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RNA-based adjuvant CV8102 enhances the immunogenicity of a licensed rabies vaccine in a first-in-human trial

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ABSTRACT

Background: We report the first-in-concept human trial of the safety, tolerability and immunogenicity when a novel TLR 7/8/RIG I agonist RNA-based adjuvant, CV8102, was administered alone or mixed with fractional doses of a licensed rabies vaccine (Rabipur®) as model antigen.
Methods: The primary objective was to assess the safety and reactogenicity of various dose levels of CV8102 alone or mixed with Rabipur® in healthy 18–40 year-old male volunteers. A secondary objective was to assess the immune-enhancing potential of bedside-mixes of CV8102 with fractional doses of Rabipur® by measuring induction of rabies virus neutralising titres (VNTs).
Results: Fifty-six volunteers received 50–100 μg CV8102 alone (n = 11), bedside-mixed CV8102 and Rabipur® (n = 20), or Rabipur® alone (n = 25; control). When given alone or mixed with Rabipur® CV8102 caused mostly Grade 1 or 2 local or systemic reactogenicity, but no related SAEs. As 100 μg CV8102 was associated with marked CRP increases further dose escalation was stopped. Combining 25–50 μg of CV8102 with fractional doses of Rabipur® significantly improved the kinetics of VNT responses; 50 μg CV8102 also improved the magnitude of VNT responses to 1/10 Rabipur® but caused severe but self-limiting influenza-like symptoms in 2 of 14 subjects.
Conclusions: Doses of 25 and 50 μg CV8102 appeared safe and with an acceptable reactogenicity profile while significantly enhancing the immunogenicity of fractional doses of rabies vaccine.
EudraCT No. 2013-004514-18.

1. Background

Novel highly purified or subunit vaccine candidates have improved safety and tolerability but are often poorly immunogenic [1]. Adjuvanted formulations increase the immune response to these vaccines, but a number of adjuvants are currently included in licensed vaccines, most of which are vehicle adjuvants including aluminium salts (alum) or oil-in-water emulsions such as MF59 [2]. Improved understanding of how innate immunity shapes adaptive immune responses has led to the development of novel adjuvants [3]. In this context, toll-like receptor (TLR) ligands have received extensive evaluation and appear to be important for the induction of optimal Th1 and Th2 immune responses [4,5]. The TLR4 agonist monophosphoryl lipid A (MPL) is a component of the AS01 adjuvant in RTS.S malaria [6] and recently approved herpes zoster (Shingrix) vaccines, and also in the AS04 adjuvant in the HPV vaccine, Cervarix. Another TLR9 agonist adjuvant, CpG 1018, is used in the recently approved hepatitis B vaccine (Heplisav-B™) [7]. Single-stranded RNA is a ligand for endosomal TLR7/8, induces innate immune responses via TLR7/8 [8,9] and can activate the RLH (RIG like helicase pathway) if it contains 5-triphosphate [10].

We developed the RNA-based adjuvant CV8102 (RNAdjuvant®, CureVac AG), a TLR 7/8 agonist and RIG I pathway activator [10], to enhance immunogenicity of poorly immunogenic antigens. It consists of non-coding, uncapped, polyU repeats-containing single-stranded RNA with a 5-triphosphate modification, complexed with a polymeric carrier, a small arginine-rich disulfide-crosslinked cationic peptide (CR12C) [10,11]. In preclinical studies CV8102 increased humoral immunity to licensed influenza vaccine in mice and domestic pigs, and to rabies vaccine in mice (Curevac data on file). Combining CV8102 with recombinant protein and antigen-derived peptides (T-cell epitopes) induced strong humoral

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and cellular immune responses [11]. These preclinical data support CV8102 as an adjuvant candidate for prophylactic and therapeutic vaccines, particularly for conditions where both Th1 and Th2 immune responses are required.

We report the first-in-human evaluation of the safety and reactogenicity of increasing doses of CV8102 administered alone and mixed with licensed rabies vaccine as model antigen, and enhancement of humoral responses to this viral antigen using the serologic correlate of protection in use at the time of the study [12].

