vomiting	0	0	1 (4.2)	1	0	0	1 (1.4)	1	0	0
General Disorders and Administration Site Conditions	**3 (12.5)**	**4**	**2 (8.3)**	**2**	**3 (12.5)**	**3**	**8 (11.1)**	**9**	**1 (5.6)**	**1**
Face oedema	0	0	1 (4.2)	1	0	0	1 (1.4)	1	0	0
Fatigue	2 (8.3)	3	1 (4.2)	1	1 (4.2)	1	4 (5.6)	5	1 (5.6)	1
Injection Site Bruising	0	0	0	0	1 (4.2)	1	1 (1.4)	1	0	0
Injection Site Paraesthesia	0	0	0	0	1 (4.2)	1	1 (1.4)	1	0	0
Injection Site Pain	1 (4.2)	1	0	0	0	0	1 (1.4)	1	0	0
Musculoskeletal and Connective Tissue Disorders	**1 (4.2)**	**1**	**1 (4.2)**	**1**	**1 (4.2)**	**1**	**3 (4.2)**	**3**	**0**	**0**
Musculoskeletal Pain	1 (4.2)	1	1 (4.2)	1	1 (4.2)	1	3 (4.2)	3	0	0
Nervous System Disorder	**1 (4.2)**	**1**	**2 (8.3)**	**2**	**2 (8.3)**	**2**	**5 (6.9)**	**5**	**5 (27.8)**	**5**
Dysaesthesia	1 (4.2)	1	0	0	0	0	1 (1.4)	1	0	0
Headache	0	0	2 (8.3)	2	2 (8.3)	2	4 (5.6)	4	5 (27.8)	5

***Table 3*: Treatment-related adverse events.**

N = number of participants with data; n = number of participants with event; m = number of events.

laboratory-related adverse events other than the anemia mentioned above (severe AE) were observed during the study.

Five COVID-19 cases were recorded during the study and all AESIs observed in the study were linked to these SARS-CoV-2 infections. One participant in the placebo group developed mild COVID-19 disease with anosmia and dysgeusia on study day 21 which resulted in disqualification for the second injection on day 28 (Figure 1 and Table 2). This participant tested positive for the presence of N-specific antibodies from day 42. One case was an asymptomatic infection identified four days after the day 56 visit in a participant in the high dose [1 inj.] group. This participant had developed moderate neutralizing antibodies after the V591 injection. The asymptomatic infection did not boost SARS-CoV-2 spike-binding and neutralizing antibodies and did not elicit detectable anti-N antibodies as measured on day 91. No

