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# Proof-of-concept of a low-dose unmodified mRNA-based rabies vaccine formulated with lipid nanoparticles in human volunteers: A phase 1 trial

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ABSTRACT

*Introduction:* In a first-in-human study immune responses to rabies virus glycoprotein (RABV-G)-mRNA vaccine were dependent on the route of administration, necessitating specialized devices. Following successful preclinical studies with mRNA encapsulated in lipid nanoparticles (LNP), we tested an mRNA-LNP formulation (CV7202).

*Methods:* In this phase 1, multi-center, controlled study in Belgium and Germany we enrolled 55 healthy 18–40-year-olds to receive intramuscular injections of 5 μg (n = 10), 1 μg (n = 16), or 2 μg (n = 16) CV7202 on Day 1; subsets (n = 8) of 1 μg and 2 μg groups received second doses on Day 29. Controls (n = 10) received rabies vaccine, Rabipur, on Days 1, 8 and 29. Safety and reactogenicity were assessed up to 28 days post-vaccination using diary cards; immunogenicity was measured as RABV-G-specific neutralizing titers (VNT) by RFFIT and IgG by ELISA.

*Results:* As initially tested doses of 5 μg CV7202 elicited unacceptably high reactogenicity we subsequently tested 1 and 2 μg doses which were better tolerated. No vaccine-related serious adverse events or withdrawals occurred. Low, dose-dependent VNT responses were detectable from Day 15 and by Day 29%, 31% and 22% of 1, 2 and 5 μg groups, respectively, had VNTs $\geq$ 0·5 IU/mL, considered an adequate response by the WHO. After two 1 or 2 μg doses all recipients had titers $\geq$ 0.5 IU/mL by Day 43. Day 57 GMTs were not significantly lower than those with Rabipur, which elicited adequate responses in all vaccinees after two doses. CV7202-elicited VNT were significantly correlated with RABV-G-specific IgG antibodies ($r^2$ = 0.8319, p < 0.0001).

*Conclusions:* Two 1 μg or 2 μg doses of CV7202 were well tolerated and elicited rabies neutralizing antibody responses that met WHO criteria in all recipients, but 5 μg had unacceptable reactogenicity for a prophylactic vaccine.

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## 1. Introduction

Recent infectious disease outbreaks caused by Zika, Ebola, chikungunya and SARS-CoV-2 viruses have highlighted the potential global threat to human health posed by emerging human infectious diseases [1]. There are increasing risks of spread of novel pathogens through human vectors with international travel as evidenced by the 2020 Covid-19 pandemic [2], or animal vectors with climate change illustrated by the increasingly wide range of dengue, chikungunya and Zika virus endemicity [3–5]. The development of new platforms to allow rapid production of novel vaccines, preferably avoiding the use of live pathogenic viruses and chemical inactivation steps which may modify the natural epitopes, is a major priority of vaccine research [1].

One promising technology with the potential to overcome many of these limitations is the use of messenger ribonucleic acid (mRNA) coding for the required antigen [6], which offers several advantages for vaccine manufacture. Production of mRNA using

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well-defined manufacturing techniques allows the same facilities to be used to prepare different mRNA molecules for vaccines against various other pathogens using the same manufacturing platform. As this may lead to lower production costs this may be of particular importance for vaccines destined for low-income countries, including rabies vaccines.

CureVac has developed a proprietary mRNA platform, RNActive®, for use in the development of safe and effective prophylactic vaccines for humans [7]. Following preclinical demonstration of the feasibility of this approach the first clinical investigation assessing the potential of RNActive® for a variety of vaccine targets used the rabies virus glycoprotein (RABV-G) as a model antigen. Use of RABV-G presents several advantages in the early stages of development: the antigen has been clearly defined and characterized, and there is a WHO-defined level of immune response that is considered adequate for the assessment of new vaccines. Furthermore, the virtually 100% fatality outcome of rabies disease means that volunteers in phase 1 trials, providing they have no history of rabies vaccination, will represent an immunologically naïve population.

We demonstrated the proof-of-concept of mRNA for human vaccines in the first-ever human clinical trial of RABV-G mRNA using an initial formulation (CV7201) with the cationic protein protamine [8]. CV7201 was generally well tolerated but the induction of adequate immune responses was dependent upon the mode of administration of the vaccine, notably requiring intradermal or intramuscular administration with specialized devices. Further preclinical research in animal models has found that formulation of the mRNA in lipid nanoparticles (LNP) protects the mRNA and enhances the immune response [9]. Preclinical studies found that CV7202, a novel mRNA-LNP formulation which includes the same mRNA antigen as CV7201 encapsulated in LNP, elicits immune responses in non-human primates comparable to those induced by licensed vaccines (CureVac, data on file). We now report on the first use of this new formulation in adult human volunteers in an ongoing phase 1 study to assess the safety and immunogenic potential of this new vaccine model, which is now being applied to other novel pathogenic viruses, notably SARS-CoV-2.

## 2. Methods

This is a non-randomized, open-label, controlled, dose-escalation, multi-center phase 1 study done at the University Hospital LMU Munich, Germany and the University of Ghent, Belgium from October 2018. The objective was to determine the safety, reactogenicity and immunogenicity of different dosages of CV7202 when administered as intramuscular injections in one- or two-dose regimens. The study protocol was approved by the respective Ethical Committees of the two institutions and registered on ClinicalTrials.gov (NCT03713086). Trial procedures were done in accordance with International Conference on Harmonization (ICH) and Good Clinical Practice guidelines, and applicable regulatory requirements. An internal safety review committee (iSRC) consisting of internal medical experts and the investigators, and an independent data safety monitoring board (DSMB) consisting of external vaccine experts reviewed safety data on a regular basis and made recommendations regarding the sequential enrolment of participants into dose escalation or de-escalation groups according to the DSMB charter and the protocol. The study is ongoing at the time of this report for long-term safety and immunogenicity follow-up, but database lock for the presented data was May 2020, at least four weeks after the second dose of experimental vaccine, CV7202.

### 2.1. Outcomes

The primary objective was assessment of safety and reactogenicity up to 28 days after administration by intramuscular injection of either a first or second dose of CV7202 administered to healthy adults in a range of increasing dosages starting at 5 μg. Main secondary objectives are ongoing evaluation of safety up to two years after vaccination, and comparison of the immune response to CV7202 with the licensed rabies vaccine, Rabipur®, administered in its recommended three dose schedule. For the latter we used proportions of each study group achieving the WHO-required level of response, a rabies-specific serum virus neutralizing titer $\geq$ 0.5 IU/ml. An exploratory objective was the characterization of the humoral immune responses in terms of the immunoglobulin IgG isotype against RABV-G.

