Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial

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Summary

Background Vaccines based on mRNA coding for antigens have been shown to be safe and immunogenic in preclinical models. We aimed to report results of the first-in-human proof-of-concept clinical trial in healthy adults of a prophylactic mRNA-based vaccine encoding rabies virus glycoprotein (CV7201).

Methods We did an open-label, uncontrolled, prospective, phase 1 clinical trial at one centre in Munich, Germany. Healthy male and female volunteers (aged 18–40 years) with no history of rabies vaccination were sequentially enrolled. They received three doses of CV7201 intradermally or intramuscularly by needle-syringe or one of three needle-free devices. Escalating doses were given to subsequent cohorts, and one cohort received a booster dose after 1 year. The primary endpoint was safety and tolerability. The secondary endpoint was to determine the lowest dose of CV7201 to elicit rabies virus neutralising titres equal to or greater than the WHO-specified protective antibody titre of 0·5 IU/mL. The study is continuing for long-term safety and immunogenicity follow-up. This trial is registered with ClinicalTrials.gov, number NCT02241135.

Findings Between Oct 21, 2013, and Jan 11, 2016, we enrolled and vaccinated 101 participants with 306 doses of mRNA (80–640 µg) by needle-syringe (18 intradermally and 24 intramuscularly) or needle-free devices (46 intradermally and 13 intramuscularly). In the 7 days post vaccination, 60 (94%) of 64 intradermally vaccinated participants and 36 (97%) of 37 intramuscularly vaccinated participants reported solicited injection site reactions, and 50 (78%) of 64 intradermally vaccinated participants and 29 (78%) of 37 intramuscularly vaccinated participants reported solicited systemic adverse events, including ten grade 3 events. One unexpected, possibly related, serious adverse reaction that occurred 7 days after a 640 µg intramuscular dose resolved without sequelae. mRNA vaccination by needle-free intradermal or intramuscular device injection induced virus neutralising antibody titres of 0·5 IU/mL or more across dose levels and schedules in 32 (71%) of 45 participants given 80 µg or 160 µg CV7201 doses intradermally and six (46%) of 13 participants given 200 µg or 400 µg CV7201 doses intramuscularly. 1 year later, eight (57%) of 14 participants boosted with an 80 µg needle-free intradermal dose of CV7201 achieved titres of 0·5 IU/mL or more. Conversely, intradermal or intramuscular needle-syringe injection was ineffective, with only one participant (who received 320 µg intradermally) showing a detectable immune response.

Interpretation This first-ever demonstration in human beings shows that a prophylactic mRNA-based candidate vaccine can induce boostable functional antibodies against a viral antigen when administered with a needle-free device, although not when injected by a needle-syringe. The vaccine was generally safe with a reasonable tolerability profile.

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Introduction

Immunisation with live and inactivated vaccines or toxoids has proven to be a highly successful strategy against many infectious diseases, illustrated by the global eradication of smallpox and imminently of poliomyelitis. Many other previously common infectious diseases have almost been eliminated by vaccination in developed countries, only emerging when immunisation programmes are interrupted.[1] However, there is a constant need for novel, rapid, and more affordable vaccine technologies to counter other infectious diseases that present as individual or public health threats in developing countries, or potential emerging global pandemic threats.[2]

One potential technology platform is the use of RNA molecules as prophylactic vaccines encoding bacterial and viral antigens,[3–5] or as therapeutic cancer vaccines when targeting tumour antigens.[6–11] Preclinical studies in animals have established that mRNA enters target cells and is translated into the encoded antigens to raise a T helper 2 immune response,[12] and when such RNA encodes antigens of rabies or influenza viruses the induced immune response is protective against viral challenge.[5,13]

We selected rabies as a model antigen to explore the mRNA technology when used as a prophylactic vaccine in humans because the population is naive to the virus unless previously vaccinated, and there is an accepted immunological surrogate of protection used in the

Research in context

Evidence before this study

We searched PubMed for published studies using the search terms "mRNA vaccine" and "clinical trial", with no restrictions in terms of search dates or language. We found three references to small clinical trials of potential protamine-stabilised mRNA therapeutic vaccines in patients with cancer, one describing the intentions of the trial and two with results. We did not find any published research on the use of mRNA in humans as a prophylactic vaccine against any infectious disease.

Added value of this study

This is the first proof-of-concept study investigating the safety and immunogenicity of a candidate prophylactic mRNA vaccine in healthy adults. We selected rabies as a model antigen because there are licensed vaccines with accepted serological correlates of protection that can serve as comparators, and the 100% fatality rate of the untreated disease means that most of the anticipated study population (ie, those with no history of rabies vaccination), would be immunologically naive because there is no risk of natural exposure. The results show that the phase 1 vaccine formulation has a reasonable safety and tolerability profile, although it induces mild or moderate adverse events in most participants, and is immunogenic when administered intradermally or intramuscularly using needle-free devices. The data support further development of better optimised formulations.

Implications of all the available evidence

Our trial shows that vaccination with a mRNA rabies candidate vaccine, CV7201, is safe irrespective of mode or route of administration, but immunogenic only when administered using needle-free devices. Although improvements are being made to the vaccine formulation to ensure the WHO-recommended virus neutralising titre level (≥0·5 IU/mL) is elicited in 100% of vaccinated volunteers, this is an important proof-of-concept for the application of mRNA technology that might be extrapolated to other potentially fatal infectious diseases. mRNA vaccines are expected to have lower production costs than inactivated rabies vaccines, are stable outside of a cold-chain, and large numbers of doses can be produced quickly.

assessment of inactivated rabies vaccines.[14,15] CV7201 is a lyophilised, temperature-stable mRNA candidate vaccine composed of mRNA encoding the rabies virus glycoprotein (RABV-G) in free and complexed form with the cationic protein protamine.[11] The mRNA drug substance was awarded the international non-proprietary name nadorameran by WHO as the first drug substance of this new class.