2. Materials and methods

2.1. Ethical approval

This phase I, proof-of-concept, first-in-human, open-label trial was conducted between Sept 8th 2014 and Oct 5th 2015 at the phase I unit of CRS Clinical Research Services, Mönchengladbach, Germany according to GCP and Declaration of Helsinki guidelines. The protocol was registered with the European Medicine Agency (EudraCT No. 2013-004514-18) and approved by the relevant regulatory authorities and IRBs. An external independent Data Safety and Monitoring Board (DSMB) provided oversight. All subjects provided written informed consent at enrolment.

2.2. Study design

The study was performed in two parts, a dosage escalation study (Part A) with two planned intramuscular injections of 50, 100, or 250 μg CV8102 given 21 days apart. A starting dosage of 50 μg CV8102 was selected based on preclinical dose toxicology studies that showed no adverse findings at 100 μg (mouse, rat) and 250 μg (mini-pig). The primary objective of part A was to determine the highest tolerable dosage and recommended dosage range of CV8102 for study part B.

In part B the primary objective was to assess safety and reactogenicity of a bedside-mix of CV8102 with fractional doses of licensed rabies vaccine, Rabipur®; secondary objectives were to assess the antigen-sparing effect of CV8102 on Rabipur® based on immune responses measured as rabies virus neutralising antibody titres (VNTs). Control groups received full (B1), 1/5 (B2), 1/10 (B3) or 1/20 (B4) doses of Rabipur® alone. Based on the outcome of study part A two groups received two 1/10 (B5) or 1/20 (B6) doses of Rabipur® administered 21 days apart as bedside-mixes with 50 μg and 25 μg doses of CV8102, respectively.

2.3. Subjects

Eligible volunteers were healthy 18–40 year-old males. Major exclusion criteria were receipt of any other vaccination within 4 weeks of first dosing, use of immunosuppressive drugs or immunoglobulins, chronic immunodeficiency or autoimmunity, acute or chronic disease, serologic evidence of hepatitis B, C or HIV infection, current alcohol or drug use, or known allergy to vaccine components. Additional exclusions for part B were any known prior vaccination with a rabies vaccine or planned travel to regions with high rabies risk.

2.4. Procedures

Both study parts involved a screening visit in the two weeks before enrolment to assess basic health parameters, vital signs (blood pressure, heart rate, ECG), clinical chemistry and verify inclusion/exclusion criteria. Participants received study administrations on days 0 and 21 and enrolment was staggered with at least two days between subsequent subjects in a dose group.

2.5. Outcome measurements - Safety

Participants recorded solicited local reactions (pain, redness, swelling, induration, ecchymosis, and itching) and systemic adverse events (AEs; fever, headache, myalgia, arthralgia, nausea, chills, and fatigue) on Days 0–7 after each administration, with severity determined according to WHO guidance [13]. Unsolicited AEs from Days 1 to 28 after each administration were recorded using MedDRA terms together with any concomitant treatment. AEs were graded 1, 2 or 3 according to FDA recommendations and classified as related or unrelated to the treatment. Blood samples for safety assessments were drawn before administration on Day 0 and at eight (Part A), ten (Part B CV8102 groups) or five (Part B control groups) additional time points up to Day 49. Laboratory safety assessments included haematology/chemistry, abnormalities qualifying as AEs if the investigator considered they were clinically significant deviations from normal ranges.

The principal investigator and sponsor medical representatives regularly reviewed AEs and SAEs, opening higher dose groups if there were no unexpected, treatment-related AEs in the previous group. Unexpected adverse reactions, severe AEs and SAEs were reviewed by the DSMB who advised on the enrolment of further subjects and opening of higher dose groups, and the enrolment of test subjects in part B (staggered vs parallel enrolment of subjects). An additional safety follow up call for documentation of any serious AE was done at 12 months.

2.6. Outcome measurements – Immunogenicity

Rabies immune responses were measured in part B on Days 0, 14, 28, and 35 as VNTs using a standardised, WHO-recommended rapid fluorescent focus inhibition test (RFFIT) with the rabies virus strain CVS-11 (German rabies reference laboratory of the Friedrich-Loeffler-Institute, Greifswald, Germany) as challenge virus in an accredited reference laboratory (University Hospital Essen, Institute of Virology, Germany). The LD_{50} was calculated by the Spearman-Karber method and resulting titres converted to international units (IU)/mL using a WHO international standard (NIBSC, Potter Bar, UK), with median titres calculated per group. Measurements of extended serum dilutions were performed on samples with results outside the validated measurement range. Seroconversion was defined as having a neutralising titre $\geq$ 0.5 IU/mL after vaccination in the previously seronegative subjects (seropositivity for rabies was an exclusion criterion).