### 2.2. Study design and participants

Eligible participants were 18–40 year-old adults of either gender who were healthy at enrolment according to medical history and examination, with a BMI $\geq$ 18.0 and $\leq$ 32.0 kg/m$^2$. Female volunteers were required to have a negative pregnancy test (serum hCG) at screening and negative urine hCG tests before vaccination on Days 1 and 29, and to agree to use approved contraception throughout the study. Male volunteers were required to use barrier contraception (condom) until three months after their last vaccination.

The main exclusion criteria included participation in any other clinical trial, receipt of other vaccines either 14 days (inactivated vaccines) or 28 days (live vaccines) before Day 1, any history of rabies vaccination, or planned travel to countries for which rabies vaccination is recommended or where there is a high risk of rabies exposure. Other exclusions included any immunosuppressive therapy within six months of study start, medication except for inhaled or nasal steroids or topically applied steroids, any history of an immunodeficient condition or potentially immune-mediated disease and any known allergy to any vaccine component.

The study was initially designed to be a dosage escalation with 5 μg as the lowest starting dosage; the first ten participants were enrolled in a staggered manner to receive the lowest anticipated dosage of CV7202, 5 μg, and this was to be followed by sequentially-enrolled groups of 16 volunteers each to receive higher dosages with the first eight participants enrolled into each group then receiving a second dose 28 days after the first. A control group enrolled without stagger received three doses of a licensed rabies vaccine according to the manufacturer's recommendations.

The planned staggered enrolment consisted of the first participant in the 5 μg group receiving their first vaccination, followed by the second and third participants two working days later. The fourth and fifth participants were then enrolled and vaccinated one week after the first. Two days after these vaccinations the iSRC and DSMB reviewed the safety data from the first five participants before agreeing to the enrolment of further participants in this group. This process was to be repeated for the next groups, with the exception that safety data for sentinel safety groups comprising the first four participants was considered by the iSRC before enrolment of the remaining participants in each group. As described in results, following observation of excess reactogenicity to 5 μg CV7202 the protocol was modified to assess lower (1 μg and 2 μg) rather than higher dosages.

#### 2.2.1. Vaccine

CV7202 is composed of mRNA encoding the RABV-G from the Pasteur strain (GenBank accession number: AAA47218.1) with four lipid components—cholesterol, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), PEGylated lipid and a cationic lipid—provided as a sterile solution in a 2 mL glass vial. The stock solution of CV7202 was mixed with 0·9% sodium chloride by a study pharmacist to produce a 10 μg/mL solution, and volumes injected were 0·5 mL, 0·2 mL and 0·1 mL for the 5 μg, 2 μg and 1 μg doses of

mRNA, respectively. The control vaccine was Rabipur® (GSK Vaccines GmbH, Marburg, Germany), a licensed, inactivated rabies vaccine containing ≥ 2·5 IU per ml reconstituted dose, administered according to the manufacturer's recommended schedule of 1, 8 and 29 days. All vaccines were administered by intramuscular injection with a standard syringe/needle in the deltoid of the non-dominant arm.

*2.2.2. Safety assessments*

Following vaccination on Day 1 participants were monitored for 4 h and were only discharged when vital parameters were within the normal ranges and similar to pre-vaccination levels. Injection site reactogenicity was assessed 1 h after vaccination on Day 1, and on Day 2 for 1 μg and 2 μg doses, or Day 3 for the 5 μg dose. Participants were provided with diary cards in which they recorded solicited local reactions (pain, redness, swelling and itching) and systemic adverse events (AE; fever, chills/shivers, nausea/vomiting, diarrhea, headache, fatigue, myalgia and arthralgia) for seven days post vaccination, and any unsolicited AEs for 28 days after each vaccination. In the two dose subsets and the Rabipur group this monitoring schedule was applied for each vaccination. All solicited and unsolicited AEs were graded according to severity, using mild, moderate or severe as categories using the FDA toxicity grading scale [10]. Serious adverse events (SAE) or adverse events of special interest (AESI; AEs with a suspected immune mediated disease etiology) were recorded throughout the study duration and reported immediately to the sponsor or investigator.

*2.3. Virus-neutralizing antibodies*

Serum samples prepared at baseline (Day 1) and subsequently on Days 8, 15, 29, 36, 43, and 57 (pre-vaccination for applicable time-points), were stored at −80 °C for assessment of immune responses. Rabies virus-specific serum neutralizing titers (VNT) were measured using the WHO-recommended rapid fluorescent focus inhibition test (RFFIT) [13] at the accredited Kansas State University Rabies Laboratory. Titers are expressed as International Units per mL (IU/mL), with a titer ≥ 0·5 IU/mL considered an adequate response to vaccination [11].

*2.4. ELISA for RABV-G-specific immunoglobulins*

Levels of RABV-G-specific IgG were measured by enzyme-linked immunosorbent assay (ELISA) at the same timepoints. Briefly, plates were coated overnight with recombinant RABV-G protein expressed in HEK293 cells diluted to 1 μg/ml in phosphate-buffered saline (PBS). Wells were blocked in phosphate-buffered saline (PBS) + 0.05% Tween-20 (PBST) + 5% skimmed milk for 1.5 h before incubation of duplicate serial dilutions of serum samples in PBST + 2% skimmed milk for 2 h at room temperature. After four washes with PBST, horseradish peroxidase (HRP)-labelled detection antibodies specific for human IgG (clone EFE 565, ThermoFischer Scientific) were added for 1 h. Plates were washed five times with PBST before colorimetric detection using tetramethylbenzidine substrate (Biolegend). Reactions were stopped with 2 N $H_2SO_4$ and absorbance read at 450 nm with 620 nm as reference wavelength. Antibody titers were calculated based on isotype control calibration curves, with titers expressed as arbitrary ELISA units per mL (U/mL).