Preclinical studies in mice and pigs have shown intradermal and intramuscular injection of CV7201 induces specific, long-lived, and protective adaptive immunity encompassing B-cell and T-cell responses.[13] In mice, CV7201 induces virus neutralising antibody titres that protect against intracranial challenge with rabies virus at up to 40 times the median lethal dose (LD_{50}). In guinea pigs, needle-free jet injection of CV7201 resulted in improved tissue distribution and antigen expression compared with needle-syringe injection, and increased immunogenicity in domestic pigs and non-human primates (CureVac AG, data on file). In this proof-of-concept in-human study we assessed the safety and immunogenicity of CV7201 in healthy adults when administered at various doses and schedules by either intradermal or intramuscular injection using needle-syringe or needle-free injector devices.

Methods

Study design and participants

This phase I proof-of-concept, open-label, uncontrolled, prospective clinical trial was done in a single site, the Department of Infectious Diseases and Tropical Medicine, Medical Centre of the University Hospital of Munich, Germany, between Oct 21, 2013, and Jan 11, 2016. Volunteers were enrolled sequentially in groups of six in a dose escalation sequence with the option to expand cohorts to 20 if at least five or six participants received the required dose of CV7201 to elicit rabies virus neutralising titres of 0·5 IU/mL or more, the WHO-recommended standard.[14,15]

Healthy 18–40-year-old men and women were screened serologically and excluded if they were positive for rabies antibodies or active viral infections (HIV, hepatitis C virus, and hepatitis B virus), or had signs of autoimmunity. Inclusion criteria included being healthy at enrolment based on physical examination and clinical laboratory assessments, having a body-mass index between 18·0 kg/m² and 32·0 kg/m², and being available for the whole study period. Major exclusion criteria included any history of rabies vaccination or intended travel to areas where rabies vaccination was recommended, or receipt of any other vaccination within 4 weeks of the first study vaccination. Further criteria included any treatment with immunosuppressive drugs or immunoglobulins, any chronic immunodeficiency, or any known allergy to vaccine components.

An independent Data Safety Monitoring Board (DSMB) assessed safety and immunogenicity results at each stage of the trial to permit the next dose escalation steps and cohort expansion. The trial has been extended to allow for long-term safety assessments after 1 and 2 years from the last vaccination.

Volunteers provided written informed consent prior to any study procedure. The protocol was approved by the Ethics Board of the Munich University Hospital and the German federal regulatory authority (Paul-Ehrlich

Institute) and conducted in accordance with Good Clinical Practice and the Declaration of Helsinki guidelines.

Procedures

The CV7201 vaccine was manufactured at CureVac AG (Tübingen, Germany), and supplied as a sterile-lyophilisate in labelled, single dose glass vials stored at 25°C or higher. CV7201 was reconstituted just before injection with Ringer-lactate solution to immunogen concentrations of 0·4 mg/mL or 0·8 mg/mL. Injections were administered in one of two schedules: a long schedule at days 0, 28, and 56 based on the preclinical data, and a short schedule at days 0, 7, and 28, which is typically used with rabies vaccinations. Participants in the expansion cohort were invited to receive a booster with the same dose and route of application 1 year after their third vaccination if the primary vaccination group they belonged to had shown satisfactory safety and immunogenicity.

CV7201 doses of 80 µg up to 640 µg were tested using intradermal or intramuscular routes of administration with needle-syringe or needle-free injector devices.[16] Three different needle-free injection devices were used: intradermal injector 1 (Tropis; PharmaJet, Golden, CO, USA), a spring-powered intradermal injector; intradermal injector 2 (Biojector 2000; Bioject Medical Technologies, Tigard, OR, USA), a carbon dioxide gas-powered intradermal injector; and intramuscular injector (Stratis; PharmaJet, Golden, CO, USA), a spring-powered intramuscular injector. The dose range was based on preclinical data, clinical experience with mRNA cancer vaccines, and by limitations imposed by injection volumes of the intradermal injectors (0·1 mL with a final concentration of 0·8 mg/mL) and intramuscular injector (0·5 mL with a final concentration of 0·4 mg/mL).

Participants were monitored for 3 h after vaccination at the trial site for onset of immediate adverse reactions. Physical examinations and vital sign measurements were done after each vaccination and follow-up visits were done on days 2, 9, 21, 30, and 91 (short schedule) or on days 2, 30, 42, 58, and 120 (long schedule). Autoimmunity assessments made at screening and at days 91 and 120 included measurement of antinuclear antibodies (ANA), thyroid-stimulating hormone (TSH), FT3 and FT4, antibodies against thyroid peroxidase, TSH receptor, and thyroglobulin if TSH was outside the normal range.

Participants recorded solicited local reactions (pain, redness, swelling, induration, ecchymosis, and itching) and systemic adverse events (fever, headache, myalgia, arthralgia, nausea, chills, and fatigue) on diary cards for days 0–7 after each vaccination. Solicited adverse events were graded for severity according to predefined criteria based on WHO guidance. Briefly, solicited local reactions (erythema, induration, and swelling) whose largest diameter was more than 100 mm and systemic adverse events that prevented normal daily activity were considered as grade 3 (severe) adverse events. All other adverse events, including solicited adverse events occurring later than 7 days after vaccination, were considered as unsolicited adverse events, and documented using MedDRA terms together with concomitant treatment at each visit. Adverse events were classified for causality as related or unrelated to the vaccination by the investigator and graded for severity as above.