In part A exploratory chemokine and cytokine measurements were performed in peripheral blood sera drawn before and 6 and 24 h after the first administration on Day 0, on Day 7, before and 6 and 24 h after the second treatment on Day 21, and finally on Day 28, using a cytometric bead array kit (BD Biosciences, Heidelberg, Germany) according to the manufacturer's instructions.

2.7. Study treatments

CV8102 is a purified, single-stranded, non-coding, long chain RNA molecule presented in a sterile aqueous, colourless solution containing the cationic peptide CR12C and trehalose, ready for mixing with an antigen. Lyophilized rabies vaccine, Rabipur® (GSK Biologicals, Wavre, Belgium) was reconstituted at the bedside in supplied diluent for Rabipur-only groups, or in CV8102 for combined groups, for intramuscular injection in volumes of 0.05–1 ml (using 1 ml BD Luer-Lok ™ syringes) in the deltoid.

2.8. Statistical analysis

This was an exploratory trial with no confirmatory proof of hypothesis so statistical sample-size estimation was not

performed. Six subjects per dose group were considered sufficient to down-select dosages which were not well tolerated. Safety analyses were performed on all subjects who received at least one study treatment and for whom any post-baseline safety data were available. Immunological evaluations were performed on all subjects who had at least one post-vaccination blood sample. Measured variables were summarised with descriptive statistics as appropriate. Between-group testing of VNTs was performed using the Mann-Whitney test or paired t tests as indicated, and correlation between CRP and VNTs was performed by Spearman's rank correlation analysis.

3. Results

3.1. Subject disposition and characteristics

Fifty-six volunteers were enrolled into two groups in part A and six groups in part B and received at least one study treatment (Fig. 1). Demographic data including age, sex, body weight, and BMI were similar across groups. In part A, 6 of 7 subjects in group AL (50 μg CV8102) completed the study, with one drop-out after the first dose due to personal reasons. All 4 subjects enrolled in group AM (100 μg CV8102) completed after the first dose with