*2.4.1. Statistics*

In this exploratory phase 1 trial we only used descriptive statistics, with no confirmatory statistical inference planned or performed. The primary outcome was analysed on the Safety Set, defined as those participants who received at least one dose of the CV7202 or the active control Rabipur® and for whom any post-Day 1 safety data are available. Immunogenicity analyses were done on the Full Analysis Set (FAS) comprising participants who provided a valid baseline sample and at least one additional blood sample for VNT analysis. Seroconversion was defined as observation of the adequate response titer (0·5 IU/mL) any time after vaccination in a subject confirmed seronegative at baseline. Geometric mean titers (GMT) of rabies-specific VNTs were calculated for each group at each study time-point with 95% confidence intervals (95% CI) as well as the percentages of each group achieving a VNT ≥ 0·5 IU/mL. GMTs and seropositivity rates of RABV-G specific IgG were calculated assigning values of half the LLoQ (780 EU/mL) for samples below that value. Spearman correlations between VNT and IgG responses to first and second doses of CV7202 were calculated. All analyses were done using SAS (version 9.4) and GraphPad Prism software.

## 3. Results

A total of 69 volunteers were screened of whom 55 were enrolled, screen failures mainly being due to inability to meet all protocol requirements (Fig. 1). Two enrolled participants did not receive any vaccination, one due to non-compliance with the protocol and one who withdrew for personal reasons. The original staggered enrolment plan was followed for the 5 μg dose group, and ten volunteers were enrolled and received one 5 μg vaccination. However, concerns over the reactogenicity profile in this group led to a temporary hiatus in the study while the reactogenicity and immunogenicity data from this group were assessed. Following an extensive root cause analysis the iSRC and DSMB recommended continuing with 1 μg and then 2 μg dosages, and the control Rabipur group. Results for all groups are reported below. Group demographics of the 53 volunteers enrolled and assigned to the four groups were similar (Table 1). One participant from the Rabipur control group was lost to follow up after Day 1, and one participant in the 5 μg CV7202 group withdrew consent at Day 15 for personal reasons. The remaining 51 participants completed study procedures through to the Day 57 visit.

*3.1. Safety*

There were no immediate reactions or AEs during the four-hour post-vaccination surveillance period in any participant, and there have been no vaccine-related SAEs or AESIs throughout the study to date. One Rabipur participant was hospitalized with appendicitis, which was not considered to be related to the vaccine. No participant withdrew from the study due to an AE.

As noted, following the administration of one dose of vaccine in the 5 μg group there was a high rate of early onset reactogenicity which affected 9 (90%) of the 10 participants (Table 2). All but one of the participants reported pain at the injection site, which was graded as severe in one case. There was only one report of any other local reaction—a case of mild redness in one participant—with no reports of injection site swelling or itching. There was a high rate of solicited systemic adverse events in this group, with 41 events reported by 9 participants, 32 (78%) of which were described as mild or moderate, but 9 (22%) of which were described as ever in 6 participants. The most frequent systemic AEs were headache, fatigue, myalgia and chills, the majority with onset within 24 h of vaccination on Days 1 or 2. Severe cases of fatigue, chills and myalgia which occurred became moderate or mild within 24 h of onset, except for one case of severe fatigue that lasted for 29 h, and a second case of severe fatigue lasting three days with onset on Day 5 that was related to an acute gastroenteritis-like illness with nausea and vomiting.