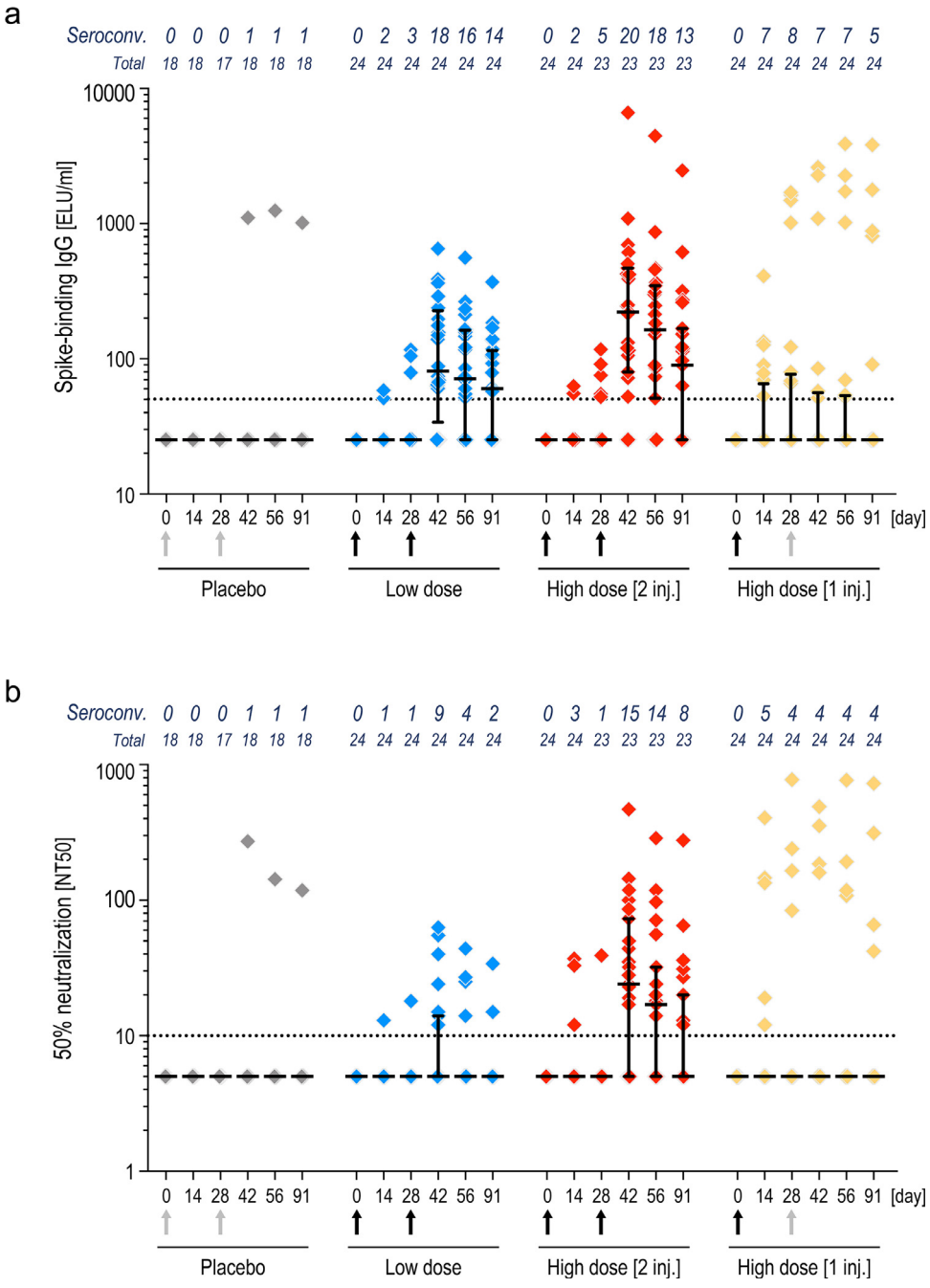

Figure 2. Spike-binding IgG antibody response (a) and neutralizing antibody response (b) to SARS-CoV-2 in trial participants per treatment group and study day.

Spike-binding IgG was measured by ELISA (Elisa units/mL [ELU/mL]), neutralizing antibodies (50% neutralizing titer [NT50]) were measured using a pseudoneutralization assay. Results of the mITT set are shown. Bars show median and interquartile ranges. The number of individuals with detectable antibody levels, defined as seroconversion (Seroconv.), and the total number of samples per timepoint (Total) are indicated above the plots. Black arrows indicate administration of vaccine, grey arrows administration of placebo. The dotted lines indicate the lower limits of quantification (a: 50.3 ELU/mL, b: 10 NT50). One participant in the placebo group seroconverted after PCR-confirmed SARS-CoV-2 infection on day 21 (no sample on day 28). One participant in the high dose [1 inj.] group was anti-N positive from day 14. One participant in the high dose [2inj.] group was discontinued before day 28.

sequence information is available regarding the SARS-CoV-2 variants causing the infections in these individuals. At the time these two cases occurred, variants of concern did not yet circulate in France and Belgium.[25] Three other cases (serology confirmed, no RT-PCR performed) occurred later, close to the end of the study, when the alpha variant was dominant.[25] Two of these cases occurred in the placebo group, the third case was in the high dose [1 inj.] group in a participant who had not developed SARS-CoV-2-specific antibodies after V591 injection. In all three cases, symptoms were mild and included anosmia and/or dysgeusia.

Potential shedding of V591 was assessed as part of the vaccine safety assessment. The regular measles vaccine is rarely shed and human to human transmission has not been reported.[26] No measles virus RNA was detected in saliva, nasal swabs, urine, and blood samples collected from the six sentinel participants (data not shown) indicating this characteristic was not changed in the recombinant measles virus V591.

None of the major protocol deviations (see above) was considered to impact the immune response to V591. Therefore, the mITT set was used for the primary immunogenicity analysis. Thirty-eight participants (81%) receiving two injections of V519 developed spike-binding IgG antibodies after the second injection (Figure 2a). Antibody levels peaked on day 42 in a dose-dependent manner (low dose vs. high dose [2 inj.], p = 0.0276). Geometric Mean Titers (GMTs) decreased over the following time points. On day 91, the spike-binding IgG level in the high dose group [2 inj.] was still statistically different from that of the placebo group (p = 0.0072) while no longer different from that of the low dose group. After one injection of V591, spike-binding antibodies were only elicited in 18 (25%) participants across all treatment groups (on day 14 and/or day 28). One participant in the placebo group developed spike-binding antibodies after COVID-19 infection between day 14 and day 28.