Sera were collected on days 0, 7, 21, 42, and 91 (short schedule) and days 0, 28, 42, 70, and 120 (long schedule), as well as pre-booster and post-booster immunisation in an expansion cohort to measure rabies virus neutralisation titres using a standardised WHO-recommended rapid fluorescent focus inhibition test (RFFIT).[14] The RFFIT was done by an accredited virology laboratory at the University Hospital Institute of Virology, Essen, Germany. RABV-G-specific IgG and IgM serum antibodies were measured by ELISA using the Platelia Rabies II Kit (Bio-Rad, Germany) according to a modified protocol for IgM.

In one cohort (80 µg of CV7201 with intradermal injector 1), peripheral blood mononuclear cells were isolated from venous blood samples drawn on days 0, 42, and 91 and stored frozen. Peripheral blood mononuclear cells samples were thawed and stimulated with overlapping 15-mer peptides covering the full open reading frame of RABV-G protein (JPT Peptide Technologies GmbH, Berlin, Germany). Cells were analysed for interferon γ, tumour necrosis factor, interleukin 2, and CD107a.

Outcomes

The primary objective of this proof-of-concept study was to assess the safety and tolerability of CV7201 in humans administered at various dose levels in different schedules by intradermal or intramuscular injection. The secondary objective was to determine the lowest dose of CV7201 necessary to elicit the WHO-recommended rabies virus neutralising antibody titre of 0·5 IU/mL in 95% or more of vaccinees.[14] Exploratory objectives included assessment of IgG and IgM antibodies and correlation with virus neutralising titres, and cellular immunity responses.

Statistical analyses

As this was an exploratory proof-of-concept phase I trial, no statistically based estimation was done. A sample size of 102–172 participants (six participants per cohort, 20 participants in each expansion cohort) was considered to be adequate to generate first-ever human clinical data on safety and immunogenicity and to down-select doses, schedules, and application route for further development. All participants who had received at least one vaccination were included in the safety set, and also in the full analysis set if they had provided blood samples at baseline and at least one additional timepoint postvaccination for immunological analysis. The per-protocol immunogenicity population included all participants who had received all three

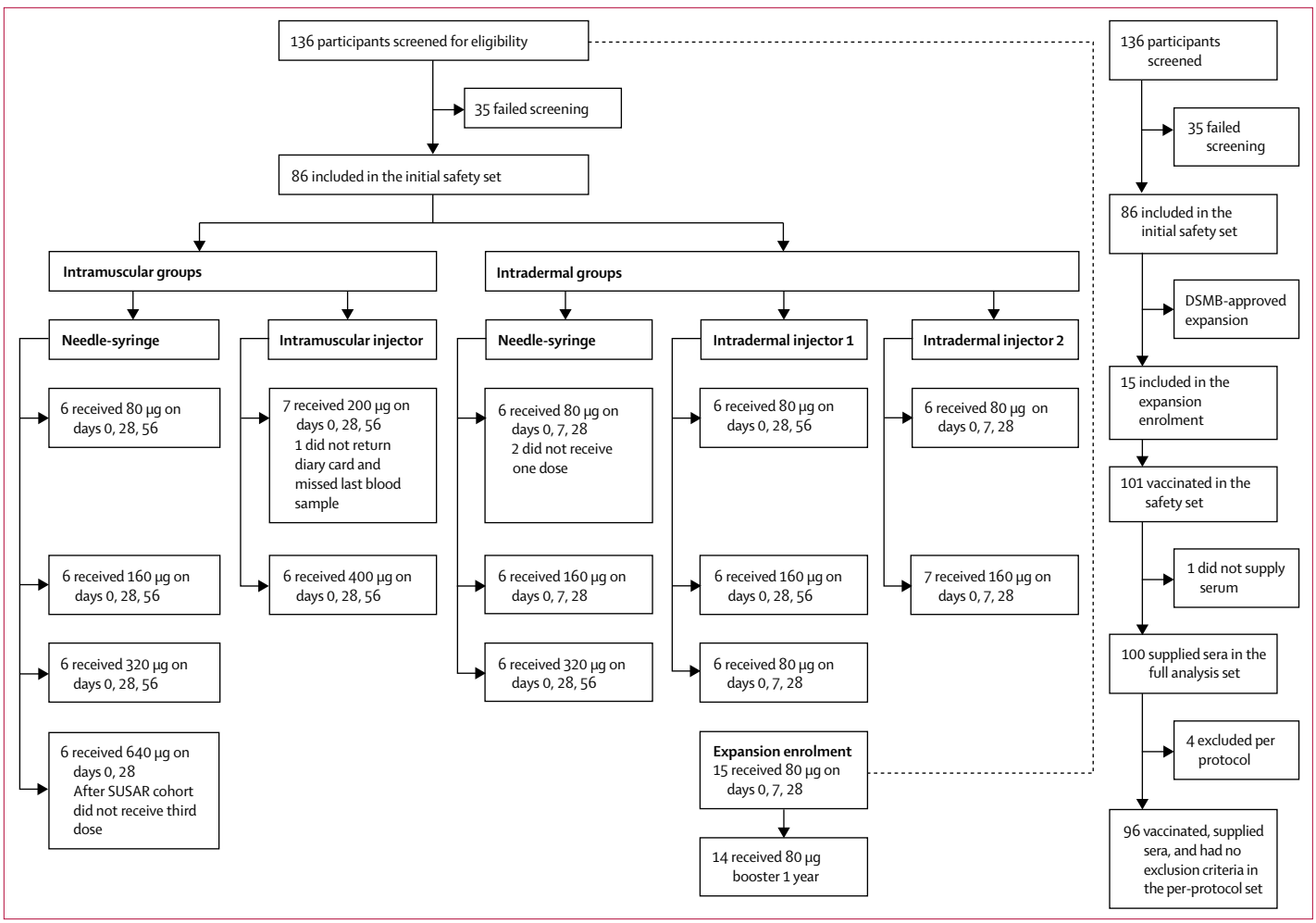

Figure 1: **Flow chart**
DSMC=Data Safety Monitoring Board.

vaccinations and for whom rabies virus neutralising titres were available at all planned timepoints and who did not have any major protocol deviations. We used SAS (version 9.4) for these analyses.