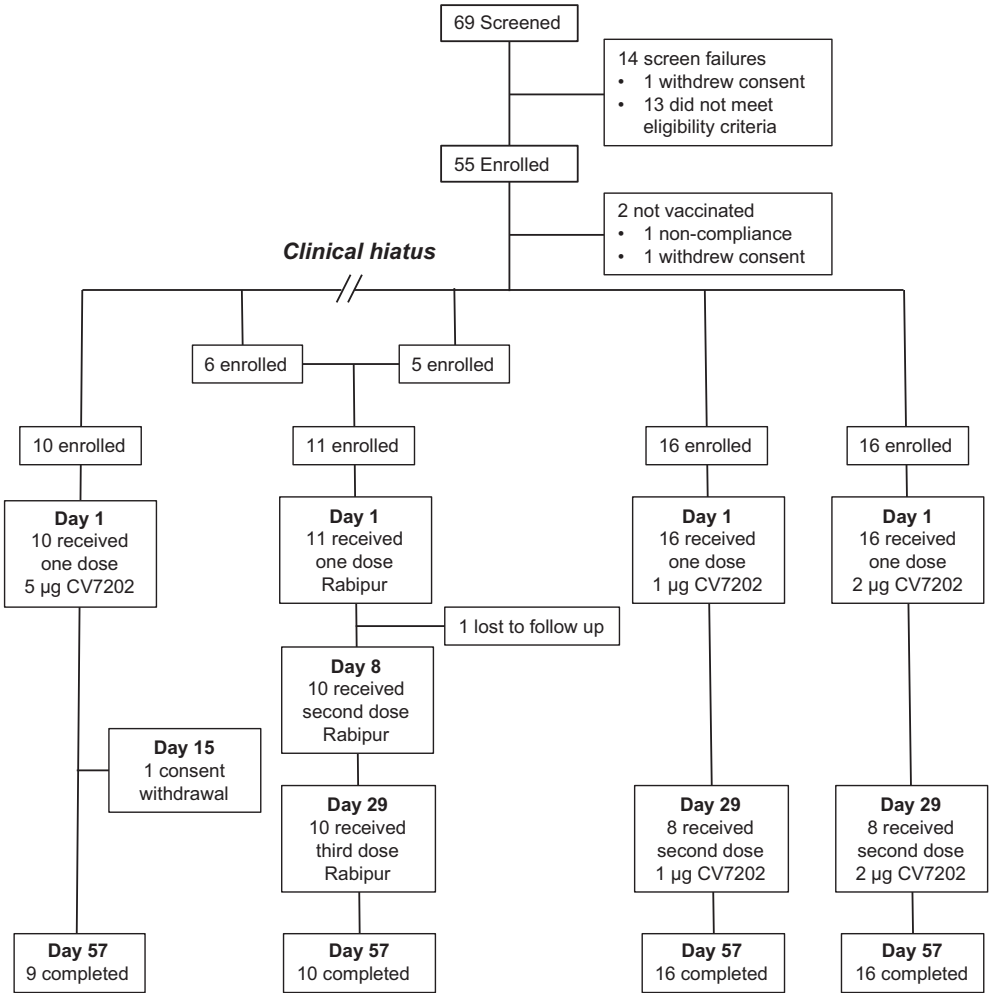


**Fig. 1.** Trial profile.

**Table 1**
Demographics of the enrolled study population.

| | CV7202 Dose groups | | | Rabipur group |
|---|---|---|---|---|
| **Parameter** | **5 µg** | **2 µg** | **1 µg** | |
| N = | 10 | 16 | 16 | 11 |
| **Age,** years | | | | |
| Mean (±SD) | 26·1 (±4·0) | 28·3 (±5·8) | 27·1 (±5·6) | 25·5 (±4.2) |
| Range | [20, 33] | [21, 38] | [19, 38] | [21, 36] |
| **Male** | | | | |
| n (%) | 5 (50) | 4 (25) | 7 (44) | 4 (36) |
| **Ethnicity** | | | | |
| White, n (%) | 10 (100) | 16 (100) | 15 (94) | 11 (100) |
| **Height,** (cm) | | | | |
| Mean (±SD) | 174 (±4·4) | 172 (±·8·4) | 171 (±8·4) | 174 (±12·4) |
| **Weight,** (kg) | | | | |
| Mean (±SD) | 72·4 (±9·2) | 72·1 (±14·4) | 70·0 (±8·9) | 71·4 (±15·1) |
| **BMI,** ($kg/m^2$) | | | | |
| Mean (±SD) | 23·8 (±2.6) | 24·4 (±3·6) | 24·0 (±2·5) | 23·2 (±2.2) |
| Range | [20, 28] | [19, 32] | [18, 28] | [20, 27] |

Unsolicited adverse events were reported by 9 (90%) of the 10 participants; 7 (70%) of these were considered to be related to vaccination, including 5 (50%) graded as severe. The most frequent of these were three cases of lack of appetite (all severe), three cases of night sweats (two severe), two cases of dizziness (one severe), and two cases of tachycardia (one severe). There were single cases of severe AEs—neck stiffness, excessive sweating, hip stiffness, presyncopal episode, hot flushes, thirst, lack of thirst, perceived dehydration, weakness, and lethargy—some in the same participant. The majority of unsolicited AEs resolved within 24 h of onset and the only medication used for treatment of such AEs was ibuprofen, used by 4 of 10 (40%) participants.

Following the study hiatus, 1 and 2 µg dosages of CV7202 were administered and were associated with a much lower reactogenic-

**Table 2**
Solicited local reactions and systemic AEs in the 5 μg CV7202 group.

| | 5 μg CV7202 (N = 10) | | | |
|---|---|---|---|---|
| **Reaction or adverse event,** n (%) | **Any** | **Mild** | **Moderate** | **Severe** |
| **Any local reaction** | 9 (90) | 3 (30) | 5 (50) | 1 (10) |
| Pain | 9 (90) | 3 (30) | 5 (50) | 1 (10) |
| Redness | 1 (10) | 1 (10) | 0 | 0 |
| Swelling | 0 | 0 | 0 | 0 |
| Itching | 0 | 0 | 0 | 0 |
| **Any solicited systemic adverse event** | 9 (90) | 7 (70) | 9 (90) | 6 (60) |
| Fever | 5 (50) | 3 (30) | 1 (10) | 1 (10) |
| Headache | 7 (70) | 0 | 7 (70) | 0 |
| Fatigue | 7 (70) | 1 (10) | 3 (30) | 3 (30) |
| Chills | 7 (70) | 2 (20) | 2 (20) | 3 (30) |
| Myalgia | 5 (50) | 1 (10) | 2 (20) | 2 (20) |
| Arthralgia | 4 (40) | 3 (30) | 1 (10) | 0 |
| Nausea/vomiting | 3 (30) | 2 (20) | 1 (10) | 0 |
| Diarrhea | 2 (10) | 1 (10) | 1 (10) | 0 |
| **Any unsolicited systemic adverse event** | 9 (90) | 6 (60) | 6 (60) | 6 (60) |

ity profile (Table 3). In the 1 μg CV7202 group there were no severe AEs reported, although 4 (25%) of the 16 participants in this group reported moderate AEs. Three unsolicited AEs were reported by two participants—one had a single episode of loose stool and another had two episodes of lower back pain—that were considered to be possibly related to the vaccination. Amongst the 16 participants of the 2 μg CV7202 group there was one (6%) report of severe injection site pain and three (19%) participants reported severe solicited systemic AEs; chills (two), headache (two) and myalgia (one). All but one of these reports occurred after the first dose, with onset on Days 1 or 2, all had improved to mild or moderate by Day 3 within 48 h and all but one had resolved by Day 4. One participant reported two severe unsolicited AEs, palpitations and tachycardia after their first 2 μg dose on the day of vaccination. All these AEs resolved without sequelae.

Each of the three doses of Rabipur were associated with injection site pain in about half of the participants (Table 3), none of which were severe, with no other local reactions. There were also cases of mild to moderate headache, fatigue and myalgia associated with Rabipur.

The major finding from the laboratory safety assessments was the observation of transient lymphopenia at Day 2, the day after vaccination in 9 of 16 and 14 of 16 in the 1 and 2 μg CV7202 groups, respectively, and 6 of 8 and 8 of 8 in those groups at Day 30, one day after the second dose. This was not observed in the 5 μg group as the first blood sampling in this group was conducted on Day 3, rather than Day 2, when lymphopenia was no longer present. These transient changes were not considered to represent toxicity but rather redistribution of lymphocytes due to the vaccine's mode of action as has been observed for other vaccines [12,13].

An anomaly which was not considered to be vaccine-related was the observation of asymptomatic, isolated elevated bilirubin levels in several young male participants recruited at the Munich site which were not temporally associated with vaccination. These were present in prevaccination (baseline) samples and post-vaccination samples in no specific pattern and with no particular association with any study vaccine. The site observed that this is "very common" in their experience with an incidence substantially (2–4x) higher than reported in most western settings and is associated with Meulengracht-Gilbert Syndrome (MGS), a benign genetic disorder which is present in approximately 10% of young men in Bavaria and affects bilirubin processing leading to elevated levels of unconjugated bilirubin in the blood [14–16]. In our study, the incidence was 5 of 45 (11%) CV-7202 vaccinated participants, and as there were no suspected MGS cases amongst Rabipur recipients, the total incidence in the study population was 5 of 53 (9%). When these five participants were interviewed for more detail two reported that episodic jaundice and/or bilirubin elevations had been present 'since youth'.