Seroconversion was defined as presence of detectable Ab levels. On day 56, one month after the second injection which was the timepoint used for an intermediate assessment of the immunogenicity of V591, the seroconversion rates for spike-binding IgG were 67% (16/24, low dose), 78% (18/23, high dose [2 inj.]), and 29% (7/24, high dose [1 inj.]). On day 91, seroconversion rates in the low dose and high dose [2 inj.] groups had decreased to 58% (14/24) and 57% (13/23), respectively, and to 21% (5/24) in the high dose [1 inj.] group (Figure 2a).

Neutralizing antibody responses (Figure 2b) followed the same kinetics as spike-binding IgG but reached lower seroconversion rates. Only 17% (4/24) of participants receiving the low dose and 61% (14/23) of participants receiving the high dose [2 inj.] had detectable neutralizing antibodies on day 56. Those frequencies decreased to 8% (2/24, low dose) and 35% (8/23, high dose [2 inj.]) by day 91. In participants who received one injection of the high dose, the seroconversion rate for neutralizing antibodies was 17% (4/24) on day 56 which remained unchanged on day 91.

Anti-measles antibody GMTs increased in response to V591 (Figure 3). The GMTs in the low dose, high dose [2 inj.], and high dose [1 inj.] groups raised from baseline to peak response (day 28 for low dose and high dose [1 inj.] group, day 56 for high does [2 inj.] group) by a factor of 1.9, 2.3, and 2.4, respectively. At the individual level, anti-measles antibody levels were boosted by a factor of 2 or more in 37% of V591 recipients after the first injection and in 44% after the second injection. As visible in the profile plots in Figure 3, the large majority of these participants (92% after 1 injection, 87% after 2 injections) had baseline anti-measles antibody levels of below 600 IU/L (corresponding to the upper limit of the second quartile of anti-MV baseline levels, see below and Figure 4).

In order to assess a potential impact of pre-existing anti-MV immunity on the immunogenicity of V591, SARS-CoV-2 spike-specific IgG Ab levels were analyzed against the anti-MV Ab levels at baseline. For this analysis, the 72 participants having received V591 (low dose, high dose [2 inj.], high dose [1 inj.]) were stratified into quartiles based on anti-measles IgG Abs titers at day 0. On day 28, after one injection, spike-binding IgG levels in individuals in the first quartile (lowest anti-MV baseline levels) were significantly higher than the spike-binding IgG levels in individuals in the third (p = 0.0184) and fourth (p = 0.0047) quartile (Fig 4). After the second injection, on day 56, the impact of pre-existing anti-MV antibody levels decreased but remained statistically significant between the first and fourth (p = 0.0314) and the second and fourth (p = 0.0218) quartile. This observation was mirrored by the number of seroconverted individuals in the respective quartiles. On day 28, 9/17, 4/18, 2/18, and 1/18 individuals per quartile 1-4, respectively, had detectable antibody levels (Figure 4). On day 56, the number of seroconverted individuals per quartile were 13, 13, 9, 6.

No detectable T cell responses were elicited by V591 (Supplementary Figure).

The immunogenicity outcomes were unchanged (data not shown) in a sensitivity analysis which was performed on the PP population with the additional restriction that for participants with confirmed COVID-19 during the trial and/or positive anti-N result, the data from the day of positive result onwards were excluded (Sensitivity PP Population).

Discussion

We report results from our Phase I clinical trial with V591, a measles-vectored COVID-19 vaccine candidate expressing a pre-fusion stabilized full-length spike protein with inactivated furin cleavage site. Two different

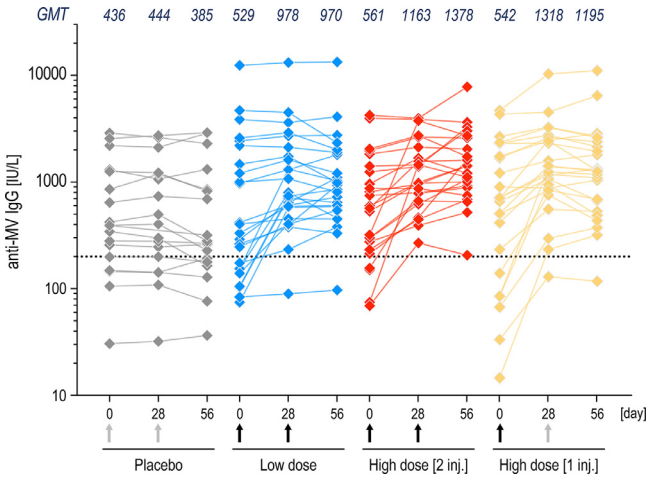

Figure 3. Anti-Measles IgG levels by participant and timepoint.