Descriptive analyses presented in standard summary tables include proportions of vaccine recipients reporting solicited adverse events, numbers displaying antirabies antibodies, and geometric mean titres (GMT) of neutralising antibodies with 95% CIs. GMTs were calculated using a value of 0·1, half the lower limit of quantitation, for those with undetectable antibodies. Post-hoc exploratory analyses included Wilcoxon rank test, Kruskal Wallis test, and Spearman or Pearson correlation analyses as indicated, using GraphPad Prism (version 6.05), and visualisation of intracellular cytokine staining data using SPICE (version 5.1).[17] Cellular immune response data were analysed using COMPASS, a computational approach to intracellular cytokine staining data analysis that summarises complex, multifunctional participant-level antigen-specific T-cell responses as functionality and the polyfunctionality scores, to assess the functional profiles of the RABV-G-specific CD4+ T cells.[18] The trial was registered with ClinicalTrials.gov, number NCT02241135.

Role of the funding source

The principal investigator (FvS) and the funder of the study designed the protocol, and UG-V had overall responsibility for the conduct of the trial. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors reviewed and agreed to submit the manuscript for publication. No honoraria, grants, or other form of payment were provided to authors, with the exception of funding needed to do the study.

Results

Of 136 volunteers screened, 101 were enrolled, 86 of whom were initially enrolled into 14 cohorts consisting of six or seven participants in each group (figure 1). After

	Intradermal							Intramuscular						All
	Needle-syringe			Intradermal injector 1*		Intradermal injector 2†		Needle-syringe			Intramuscular injector‡			
Dose (μg)	80	160	320	80§	160	80	160	80	160	320	640	200	400	..
n	6	6	6	27§	6	6	7	6	6	6	6	7	6	101
Mean age (years)	30·8 (4·6)	30·3 (7·4)	28·7 (4·4)	25·7 (5·0)§	24·0 (1·1)	29·3 (5·8)	22·1 (3·1)	27·2 (5·8)	24·5 (4·4)	24·7 (5·9)	23·7 (4·7)	22·9 (3·0)	26·2 (6·7)	25·9 (5·3)
Men	1 (17%)	3 (50%)	3 (50%)	13 (48%)§	4 (67%)	5 (83%)	4 (57%)	4 (67%)	4 (67%)	4 (67%)	2 (33%)	1 (14%)	3 (50%)	50 (50%)
BMI (kg/m²)	24·2 (2·2)	23·3 (2·3)	25·6 (4·9)	24·2 (3·7)§	20·9 (0·6)	25·1 (3·6)	23·7 (1·4)	24·0 (4·1)	23·1 (4·5)	22·4 (2·5)	23·2 (2·7)	22·7 (4·2)	25·5 (4·2)	23·8 (3·4)

Data are n (%) or mean (SD), unless otherwise stated. BMI=body-mass index. *Tropis. †Biojector 2000. ‡Stratis. §The expansion cohort.

Table 1: **Demographic overview of all participants (safety set) including the expansion cohort**

safety and immunology analysis, the 80 μg of CV7201 intradermal cohort vaccinated with intradermal injector 1 was expanded by an additional 15 participants. Of those, 14 received a booster vaccination 1 year after their third study vaccine administration. Only two participants were replaced because of dropouts in accordance with the protocol. Demographics of each study group were similar (table 1). All volunteers but one were white. The 101 participants who received a total of 306 vaccinations constitute the full safety set for safety analyses, and the 100 who supplied sera for virus neutralising titre analyses form the full analysis set for immunogenicity, of whom four were excluded from the per-protocol set.

Safety data were available from 101 participants, but one did not return their diary for solicited adverse events. Vaccination with CV7201 was generally safe irrespective of route or device. There were no anaphylactic reactions, clinically relevant abnormalities, or shifts from haematology baselines or vital signs related to vaccination. Significant laboratory abnormalities (all grade 1 or 2) at a single timepoint documented as adverse events were seen in 12 (12%) of 101 participants; of these, nine were intradermal and three were intramuscular recipients (appendix). Two participants experienced transient decreases in white blood cell count and granulocytes assessed as temporally related, although one was probably due to a diagnosed sinusitis. There were no significant increases in autoimmune parameters (ANA or thyroid autoantibodies) assessed as related. One female participant was diagnosed with mild increase in TSH and thyroid autoantibodies consistent with autoimmune thyroiditis 1 year after dose 3 and was excluded from the booster vaccination.

There were three serious adverse events, one of which was assessed as possibly related to the study vaccination by the investigator. A case of transient, moderate (grade 2) Bell's palsy was reported as a suspected unexpected serious adverse reaction 7 days after a second 640 μg dose of CV7201 was administered intramuscularly by needle-syringe. Although the independent DSMB assessed the case to have unknown causality in line with the general incidence,[19] the planned third vaccinations in this study group at day 56 were not administered as a precautionary measure. Two other serious adverse events, a nasal septum deviation that required surgery, and a campylobacter infection requiring admission to hospital, both in participants given 80 μg intradermal vaccines of CV7201, were considered to be unrelated to the study vaccine. All three serious adverse events resolved without sequelae.