In three of these five subjects, we observed isolated, elevated total bilirubin elevations prior to the first vaccination (either at screening or at baseline immediately prior to vaccine administration. Three of the five had one or more bilirubin elevations that were marked as clinically significant during the conduct of the study, two of which were present at baseline. The third described previous clinically significant elevations which were added to their medical history.

Split bilirubin analysis was requested in the three cases of clinically significant bilirubin elevation to substantiate the suspected MGS: one participant had a split bilirubin typical for MGS and in combination with a history of raised elevations the diagnosis of MGS was marked in the medical history without genetic confirmation. In a second suspected MGS participant split bilirubin was requested at a visit when total bilirubin was normal and there were no further elevations observed. Because of a childhood history of intermittent icterus on further questioning, suspected MGS was marked in their medical history. Split bilirubin analysis will be done at any future study visit where total bilirubin is elevated and the subject will be referred to their GP for further testing. In the third participant with clinically significant elevation of bilirubin results of the split bilirubin analysis was not typical for MGS, but the participant had a recent symptomatic acute CMV illness. Considering the pattern of bilirubin elevations, overall clinical picture and prevalence in Munich, *per exclusionem* MGS is still suspected in this participant who has been referred to their GP for further testing. The results are as yet unknown.

Split bilirubin levels will be analyzed for both participants with non-clinically significant bilirubin elevations at their next scheduled site visit. Until then, both subjects remain suspected for MGS and might be referred for further testing.

### 3.2. Immunogenicity – Neutralizing antibodies

VNT responses were detected in all four study groups as illustrated in Fig. 2. Following a 5 μg dose of CV7202, VNT levels $\geq$ 0·5 IU/ml were observed from Day 29 in two of nine (22%) participants and these responses were maintained up to Day 57, the last timepoint assessed (Fig. 3).

The 1 μg and 2 μg CV7202 groups also displayed small detectable responses following the first dose. These were more pronounced in the 2 μg group in which 5 of 16 (31%) had VNT

**Table 3**
Numbers (%) of participants reporting solicited local reactions and systemic adverse events (AE) in the 7 days after receiving CV7202 or Rabipur.

| | 5 µg CV7202 | 1 µg CV7202 | | 2 µg CV7202 | | Rabipur | | |
|---|---|---|---|---|---|---|---|---|
| **Reaction or AE,** n (%) | **Dose 1** | **Dose 1** | **Dose 2** | **Dose 1** | **Dose 2** | **Dose 1** | **Dose 2** | **Dose 3** |
| **N =** | **10** | **16** | **8** | **16** | **8** | **11** | **10** | **10** |
| **Any solicited local reaction** | **9 (90)** | **13 (81)** | **5 (63)** | **15 (94)** | **6 (75)** | **5 (45)** | **6 (60)** | **5 (50)** |
| Pain | 9 (90) | 13 (81) | 5 (63) | 15 (94) | 6 (75) | 5 (45) | 6 (60) | 5 (50) |
| Redness | 1 (10) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Swelling | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Itching | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Any solicited systemic AE** | **9 (90)** | **11 (69)** | **5 (63)** | **13 (81)** | **7 (88)** | **8 (73)** | **5 (50)** | **4 (40)** |
| Fever | 5 (50) | 0 (0) | 1 (13) | 3 (19) | 2 (25) | 0 (0) | 0 (0) | 0 (0) |
| Headache | 7 (70) | 8 (50) | 4 (50) | 11 (69) | 5 (63) | 4 (36) | 2 (20) | 3 (30) |
| Fatigue | 8 (80) | 6 (38) | 4 (50) | 9 (56) | 5 (63) | 5 (45) | 5 (50) | 1 (10) |
| Chills | 7 (70) | 0 (0) | 2 (25) | 6 (38) | 2 (25) | 0 (0) | 0 (0) | 1 (10) |
| Myalgia | 5 (50) | 6 (38) | 4 (50) | 7 (44) | 5 (63) | 2 (18) | 0 (0) | 0 (0) |
| Arthralgia | 4 (40) | 3 (19) | 3 (38) | 3 (19) | 1 (13) | 0 (0) | 0 (0) | 0 (0) |
| Nausea/vomiting | 3 (30) | 1 (6) | 0 (0) | 1 (6) | 1 (13) | 1 (9) | 0 (0) | 0 (0) |
| Diarrhea | 2 (20) | 1 (6) | 0 (0) | 1 (6) | 2 (25) | 0 (0) | 0 (0) | 0 (0) |
| **Any unsolicited systemic AE** | **9 (90)*** | **9 (56)** | **4 (50)** | **14 (88)** | **5 (63)** | **4 (36)** | | **4 (40)** |
| Related to vaccination | 7 (70) * | 2 (13) | 1 (13) | 9 (56) | 2 (25) | 1 (9) | | 0 (0) |
| **Any medically attended AE** | **1 (10)** * | **4 (25)** | **1 (13)** | **2 (13)** | **1 (13)** | **0 (0)** | | **0 (0)** |

* Events reported over the whole 56-day study period.