Anti-measles IgG ELISA results (mITT set) are indicated in international units/liter [IU/L], results below 200 IU/L (dotted line) are considered negative. Solid lines connect samples from the same participant. Black arrows indicate administration of vaccine, grey arrows administration of placebo. One participant in the high dose [2 inj.] group was discontinued before the second injection, the GMT of this group at baseline considering only the 23 participants who completed the study is 595 IU/L.

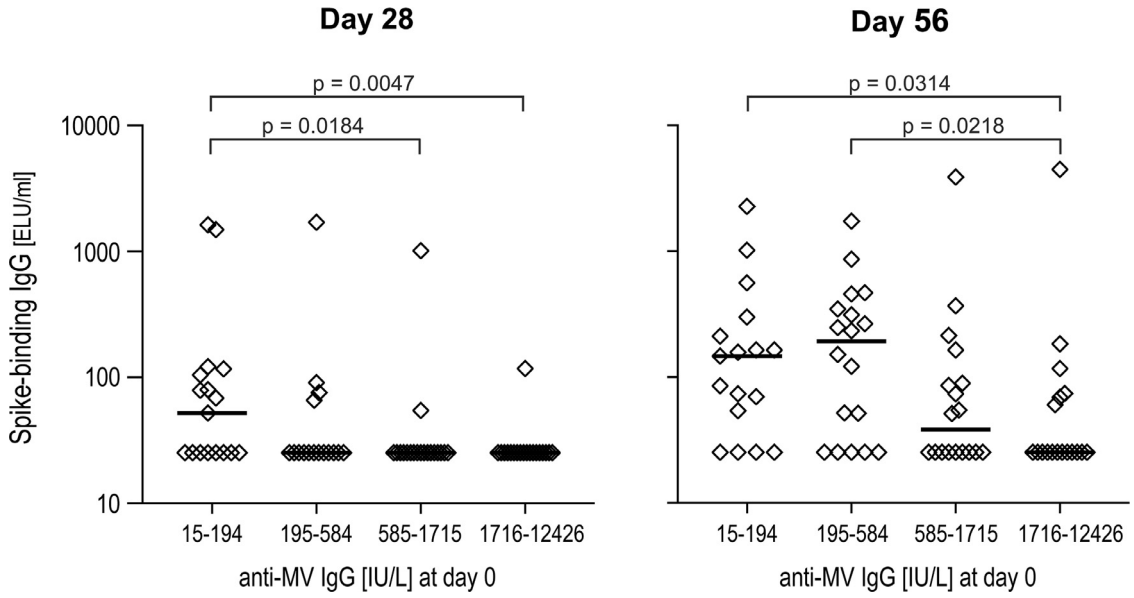

Figure 4. Impact of pre-existing anti-measles antibody levels on V591 immunogenicity.

All participants (mITT set) having received V591 across the different groups (low dose, high dose [2 inj.], high dose [1 inj.]) were stratified into quartiles based on anti-measles antibody levels on day 0. SARS-CoV-2 spike-binding IgG levels on day 28 and day 56 are plotted for each V591 recipient according to quartile allocation. In quartile 1, results of 17 individuals are reported as one participant who was stratified into this quartile was discontinued before day 28. The other three quartiles show results from 18 individuals. Quartiles were compared using an LS means (i.e. GMT) comparison in the MMRM model. Statistically significant differences between quartiles are indicated. Bars indicate the median.

dose levels were evaluated, administered twice by intramuscular injection. The higher dose level was also tested as single injection. All regimens of V591 were well tolerated. No SAEs were reported. However, the immunogenicity of V591 was low.