In the 7 day solicitation period after vaccination with a total of 306 doses, there were 248 injection site reactions reported by 60 (94%) of 64 participants vaccinated intradermally, and 36 (97%) of 37 participants given intramuscular vaccines (table 2). All reactions were transient and described as mild-to-moderate in severity; no grade 3 (prevented normal daily activity) local reactions were reported, and all resolved spontaneously within the observation period. Solicited systemic adverse events (mainly fever, headache, and chills) were less frequent, with 205 events reported by 50 (78%) of 64 participants vaccinated intradermally and 29 (78%) of 37 participants vaccinated intramuscularly, ten events (5%) of which, in eight volunteers, were described as grade 3. Seven of these events were in the intradermal groups: severe nausea, headache (three cases), chills (2 cases), and a high fever, while one volunteer in the intramuscular group reported severe myalgia and arthralgia, and another had severe fatigue. These reactions were found across the dose ranges and were not associated with any specific device or mode of administration. Rates of solicited adverse events did not increase with subsequent doses during the primary series, nor were they increased after the booster dose 1 year later (table 2).

See Online for appendix

315 unsolicited adverse events were reported by 90 (89%) of the 101 participants between the first vaccination (day 0) and days 90 or 120, of which 151 events in 63 (62%) participants were considered as possibly related to vaccination (appendix). 18 (12%) of these 151 events were considered severe (headache, vomiting,

	Intradermal						Intramuscular					
	Needle-syringe			Intradermal injectors			Needle-syringe				Intramuscular injector	
Dose (μg)	80	160	320	80	160	80 Boost†	80	160	320	640	200	400
n	6	6	6	33	12*	14†	6	6	6	6	7	6
Local reactions												
Pain	6 (100%)	5 (83%)	6 (100%)	28 (85%)	12 (100%)	10 (71%)†	5 (83%)	6 (100%)	6 (100%)	6 (100%)	7 (100%)	6 (100%)
Haematoma	3 (50%)	5 (83%)	6 (100%)	1 (3%)	0	0†	0	0	0	0	0	0
Erythema	6 (100%)	6 (100%)	6 (100%)	22 (67%)	9 (75%)	5 (36%)†	0	1 (17%)	2 (33%)	2 (33%)	4 (57%)	3 (50%)
Swelling	5 (83%)	4 (67%)	4 (67%)	9 (27%)	2 (17%)	1 (7%)†	0	1 (17%)	1 (17%)	0	4 (57%)	4 (67%)
Pruritus	5 (83%)	4 (67%)	5 (83%)	12 (37%)	9 (75%)	4 (29%)†	0	1 (17%)	0	0	3 (43%)	1 (17%)
Induration	0	0	0	4 (12%)	0	0†	0	0	0	0	0	1 (17%)
Systemic adverse events												
Fever	1 (17%)	1 (17%)	1 (17%)	0	0	0†	0	0	0	0	0	1 (17%)
Fatigue	5 (83%)	4 (67%)	6 (100%)	16 (49%)	2 (17%)	5 (36%)†	2 (33%)	2 (33%)	3 (50%)	6 (100%)	4 (57%)	4 (67%)
Chills	2 (33%)	2 (33%)	5 (83%)	2 (6%)	0	1 (7%)†	1 (17%)	1 (17%)	1 (17%)	3 (50%)	1 (14%)	2 (33%)
Headache	3 (50%)	5 (83%)	5 (83%)	21 (64%)	6 (50%)	5 (36%)†	1 (17%)	2 (33%)	2 (33%)	6 (100%)	4 (57%)	4 (67%)
Nausea	0	1 (17%)	1 (17%)	6 (18%)	1 (8%)	3 (21%)†	0	0	1 (17%)	3 (50%)	0	2 (33%)
Myalgia	3 (50%)	4 (67%)	5 (83%)	9 (27%)	3 (25%)	1 (7%)†	3 (50%)	1 (17%)	0	3 (50%)	2 (29%)	2 (33%)
Arthralgia	1 (17%)	3 (50%)	4 (67%)	3 (9%)	1 (8%)	2 (14%)†	1 (17%)	1 (17%)	0	3 (50%)	0	1 (17%)

Data are n (%). *One participant did not provide diary information. †The expansion cohort.

Table 2: **Participants reporting solicited injection site reactions or systemic adverse events after any dose in the different study cohorts**

	Vaccination schedule (days)	Detectable VNTs (≥0·2 IU/mL)	VNTs ≥0·5 IU/mL	GMTs* (IU/mL)	Range of titres (IU/mL)
Intradermal					
Needle-syringe					
80 μg dose	0, 7, 28	0/6	0/6	..	..
160 μg dose	0, 7, 28	0/6	0/6	..	..
320 μg dose	0, 28, 56	1/6	0/6	..	..
Injector 1†‡					
80 μg dose	0, 7, 28	21/21	17/21	0·83 (0·51–1·34)	0·1–8·5
80 μg dose	0, 28, 56	6/6	5/6	0·66 (0·35–1·22)	0·3–1·1
160 μg dose	0, 28, 56	4/6	3/6	0·39 (0·11–1·35)	0·1–1·4
Injector 2 dose†‡					
80 μg dose	0, 7, 28	3/6	2/6	..	..
160 μg dose	0, 7, 28	6/6	5/6	1·27 (0·39–4·18)	0·3–8·5
Intramuscular					
Needle-syringe					
80 μg dose	0, 28, 56	0/6	0/6	..	..
160 μg dose	0, 28, 56	0/6	0/6	..	..
320 μg dose	0, 28, 56	0/6	0/6	..	..
640 μg dose	0, 28§	0/6	0/6	..	..
Injector¶					
200 μg dose	0, 28, 56	6/7	3/7	..	
400 μg dose	0, 28, 56	4/6	3/6	0·44 (0·10–1·90)	0·1–2·3

DSMB=Data Safety Monitoring Board. GMTs=geometric mean titres. VNTs=virus neutralising titres. *Values shown with 95% CIs in parentheses for the full analysis set. †Tropis. ‡Biojector 2000. §A third dose was not given following DSMB decision. ¶Stratis.··=not determined.