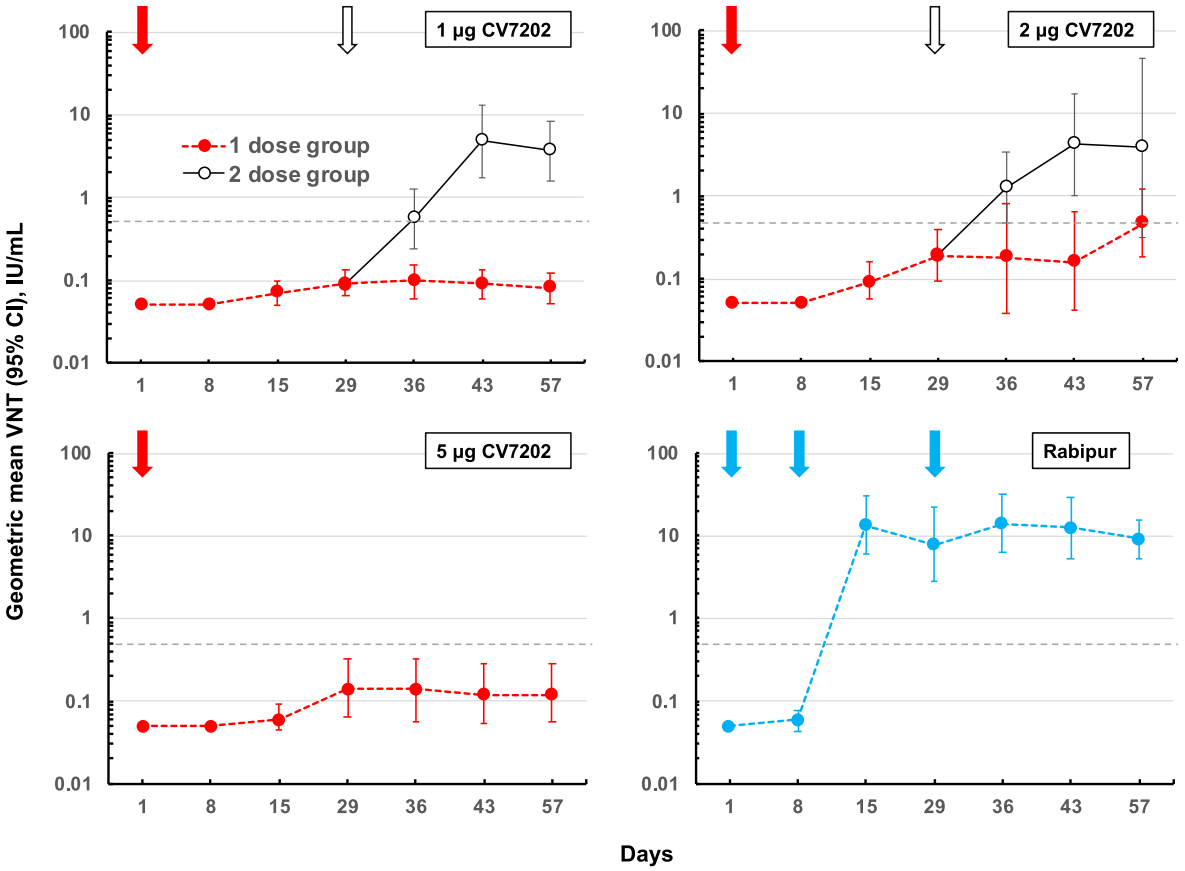


**Fig. 2.** Geometric mean virus neutralizing titers (with 95% CI) in the four study groups after immunization (indicated by arrows) with CV7202 or Rabipur. Dashed line indicates level considered adequate by the WHO (0.5 IU/mL).

levels ≥ 0·5 IU/ by Day 29, whereas no participants in the 1 µg group displayed an adequate response (0·5 IU/mL) by Day 29 after one dose (Fig. 3). Responses were markedly increased following the second dose on Day 29 such that 5 of the 8 (63%) participants who received a second dose of 1 µg and 7 of 8 (83%) participants who received a second dose of 2 µg had titers ≥ 0·5 IU/mL at Day 36. All participants (100%) in both the 1 µg and 2 µg groups reached this level at Day 43 (Fig. 3). GMTs were higher than 0·5 IU/mL at Day 36 in both groups and were further increased at Days 43 and 57. Peak GMTs were achieved at Day 43 with 1 µg (4·8 IU/mL [95% CI:1·77–13·0]) and 2 µg (4·2 IU/mL [1·02–17·2]) of CV7202. All participants in the Rabipur group had titers ≥ 0·5 IU/mL by Day 15, 7 days after the second vaccination and this 100% rate was maintained up to Day 57.

Rabipur achieved a peak GMT of 13·5 IU/mL [5·95–30·6]) IU/mL at Day 15, 7 days after the second dose. The GMT did not further

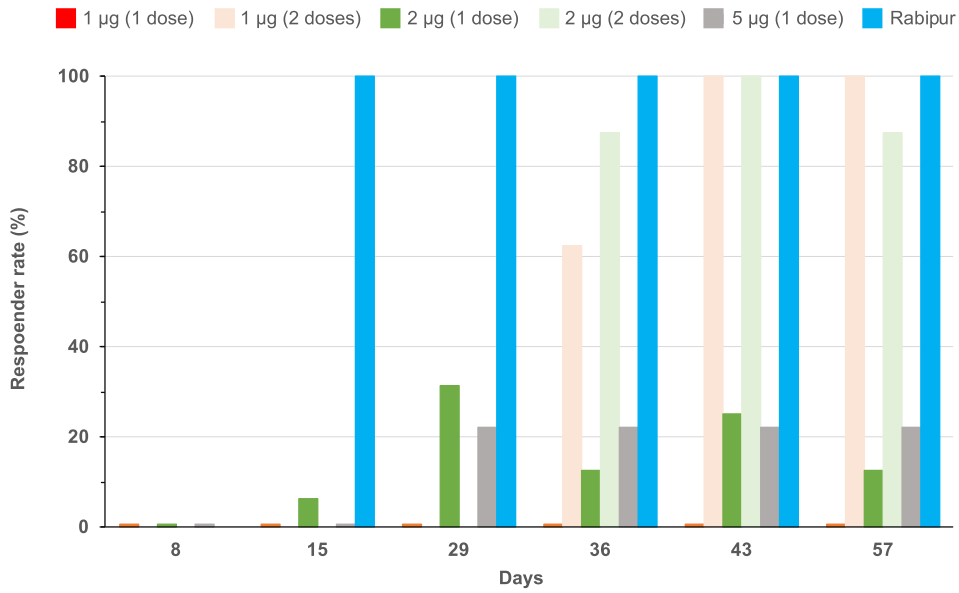


**Fig. 3.** Responder rates (percentages of each group with a VNT ≥ 0.5 IU/mL) in the four study groups after immunization with CV7202 or Rabipur. Rates represent the numbers of participants achieving the protective VNT of 0.5 IU/mL. The 1 and 2 µg CV7202 groups consisted of 16 participants each for Days 8, 15 and 29, and 8 participants each for Days 36, 43 and 57. The 5 µg CV7202 group consisted of 10 participants for Days 8 and 15, 9 participants for Days 29, 36, 43 and 57. The Rabipur group had 10 participants at each timepoint.

increase following a third dose of Rabipur but was maintained at 9·1 IU/mL through to Day 57. Day 43 GMTs after two doses of CV7202 were not statistically significantly lower than those achieved with three doses of Rabipur (p = 0.2831 for 1 µg, p = 0.3507 for 2 µg; Mann-Whitney test).