AEs were reported by 63% of participants receiving V591 with no significant difference to placebo recipients and were predominantly mild or moderate. This safety profile was overall consistent with the safety profile of MV-CHIK in the Phase II trial.[20] Treatment-related

AEs were reported by 21% of participants receiving V591, in equal distribution between the treatment groups, and 33% of participants receiving placebo. Considering that both systemic and local AEs were included this rate was low. The very low frequency of local reactions elicited by V591 is one notable difference to the MV-CHIK vaccine or other COVID-19 vaccines. Only 4% of participants receiving V591 reported local AEs, compared to 52% of participants reporting injection site tenderness and 33% experiencing injection site pain in the MV-CHIK Phase II trial[20] and ≥ 59% individuals reporting injection site pain upon the first injection of ChAdOx1 nCoV-19 or BNT162b in a UK community study.[27]

While a firm correlate of protection against COVID-19 infection has not yet been established, neutralizing antibodies are emerging as predictive of immune protection.[28] On day 56, the timepoint of an intermediate analysis, the seroconversion rate for neutralizing antibodies was 61% in the group with the best performing regimen, i.e. high dose [2 inj], and only 17% in participants receiving the low dose. After one injection of V591, only 25% of participants responded with detectable neutralizing antibodies across all groups. A similar observation was made by Merck Sharpe & Dohme (MSD) in an independent clinical trial performed with V591 (NCT04498247), see the companion publication by Vanhoutte et al.[29], confirming that this finding was not specific for our trial. The seroconversion rates elicited by V591 were lower than those previously obtained in Phase I and Phase II trials with the measles-vectored chikungunya vaccine candidate (MV-CHIK).[19,20] MV-CHIK administered at similar dose levels as V591 resulted in seroconversion of 90% of participants after one immunization and 100% after two immunizations. In addition, MV-CHIK elicited high chikungunya virus neutralizing antibody titers, demonstrating that the measles vector technology is able to elicit strong immune responses in humans. In spite of this, the magnitude of the neutralizing antibody response elicited by V591 was found to be lower than in convalescent individuals (for comparison: the antibody levels of the participant in the placebo group after COVID-19 infection (Figure 2) are similar to the GMT of a panel of convalescent samples measured in the same assay, see the companion publication by Vanhoutte et al.[29]) In contrast, at the time when the intermediate analysis was performed, neutralizing antibody levels in individuals immunized with authorized COVID-19 vaccines were reported to be equivalent or higher than those in convalescent people.[8,9,28,30] Based on these results, further development of V591 was abandoned.

It is important to note that in parallel to the lower than expected anti-spike immune response, the anti-MV boost elicited by V591 was also lower than previously observed with MV-CHIK. At similar dose levels as used for V591, and in a participant population with similar anti-MV baseline GMTs, MV-CHIK elicited an approximately 3-fold increase of the anti-MV GMT in the low dose group and a 5-fold increase in the high dose group in the Phase II trial[20], whereas the anti-MV GMT increase upon V519 injection was approximately 2-fold in the low dose group and below 2.5-fold for both of the high dose groups. This suggests a generally limited immunogenicity of V591 in humans rather than a specific poor immunogenicity of the spike antigen.

It is unclear at this time why V591 showed this limited immunogenicity in humans, while during the pre-clinical development V591 elicited strong and sustained immune responses in pre-clinical animal models even after one injection (preclinical data for V591 will be reported separately). Investigational studies are currently ongoing to understand the underlying mechanistic reasons. Previously developed MV-CHIK[12], MV-Lassa[18], and MV-Zika[17] are derived from the MV Schwarz backbone, as is V591. One noteworthy feature distinguishing V591 from these other MV-based clinical candidates is the fact that the heterologous SARS-CoV-2 spike antigen was functionally inactivated and stabilized by mutations, as these modifications were shown to greatly enhance anti-SARS-CoV-2 immunogenicity in the pre-clinical evaluation. Two other measles vector-based candidates against SARS-CoV-2 in preclinical development have been described. One of these candidates is based on the Edmonston backbone expressing a trimerized soluble ectodomain of the spike protein, pre-fusion stabilized and with inactivated furin cleavage site similar to the type of mutations used in V591.[31] The other pre-clinical candidate expresses the full-length unmodified spike protein in the Moraten backbone.[32] It will be interesting to compare the immunogenicity of these candidates to the results obtained with V591 when they enter clinical development.