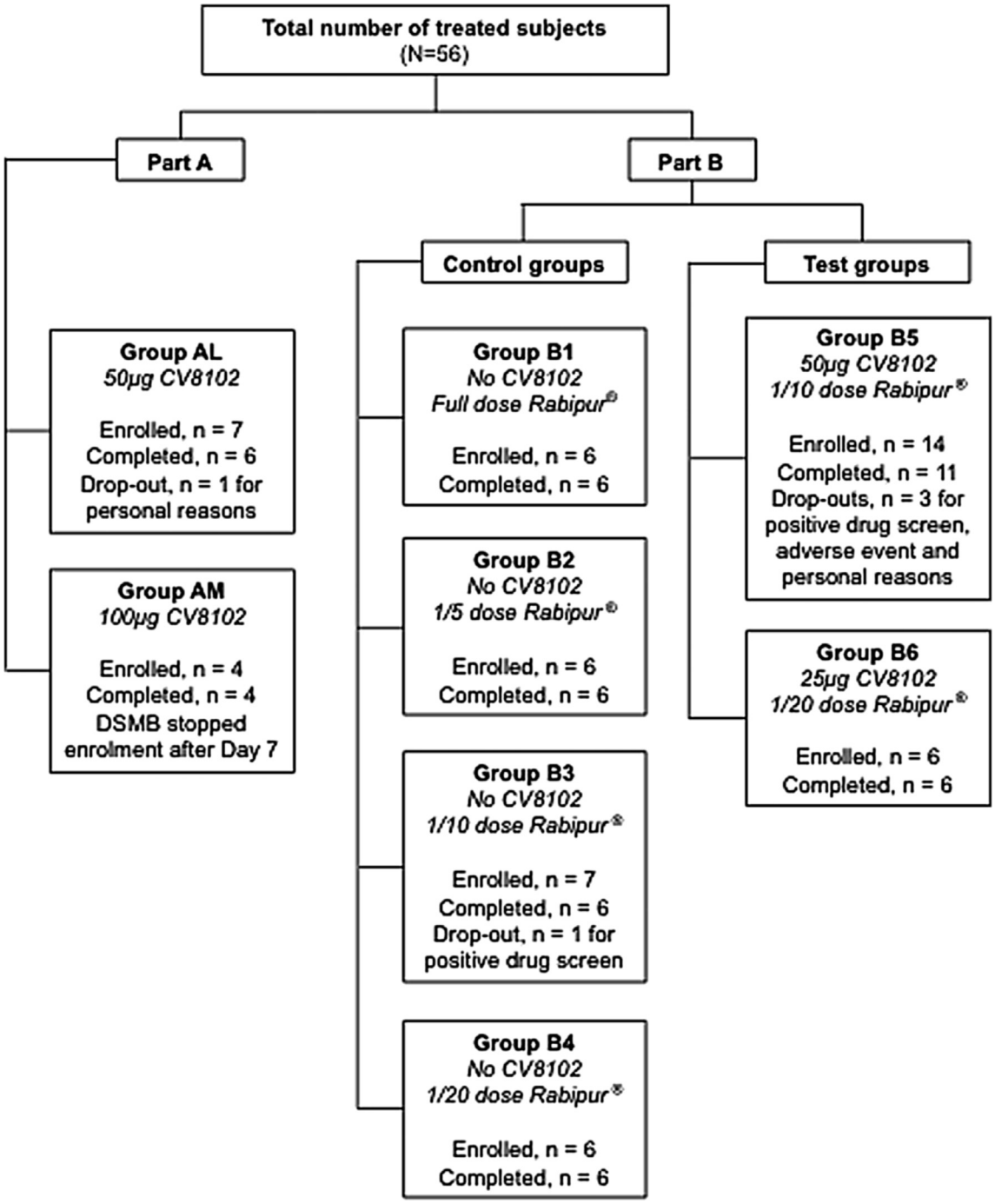

Fig. 1. Flow chart of the study.

further AM group enrolment being stopped due to transient marked CRP increases in two subjects after the first dose and no further dose escalation was done.

In part B 45 volunteers were enrolled into six groups; 25 subjects in four control groups (B1–B4) received Rabipur alone (full, 1/5, 1/10, or 1/20 of the licensed dose), and 20 subjects in two groups received bedside-mixes of CV8102 and Rabipur®. Group B5 (50 μg CV8102 and 1/10 Rabipur®) was expanded from the 6 subjects originally planned to 14 subjects on the recommendation of the DSMB. Six subjects in Group B6 received 25 μg CV8102 and 1/20 Rabipur®.

Four subjects discontinued after one vaccination, one from B3 (1/10 Rabipur® group) due to a positive drug screening test, and three from B5 (1/10 Rabipur® + 50 μg CV8102 group) due to a positive drug screening test, an unrelated SAE (dizziness) and personal reasons, respectively (Fig. 1).

3.2. Part A: Safety of CV8102 alone

No SAEs or severe AEs were reported in study part A up to the end of the study at day 365. After one 50 μg CV8102 dose (group AL) six of seven subjects experienced mostly grade 1, self-resolving solicited AEs; five cases of local pain, two of fatigue, two of headache and one of fever. Incidence and severity did not increase after the second dose. All four subjects given one 100 μg CV8102 dose (group AM) reported grade 2 local pain on Days 0 and 1, three reported grade 1 or 2 fatigue, two grade 1 or 2 headache, two grade 1 myalgia/arthralgia, and one reported grade 1 nausea and another grade 1 fever.

In groups AL and AM, 6 of 11 (54.5%) subjects reported grade 1 or 2 unsolicited AEs, all considered unrelated to study treatment, which resolved without sequelae and without study discontinuation. Laboratory assessment did not find any relevant changes in haematological or clinical chemistry parameters, other than transient mild changes in leukocyte populations, an expansion of neutrophils and monocytes and reduction of lymphocytes 1 day after treatment (not shown). Dosage-dependent increases in C-reactive protein (CRP) in the two days following CV8102 administration (Fig. 2A) was particularly marked in two subjects after 100 μg CV8102, peaking on Day 2 at 49.3 and 51.9 mg/L (ULN < 5.0 mg/L), respectively. All CRP values decreased to baseline within 7 days, and increases were not related to the severity of clinical adverse events.