### 3.3. Immunogenicity – ELISA RABV-G-specific immunoglobulin antibodies

As illustrated in Fig. 4 anti-RABV-G IgG antibodies displayed the same pattern of responses as VNT. There were detectable increases after one dose with 6 of 16 (38%), 11 of 16 (69%) and 8 of 9 (89%) participants in the 1, 2 and 5 µg dosage groups developing low levels of RABV-G-specific IgG, respectively. GMTs were 853 U/mL (95% CI: 455–1599), 1581 U/mL (899–2780), and 2409 U/mL (1113–5215) at Day 29 after the first dose in the 1, 2, and 5 µg CV7202 groups, respectively, and these levels did not further increase in one dose groups. Much larger increases were observed after second vaccinations, peaking at 34,186 U/mL (13253–88185) and 20,707 U/mL (5592–76678) at Day 43 in the 1 and 2 µg groups, respectively. There were highly significant positive Spearman correlations between VNT and IgG titers (Fig. 5), particularly after two doses of CV7202 ($r^2$ = 0·8319, p < 0·0001).

An IgG response was not detected one week after the first Rabipur vaccination; GMTs were 461 and 464 at Days 1 and 8, respectively, but rapidly increased to 12,460 U/mL (95% CI: 6575–23611) at Day 15, 7 days after the second dose. A further incremental increase to 33,373 U/mL (21236–52447) was observed after the third dose and this level was sustained to Day 57. As for the VNT, RABV-G IgG GMTs at Day 43 after two doses of CV7202 were not statistically significantly lower than those achieved with three doses of Rabipur (p = 0·9654 for 1 µg, p = 0·2031 for 2 µg by Mann-Whitney test).

## 4. Discussion

Following demonstration that encapsulation of RABV-G mRNA in lipid nanoparticles improved immune responses in animal models [9], we assessed the safety, reactogenicity, and immunogenicity of CV7202, a novel mRNA-LNP formulation, in adults in comparison with a licensed rabies vaccine. Following observations of high reactogenicity when using the 5 µg dose of CV7202 we found 1 and 2 µg dosages were better tolerated, with no safety concerns and two doses elicited immune responses in terms of neutralizing activity and IgG antibodies that were comparable with three doses of licensed rabies vaccine. Preliminary investigation of the 5 µg response suggest that high innate immune responses driven by type 1 interferon and cytokines and strong induction of toll-like receptor signaling pathways observed in most participants, might have contributed to unfavorable reactogenicity and immunogenicity profiles. This will be investigated in further studies.

In this small trial CV7202 appeared to be safe, with no vaccine-related SAEs or withdrawals due to AEs. Although over half the recipients of the highest dose of CV7202 reported severe solicited systemic or unsolicited AEs, the reactogenicity profiles of the lower doses of CV7202 (1 and 2 µg) were more acceptable. The 2 µg dose elicited a limited number of severe AEs in the first 24 h post-vaccination. Local reactions to these dosages consisted almost exclusively of transient mild to moderate injection site pain. Systemic AEs mainly consisted of transient mild or moderate headache, fatigue and chills, and any cases that were described as initially severe rapidly moderated and resolved, most within 48–72 h and all within the 7-day reporting period. There were no major changes in reactogenicity after the second dose when compared with the first, although the numbers of participants are small. This contrasts with recent reports of mRNA-LNP vaccines against the SARS-CoV-2 virus responsible for the COVID-19 pandemic which had similar rates of reactogenicity and in which reactogenicity noticeably increased after a second dose [17,18].

Importantly, we showed that all recipients, despite the low amount of mRNA included in the vaccine, had functional antibody responses after two 1 or 2 µg doses of CV7202, with GMTs of both rabies-specific neutralizing and RABV-G-specific IgG antibodies that were not significantly lower than those observed after three doses of licensed rabies vaccine. The neutralizing response profile of CV7202 displayed a strong correlation with production of RABV-G-specific IgG antibodies following two doses. There were