Table 3: **Immunogenicity for the different study groups 14 days after the third vaccination, with GMTs in groups with more than 50% responders, by injection route and application device**

and nasopharyngitis being the most frequent), but of these, only one case of pyrexia was assessed as being related to the study vaccination, although four others did lead to delay or withdrawal from the study after assessment by the DSMB. Unsolicited adverse events comprised typical disorders observed in vaccine trials, only the following were reported by 5% or more of the participants: nasopharyngitis, headache, oropharyngeal pain, vertigo, and rhinitis as well as ongoing injection site reactions after the 7 day solicitation period.

Immunogenicity assessments were done on the full analysis set population (100 of 101 participants vaccinated) because only four participants were excluded from the per-protocol analyses: three because they did not receive all three vaccinations, two were from the 80 μg of CV7201 intradermal needle-syringe group, one was from the 200 μg of CV7201 intramuscular injector cohort who also missed the day 120 timepoint, and one was from the 640 μg of CV7201 intramuscular group whose day 70 sample was taken on day 56.

Analyses of functional antibody titres against rabies virus revealed clear differences between the cohorts vaccinated with needle-syringe and those in which injector devices were used. In cohorts who received intramuscular vaccinations by needle-syringe there were no detectable antibody responses with CV7201 doses from 80 μg to 640 μg. By contrast, ten (77%) of 13 volunteers who received either 200 μg or 400 μg of CV7201 vaccines by intramuscular injector developed detectable virus neutralising titres, and half exhibited virus neutralising titres of 0·5 IU/mL or more, with no apparent difference between the doses (table 3).

A similar pattern was observed when the candidate vaccine was given intradermally. After needle-syringe administration there was a detectable antibody response (but below the WHO threshold) in one of six participants given the highest dose (320 µg mRNA), but no antibodies were detected with lower doses. By contrast, 40 (89%) of 45 participants who received 80 µg or 160 µg by intradermal administration with an injector device raised antibodies after vaccination, and 32 (71%) of 45 developed titres of 0·5 IU/mL or more. There were no apparent differences in response between the two intradermal devices, nor between dose levels or vaccination schedules; For those who received the 80 µg dose, 30 (91%) of 33 participants seroconverted and 24 (73%) of 33 had titres of 0·5 IU/mL or more, compared with ten (83%) of 12 participants and eight (67%) of 12 who received the 160 µg dose. In the short schedule, 30 (91%) of 33 participants seroconverted and 24 (73%) of 33 had titres of 0·5 IU/mL or more, whereas in the long schedule ten (83%) of 12 particpants and eight (67%) of 12 seroconverted and had titres of 0·5 IU/m or more, respectively (table 3).

As per protocol, the 21 individuals from the expansion cohort given 80 µg with intradermal injector 1 were recalled 1 year after their last dose to determine antibody persistence and boostability. After the initial vaccinations, 17 of these 21 participants had titres of 0·5 IU/mL or more 4 weeks after priming (table 3, figure 2), but 1 year later, only two of the 14 who returned still had detectable antibodies, and neither had a titre of 0·5 IU/mL or more. An increase in GMT was already detectable 7 days after a booster dose owing to responses in six of the 14 participants (figure 2B). At day 28 post-boost, 13 (93%) of the 14 were seropositive, with a GMT of 0·73 IU/mL (95% CI 0·36–1·46), similar to the post-primary GMT. However, only eight (57%) of 14 participants achieved titres of 0·5 IU/mL or more of neutralising antibodies (figure 2B).

We measured RABV-G-specific IgM and IgG antibodies in the expanded cohort to determine their putative contributions to the virus-neutralising activity of the sera. In most participants, RABV-G-specific IgM titres peaked at day 21 with a good correlation with the virus neutralising titres (appendix). Class-switched RABV-G-specific IgG antibodies peaked later at day 42 with only a modest correlation with day 42 virus neutralising titres (appendix), suggesting that the virus neutralising activity of day 42 sera was mediated by both RABV-G-specific IgG and IgM antibodies, as seen with inactivated vaccines. After the 1 year boost there was no change in RABV-G-specific IgM antibody levels, but there was a rapid rise in RABV-G-specific IgG antibodies that were strongly correlated with virus neutralising titre levels. This predominantly IgG response, together with the rapid response after 7 days in six of 14 participants is indicative of an established immune memory response during the initial vaccination schedule (appendix).