Transient increases in some inflammatory mediators, primarily chemokines, were also apparent at early time points after administration of CV8102 alone: CCL11 levels peaked at 6 h, CCL4 and IP-10 levels peaked at 24 h after treatment (Fig. 3). Mean CCL11 levels returned to baseline within 24 h and CCL4 and IP-10 essentially normalised by Day 7. A minor increase in pro-inflammatory IL-6 was observed in 2 subjects, but no correlation of increased inflammatory mediator levels with CRP increases were observed. Most other factors showed minor increases over detection limits or remained at undetectable levels (data not shown).

3.3. Part B: Safety of Rabipur® and CV8102/Rabipur® bedside-mix

In Part B 25 participants received 50 doses of Rabipur® alone as controls and 20 received 37 doses of mixed CV8102/Rabipur®. All

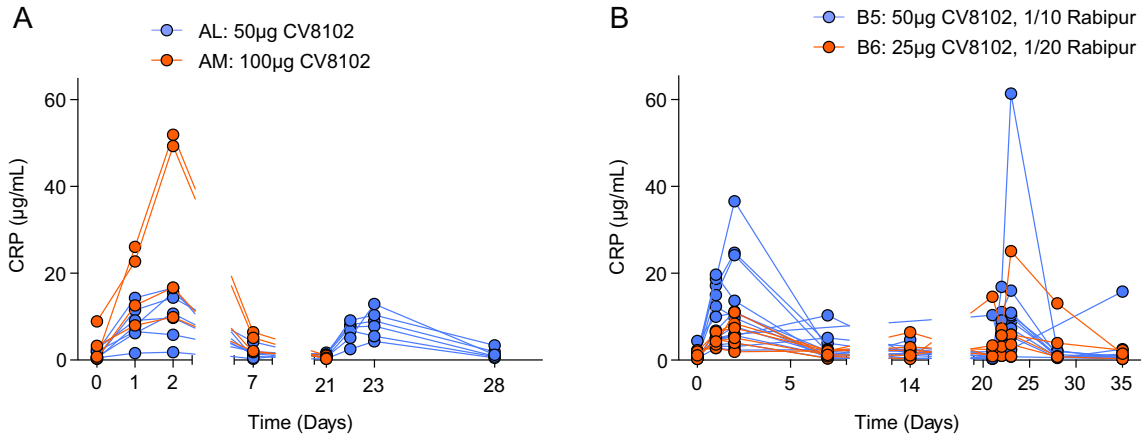

Fig. 2. C-reactive protein (CRP) levels in participants after each of two 50 μg doses (group AL) or one 100 μg dose (group AM) of CV8102 alone [panel A]; or each of two doses of 50 μg CV8102 with 1/10 dose Rabipur (group B5) or 25 μg CV8102 with 1/20 dose Rabipur (group B6) [panel B]. Upper limit of normal is < 5.0 mg/L.