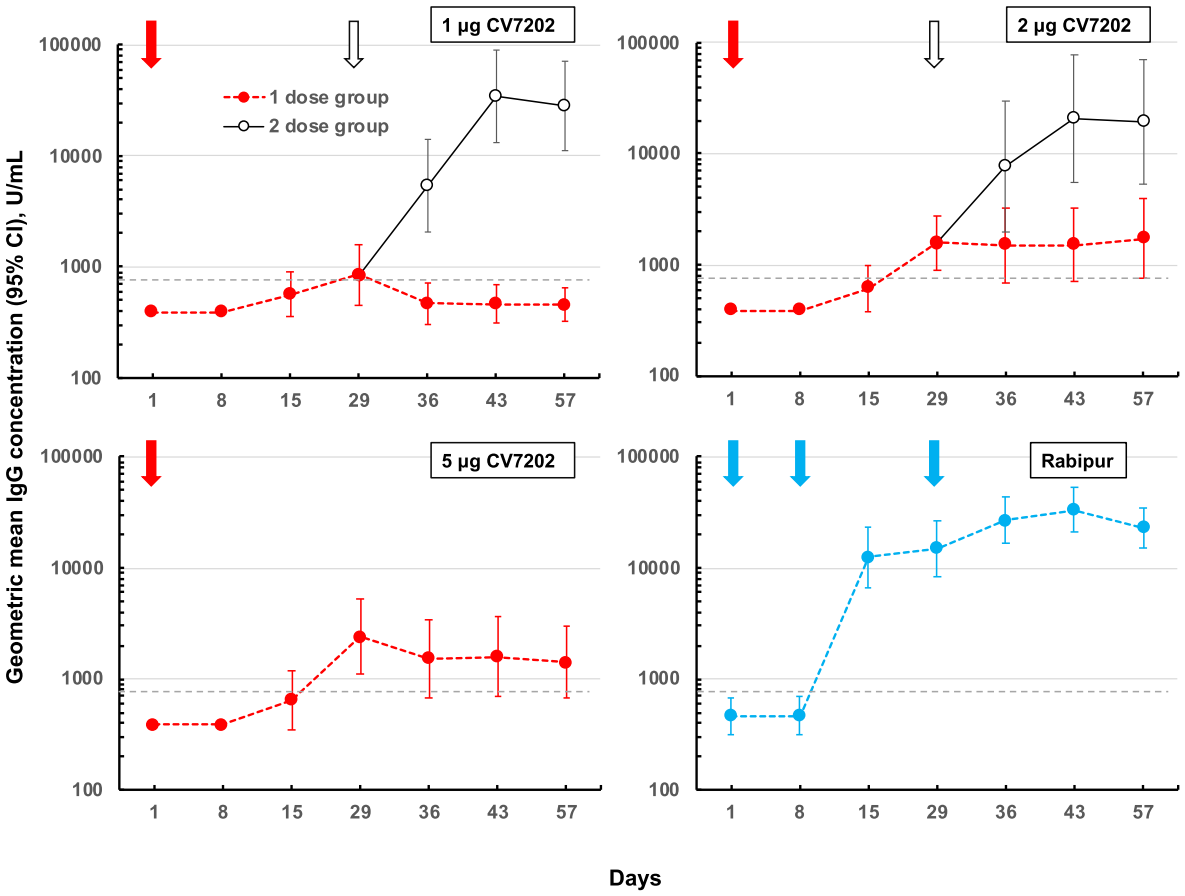


**Fig. 4.** GMTs (with 95% CI) of RABV-G-specific Ig responses assessed by ELISA. IgG concentrations after immunization with one (red arrow) or two (open arrow) doses of CV7202 or three doses of Rabipur (blue arrows). Dotted lines indicate LLOQ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

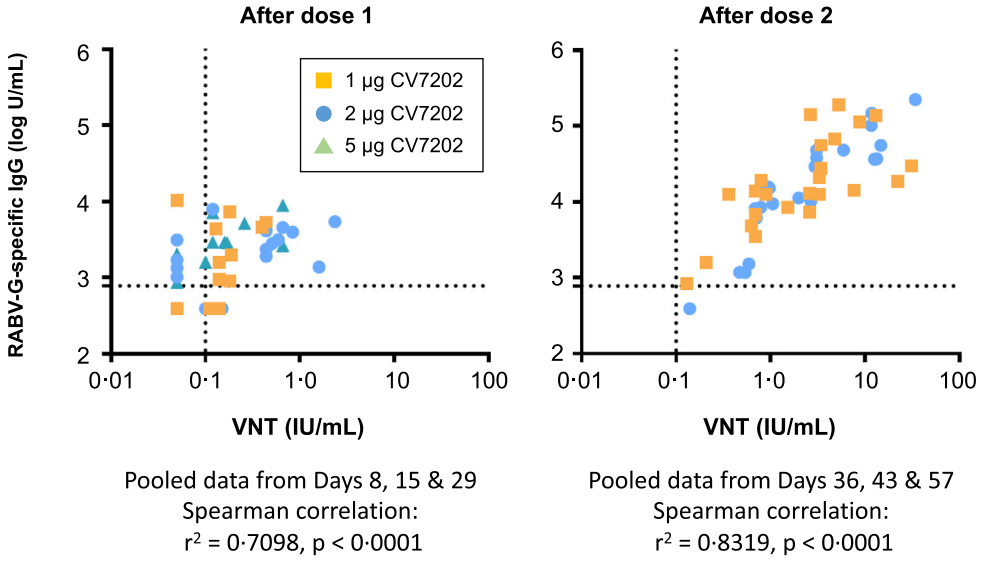


**Fig. 5.** Correlation of titers of RABV-G-specific neutralizing activity (VNT) and IgG antibodies after one or two doses of CV7202.

also transient increases in IgM antibodies but not IgA after second doses of Rabipur and CV7202 (data not shown), but direct comparison of the kinetics of these responses is complicated by the different vaccination schedules—1 and 8 days for licensed vaccine and 1 and 29 days for CV7202—and no second 5 µg dose. The large IgG responses evident 7 days after the second vaccination, and their direct correlation with the neutralizing response suggest the first dose of lower dosages of CV7202 had primed B cells to respond to the second vaccination with an anamnestic response. Although responses with two doses of 1 or 2 µg CV7202 were not signifi-

cantly lower than those induced by three doses of Rabipur, it may be interesting to compare CV7202 and Rabipur responses when used in the same schedule of three doses at Days 1, 8 and 29.

This interim report presents the immune responses up to four weeks after the second dose, but participants will be monitored for two years to assess long-term safety and persistence of the immune response. Further investigations of antibody responses after a booster vaccination, possibly with lower doses, will be necessary to determine whether long-term immune memory has developed, together with a qualitative comparison of avidity, IgG subclasses and B cell responses for CV7202 and the licensed vaccine.

This investigation of mRNA rabies vaccination was performed to determine the validity of the mRNA-LNP platform as a potential approach for viral vaccines. As already mentioned, using rabies as a model antigen presents several advantages in this early development program, but the principles being investigated can be applied to other viral pathogens. Although initial studies of mRNA vaccines were targeted against tumors for cancer immunotherapy [19,20], their potential for prophylactic use has led to an increasing amount of research into this aspect, notably the recent focus on vaccines against SARS-CoV-2 during the Covid-19 pandemic [21]. Several mRNA vaccine candidates using this approach have been reported to be in development [22,23]. Our initial studies of the mRNA technology using rabies mRNA, CV7201, found that there was potential for a human mRNA rabies vaccine, but the mode of delivery of the molecule was critical to elicit an adequate immune response due to its known instability in physiological conditions [8]. Intracellular delivery of oligonucleotides can be improved by encapsulating them in lipid nanoparticles (LNP) [9,23], and preclinical studies showed that protecting the mRNA by lipid encapsulation significantly enhanced the response to the CV7201 [9,24]. Studies to develop efficacious COVID-19 vaccines using mRNA have employed larger quantities of mRNA in LNP-formulations, 10–100 μg [17] or 25–250 μg [18], from which final doses of 30 μg and 100 μg were selected, respectively.

Having previously demonstrated the concept of mRNA vaccination in humans using rabies glycoprotein as antigen we have now confirmed the preclinical observations that lipid encapsulation also protects the mRNA molecule in humans and enhances the generation of protective immune responses after two doses in a similar manner to the inactivated control vaccine. It will be necessary to determine the duration of the immune response, and, for less pathogenic agents than rabies virus, potentially the efficacy of the response in a challenge model (e.g. prophylactic influenza vaccine development). Further studies are underway to apply this mRNA technology to other antigens, notably the development of a vaccine against the SARS-CoV-2 virus responsible for COVID-19 (ClinicalTrials.gov NCT04449276, EudraCT 2020-001286-36). Our observations of a significant immune response with extremely low amounts of mRNA, show the technology implemented for the CV7202 development holds the promise to allow protection of a large part of the population.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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