One contributing factor might be the fact that pre-existing anti-measles immunity showed a statistically significant impact on the response to V591 in the clinical trial. Although the power of the statistical comparisons is limited due to the small sample size of the study, the trend in this study is quite striking. Thirteen (81%) of 16 participants with detectable SARS-CoV-2 spike-binding antibody levels on day 28 after the first injection of V591 were in the first (lowest) two quartiles of anti-measles antibody levels at baseline (anti-MV IgG 15-584 IU/L). Nine of those (56% of seroconverted individuals) were in the first (lowest) quartile (anti-MV IgG 15-194 IU/L) and their spike-binding antibody levels were significantly higher than those in the third (anti-MV IgG 585-1715 IU/L) and fourth quartile (anti-MV IgG 1716-12424 IU/L). After the second injection of V591, the impact was less pronounced but GMTs of spike-binding IgG of participants in the first and second (anti-MV IgG 195-584 IU/L) quartile were still statistically higher than those of participants in the fourth quartile. This was reflected by the fact that 63% of all

seroconverted individuals on day 56 were in the first two quartiles. This observation also sheds light on the seemingly better response to the first injection of V591 in the high dose [1 inj.] group compared to the high dose [2 inj.] group, since the former group happened to include the participants with the lowest anti-MV IgG levels at baseline (Figure 3) who developed anti-SARS-CoV-2 antibody levels which were among the highest. While an impact of pre-existing anti-vector immunity is not uncommon and has been observed with an adenovirus-vectored COVID-19 vaccine[10,33], the finding in our trial was unexpected based on results with the MV-CHIK vaccine for which no impact of preexisting anti-measles immunity on the anti-CHIK immunogenicity of the vaccine was found in the Phase I and II trials[19,20].

This trial had several limitations. First, the study size was small, as typical for a Phase I study. The number of participants was estimated to detect general differences and no formal sample size calculation was performed. Thus, the statistical power of the performed comparisons is limited as highlighted above. In addition, rare serious adverse events or adverse events of interest might not have been captured. Second, the participants in this first-in-human study were healthy young adults. Therefore, the results are not easily generalizable to the entire population. Third, mandatory confirmation of COVID-19 cases by RT-PCR and collection of sequence information were not part of the study protocol as at the time the study was planned, SARS-CoV-2 variants were not yet a major concern. Thus, information about the variants causing the COVID-19 cases detected during the course of the study is not available.

In conclusion, our Phase I clinical trial results showed that V591 was well tolerated but induced insufficient immune responses against SARS-CoV-2 to support further development. Current efforts to investigate potential reasons and underlying mechanisms for the limited immunogenicity of the V591 candidate and the sensitivity to pre-existing anti-MV immunity will inform the development of future measles vector-based candidates.

Contributors

OL was the coordinating principal investigator and led the clinical conduct at the CIC Cochin-Pasteur, PJB was the principal investigator at CPU. ML and LBLN were study sub-investigators at CIC Cochin-Pasteur, and JK at CPU. CA, MAA, CD, YT, RT, KR, CG, PJB, and OL designed the study. CA, MAA, YT, AG, NJ, ML, JK, LBLN, RT, and CG were involved in the management of the study. CA and MAA led the regulatory-relevant activities. NE, AM, CG, JB, MG, ZC, HT, and DB developed the vaccine candidate. MM was responsible for manufacturing of V591. NE, AM, HT, DB, and JB generated and standardized materials for the shedding assay. CG, RT, AD, and NE contributed to the immunological plan and assessment. BJ led the work for the statistical analysis. CG and MAA verified the underlying data and CG, MAA, KR, RT, CD, NE, AM, PJB, and OL interpreted the data. CG, MAA, NE, AM, RT, and OL did the primary writing and editing of the manuscript. All authors read and approved the final version of the manuscript.

Data sharing statement

The data cannot be made publicly available because of ethical and regulatory restrictions on participant privacy. However, a pseudonymized individual study dataset will be made available after the scientific data from the development of this candidate vaccine have been fully published, and on request directed to the corresponding author, subject to the provisions of the European General Data Protection Regulation and the French Laws and pursuant to the sponsor's policies and procedures.

Declaration of interests

KR, RT, YT, AG, MM are employees of Themis Bioscience GmbH, a subsidiary of Merck & Co. Inc., Kenilworth, NJ, USA. KR, RT, MM possess stock options of Merck & Co. A patent application including the design of V591 has been filed by the Institut Pasteur and is part of a licensing agreement between the Institut Pasteur and Themis/MSD, NE and CG are inventors. CIC Cochin-Pasteur, SGS, INSERM, Bioaster received payment to conduct the study. All other authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2021.103810.

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