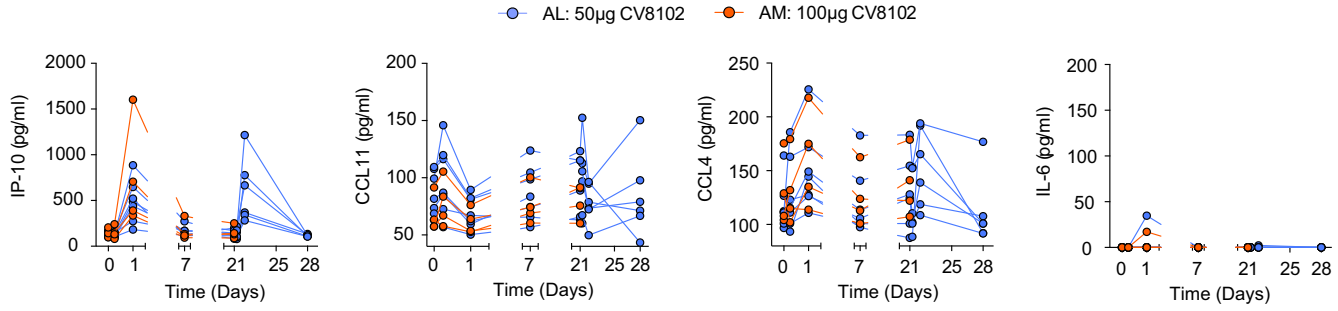

Fig. 3. Effects of two doses of 50 μg (group AL) or one dose of 100 μg (group AM) CV8102 on serum chemokine and cytokine levels. The following soluble factors were measured in blood sera using cytokine bead array according to the manufacturer's instructions: IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, IL-17F, IFN-a, IFN-g, TNF-a, CCL2, CCL3, CCL4, CCL5, CCL11, CXCL8 (IL-8), CXCL9, CXCL10 (IP-10), CXCL11, CX3CL1, VEGF, GzmA, and GzmB. Only those shown, IP-10, CCL11, CCL4 and IL-6, showed treatment-associated changes.

were assessed for safety up to one year after the last dose. There were no SAEs or severe AEs in Rabipur® controls or 25 μg CV8102 and 1/20 Rabipur® recipients (group B6). One SAE after a dose of 50 μg CV8102 and 1/10 Rabipur® (group B5) was hospitalisation for diagnostic evaluation of grade 2 dizziness accompanied by grade 3 headache 7 days after the first vaccination, but symptoms resolved completely after symptomatic treatment. Although the SAE was considered to be unrelated to vaccination the second vaccination was not given.

All solicited local reactions and systemic AEs after Rabipur® alone were grade 1, except for grade 2 injection site pain in two subjects after their second full dose (Table 1). In group B5 (50 μg CV8102 mixed with 1/10 Rabipur®) injection site pain occurred after 86% of first doses, including one grade 3 case, and after 64% of second doses. Solicited systemic AEs were less common; headache was reported by 4 of 14 (29%) and 5 of 11 (45%) subjects after first and second vaccinations, respectively, with two reports of grade 3 headache after each dose. Myalgia/arthralgia and fatigue were reported by 4 of 14 subjects (29%) of Group 5 after their first dose, with one grade 3 case of each, and 3 of 11 subjects (27%) after the second dose, with two grade 3 cases of each. In contrast, in Group B6 given 25 μg CV8102 and 1/20 Rabipur® 66% and 33% reported grade 1 or 2 injection site pain after the first and second doses respectively, and the only reports of systemic AEs were two cases of headache after the first dose, graded 1 and 2 respectively.

Fifteen unsolicited AEs reported during the 49 day observation period by 13 of 45 (29%) subjects were mainly grade 1 (n = 9) or grade 2 (n = 4) general disorders such as back pain or nasopharyngitis; all were considered unrelated to vaccination. Two events, local rash at the injection site and increased CRP, were considered to be treatment-related. CV8102 dosage-dependent increases in CRP observed in Part A also occurred with CV8102/Rabipur® mixes (Fig. 2B). One subject in group B5 (50 μg CV8102 with 1/10 Rabipur®) had a peak CRP of 61.4 mg/L on Day 23, two days after the second dose, which was considered clinically significant and related to the treatment. This subject also experienced grade 2 headache and myalgia, and grade 1 influenza-like symptoms. All symptoms resolved spontaneously, CRP values returning to normal limits by Day 28. No CRP assessments were performed following Rabipur® alone.

3.4. Immunogenicity

In part B, two fractional doses of Rabipur® in Groups B1–B4 elicited dose-dependent increases in rabies virus neutralising antibody titres (VNT) (Fig. 4A). CV8102 enhanced the kinetics of the VNT responses when expressed as the Day 14 seroconversion rate, i.e. the percentage achieving a VNT $\geq$ 0.5 IU/mL, the WHO protective threshold [12]. After 1/20 and 1/10 Rabipur® doses 33.3% and 57.1% seroconverted at Day 14, respectively, increasing to 83.3% and 85.7% when mixed with CV8102 (Table 2, Fig. 4B, C). All subjects, except one in the B2 group (1/5 Rabipur® dose), had seroconverted one week after a second vaccination.