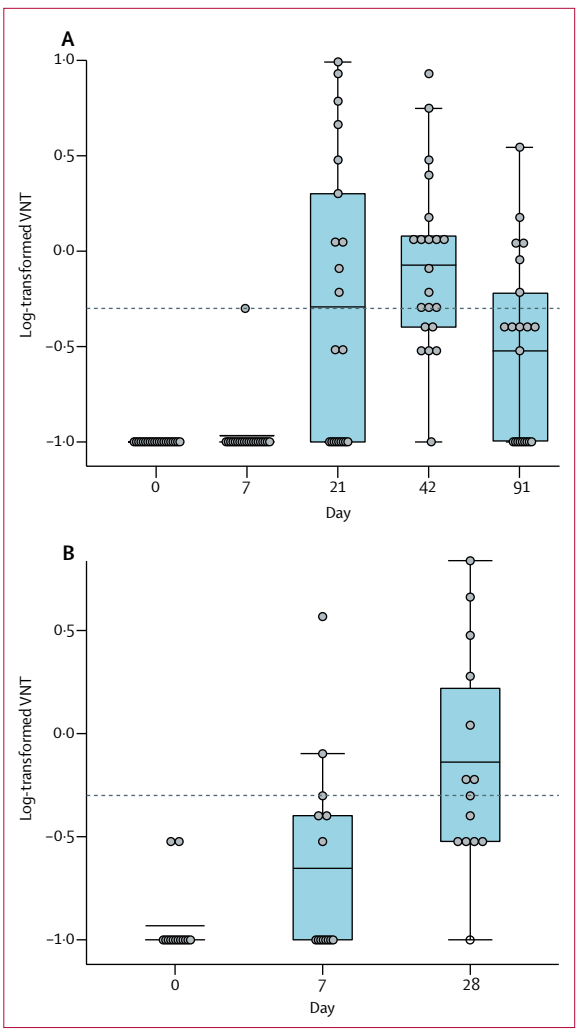

Figure 2*: Humoral immune responses in the expanded cohort immunised intradermally with 80 µg doses of CV7201 administered with intradermal injector 1

(A) Serum neutralising antibody responses to the three primary vaccinations (n=21). (B) Serum neutralising antibody responses to the 1-year booster (n=14). Each dot represents a participant. Boxes depict geometric mean titres and IQRs; whiskers extend from the hinge to the highest or lowest value within 1·5 × IQR of the respective hinge, and the dashed line marks the protective threshold. VNT=virus neutralising titre. *Injecter 1 is Tropis.

Intracellular cytokine staining assays were done on peripheral blood mononuclear cell samples isolated at baseline and at days 42 (n=21) and 91 (n=15) from 21 participants in the expanded cohort (80 µg with intradermal injector 1). We used COMPASS, which summarises multifunctional subject-level antigen-specific T-cell responses as functionality and the poly-functionality scores. Both scores of RABV-G-specific CD4+ T cells were transiently increased at day 42 compared with baseline (figure 3A, B) indicating the induction of rabies-specific T-cell responses post vaccination. As expected, these responses declined to baseline levels by day 91 (63 days after third vaccination), which is consistent with the contraction and memory

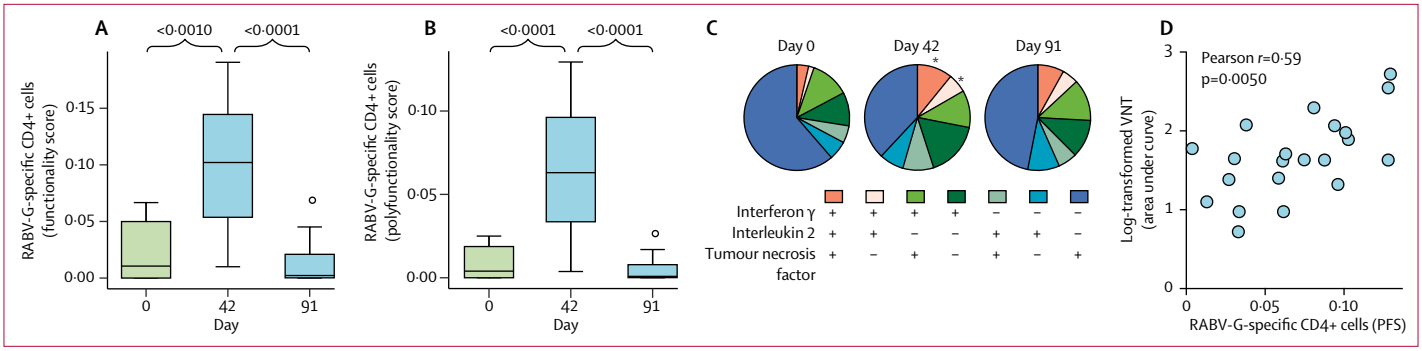

Figure 3: **Cellular immune responses in the expanded cohort immunised intradermally with 80 µg doses of CV7201 administered with intradermal injector 1***
n=21 for days 0 and 42; n=15 for day 91. (A–B) RABV-G-specific CD4+ T cell responses at baseline and at day 42. Intracellular cytokine staining data were analysed using COMPASS and summarised as functionality (A) or polyfunctionality (B) score. Boxes show median values and IQRs, whiskers extend from the hinge to the highest or lowest value within 1·5 × IQR of the respective hinge. Statistical significance between timepoints was tested by the Kruskal-Wallis test. (C) Relative proportions of RABV-G-specific CD4+ T cells producing any combination of interferon γ, tumour necrosis factor, or interleukin 2. Wilcoxon rank test was used to assess statistical significance between timepoints. (D) Pearson correlation between log-transformed virus neutralising titre and RABV-G-specific CD4+ polyfunctionality scores at day 42. VNT=virus neutralising titre. RABV-G=rabies virus glycoprotein. PFS=progression-free survival. *denotes p<0·01. *Injecter 1 is Tropis.

phase of the immune response. This finding was corroborated by SPICE analysis showing an increase of polyfunctional RABV-G-specific CD4+ T cells (figure 3C). There was a robust correlation between humoral and cellular immune responses (figure 3D). These data show that the mRNA vaccine can induce virus-specific cellular immune responses in previously naive participants.

Discussion

We report the safety and immunogenicity of a first-in-human proof-of-concept trial of a candidate mRNA-based prophylactic vaccine in a naive population. This technology platform offers many advantages over present cell-based, inactivated or purified antigen or toxoid vaccines. mRNA vaccines are potentially quicker and more cost-efficient to produce, and more agile and flexible to allow rapid development of vaccines against newly emerging diseases in large quantities. The vaccine product is stable at 5–25°C for 36 months and at 40°C for 6 months (in-house data). In this proof-of-concept study we used mRNA coding for the rabies virus glycoprotein as a model antigen to explore various modalities that influence vaccine response including dose, schedule, mode of application, and route (intradermal or intramuscular) because it has an established serological correlate of protection, and the fatal nature of untreated rabies infection means a study population will not been primed by natural exposure.

We showed that mRNA vaccination is safe and has a reasonable tolerability profile. Although most volunteers reported local reactions to vaccination, all were mild or moderate, self-resolving, and dose-independent. Severe (grade 3) solicited systemic adverse events were transient and resolved without sequelae, including the only serious incident where the causality could not be fully clarified: a case of transient Bell's palsy with full recovery occurring 7 days after a second vaccination with 640 µg CV7201. The single event of autoimmune thyroiditis reported 1 year after the last dose is in line with the general frequency in the population and there were no other autoimmune events. These findings are consistent with the expected safety profile of a vaccine and related stimulation of the immune system. In terms of safety, no relevant differences could be seen either for the route of administration (intradermal *vs* intramuscular) or for needle-syringe versus needle-free administration.

When assessing the effects of different vaccination routes, devices, schedules, and vaccination doses on the induction of anti-rabies virus neutralising antibodies, the key finding was that vaccination by needle-syringe failed to induce an adequate antibody response, independent of dose or route of administration. Use of needle-free devices provided superior results, whereas dose, schedule, and route (intradermal or intramuscular) had limited effects on the induced immune responses. The candidate vaccine induced WHO-recommended levels of neutralising antibodies in most participants when administered intradermally with a needle-free device, but at the doses tested, seemed less immunogenic by intramuscular injection with an injector device. However, the differences were modest and the small cohort sizes do not allow for definitive conclusions.

Conflicting data have been reported as to whether the use of needle-free devices can enhance the immunogenicity of vaccines. In animal models and human studies with other licensed vaccines it has been observed that vaccination delivered by needle-free injection systems induces immunity that is equivalent to or superior to delivery by a conventional needle-syringe injection.[13,20–23] On the other hand, DNA-based vaccines administered by jet injection were more immunogenic in animal studies[24] and a more favourable outcome was reported using needle-free administration in a DNA vaccine trial followed by rAd5 boost.[25] Needle-free administration generally leads to broader dispersion patterns of the vaccine, which could recruit more immunocompetent cells at the injection site or enable more efficient cellular uptake of

nucleic acid-based vaccines, increasing antigen expression levels. In contrast to classical vaccines containing protein as an antigen, the efficacy of nucleic acid-based vaccines relies on the translation of the antigen by cells for which optimum uptake is crucial. This might explain the superiority of needle-free injection in our trial, which led to a ten-fold increase in the number of cells loaded with mRNA in preclinical models.

Although the mRNA vaccine candidate was able to induce antibody responses, further improvements are imperative for the development of a viable vaccine. Whereas all 21 participants given 80 µg of CV7201 with intradermal injector 1 had detectable virus neutralising titres at one or more visits within 91 days of post-prime vaccination, 1 year later, only two of 14 still had detectable antibodies. The rapid increase (7 days) of virus neutralising titres in some participants after the 1 year booster vaccination, which was strongly associated with RABV-G-specific IgG levels, indicated the presence of class-switched, RABV-G-specific memory B cells. Nonetheless, this only led to virus neutralising titres of 0·5 IU/mL in eight of 14 participants. We also show for the first time the induction of virus-specific T-cell responses by a prophylactic mRNA-based vaccine. The magnitude of the cellular immune response was relatively low, but our functional T-cell assays were done on peripheral blood mononuclear cell samples that for logistical reasons were derived 2 weeks after the third vaccination, while induction of vaccine-specific human CD4+ and CD8+ effector T cells were shown to peak approximately 1 week after peak antigen load in response to various vaccines.[26,27] Thus, the magnitude and longevity of the immune responses, particularly for an invariably fatal infection such as rabies, are currently inadequate. Studies of an optimised vaccine formulation in non-human primates have produced promising findings (manuscript in preparation) and a follow-up clinical trial in humans is currently in preparation.

We did this first-in-human study with a prophylactic mRNA candidate vaccine to understand the effect of dose, schedule, route, and mode of administration on the immune response in an unprimed population. Our study provides the first proof-of-concept that an mRNA-based prophylactic vaccine is reasonably safe and capable of inducing rabies antibodies in humans, and provides important guidance for improvements in this technology. Further development to increase antibody titres after priming, with better persistence and high rates of immune memory, might not only result in cheaper, temperature-stable rabies vaccines to meet the present public health needs for such a vaccine, but also could lay the foundation to expand this novel vaccine platform to other infectious diseases where rapid supply is critical.

Contributors

MA, RC, KTM, FvS, and UG-V conceived and designed the trial. MA, RC, KTM, MF-M, FvS, and UG-V contributed to the protocol and design of the study. AG and UG-V provided management and oversight of the trial as sponsor representatives. UG-V coordinated the study design process and implementation of the trial on behalf of the sponsor. FvS was the Coordinating Investigator. MA, AG, and KTM contributed to the implementation of the trial. HSH and SDK contributed to immunological data collection, analysis, and interpretation of the data. LB, GF, RG, and HSH did computational analyses of the immunogenicity data. MA, MAB, RC, HSH, MF-M, KTM, FvS, IH, and UG-V contributed to data interpretation. MAB did the literature search. MAB, AG, HSH, KTM, FvS, and UG-V wrote the first draft and all authors contributed to, and approved, the final manuscript.

Declaration of interests

MAB, AG, HSH, SDK, MF-M, and UG-V are full-time employees and IH is the CEO of the study sponsor, Curevac AG. RC is chairman of the CureVac Scientific Advisory Board and member of the supervisory board. KTM is a medical consultant to CureVac, dedicated to this trial. MA, LB, and FvS have received study funds through their universities for performing the study. GF and RG declare no competing interests.

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