Many subjects displayed VNTs above the upper limit of quantification (ULOQ), limiting our ability to compare the magnitude of the humoral immune responses across groups, so we performed a post-hoc exploratory analysis using an extended dilution range of available remaining sera from Groups B1, B3 and B5 (full and 1/10 Rabipur® ± 50 μg CV8102 groups). In this assay all 6 (100%) subjects given full-dose Rabipur® seroconverted by Day 14, compared with 4 of 7 (57.1%) given a 1/10 dose, and 13 of 14 (92.9%) subjects given the 1/10 dose mixed with 50 μg CV8102 (Table 2). All subjects in all three groups seroconverted one week after their second dose. Median VNTs after full and 1/10 Rabipur® doses were 2.9 and 1.1 IU/mL at Day 14, increasing to 9.8 and 2.1 IU/mL at Day 28 and to 23.2 and 2.9 IU/mL at Day 35, respectively (Fig. 4D). Mixing 1/10 dose with 50 μg CV8102 increased median titres although not to the full dose levels, at 2.7, 6.5 and 8.5 IU/mL at Days 14, 28 and 35, respectively.

Due to the robust immunostimulatory nature of CV8102 observed in part A, we investigated whether early increases in CRP levels correlated with the VNTs in subjects receiving the combination of Rabipur® and CV8102 (cohorts B5 and B6). We identified a modest association between CRP levels and VNTs at Day 35 on Day 1, which was significant at Day 2 (Fig. 5).

Table 1
Overview of solicited adverse events after first and second doses, and unsolicited adverse events in study groups in part B of the study (safety set).

		Solicited AEs according to group after the first and second dose administrations, n (%)											
Group		B1		B2		B3		B4		B5		B6	
Rabipur®		Full dose		1/5 dose		1/10 dose		1/20 dose		1/10 dose		1/20 dose	
CV8102		None		None		None		None		50 μg		25 μg	
	Grade	Dose 1 (N = 6)	Dose 2 (N = 6)	Dose 1 (N = 6)	Dose 2 (N = 6)	Dose 1 (N = 7)	Dose 2 (N = 6)	Dose 1 (N = 6)	Dose 2 (N = 6)	Dose 1 (N = 14)	Dose 2 (N = 11)	Dose 1 (N = 6)	Dose 2 (N = 6)
Local reaction													
Pain	1	1 (16.7)	1 (16.7)	–	1 (16.7)	2 (28.6)	1 (16.7)	–	1 (16.7)	3 (21.4)	4 (36.4)	1 (16.7)	1 (16.7)
	2	–	2 (33.3)	–	–	–	–	–	–	8 (57.1)	3 (27.3)	3 (50.0)	1 (16.7)
	3	–	–	–	–	–	–	–	–	1 (7.1)	–	–	–
Systemic adverse events													
Headache	1	1 (16.7)	–	–	–	–	–	–	1 (16.7)	1 (7.1)	–	1 (16.7)	–
	2	–	–	–	–	–	–	–	–	1 (7.1)	3 (27.3)	1 (16.7)	–
	3	–	–	–	–	–	–	–	–	2 (14.3)	2 (18.2)	–	–
Myalgia/arthralgia	1	–	–	–	–	1 (14.3)	–	–	–	2 (14.3)	–	–	–
	2	–	–	–	–	–	–	–	–	1 (7.1)	1 (9.1)	–	–
	3	–	–	–	–	–	–	–	–	1 (7.1)	2 (18.2)	–	–
Fatigue	1	–	–	–	–	1 (14.3)	–	–	–	2 (14.3)	–	–	–
	2	–	–	–	–	–	–	–	–	1 (7.1)	1 (9.1)	–	–
	3	–	–	–	–	–	–	–	–	1 (7.1)	2 (18.2)	–	–
Chills	1	–	–	–	–	–	–	1 (16.7)	1 (16.7)	–	2 (18.2)	–	–
	2	–	–	–	–	–	–	–	–	–	1 (9.1)	–	–
Nausea	1	–	–	–	–	–	–	1 (16.7)	1 (16.7)	–	–	–	–
Fever	1	–	–	–	–	–	–	–	–	1 (7.1)	1 (9.1)	–	–
Unsolicited adverse events (all doses)													
No. of Events (No. Subjects)		3 (2)		1 (1)		1 (1)		0		5 (4)		5 (5)	

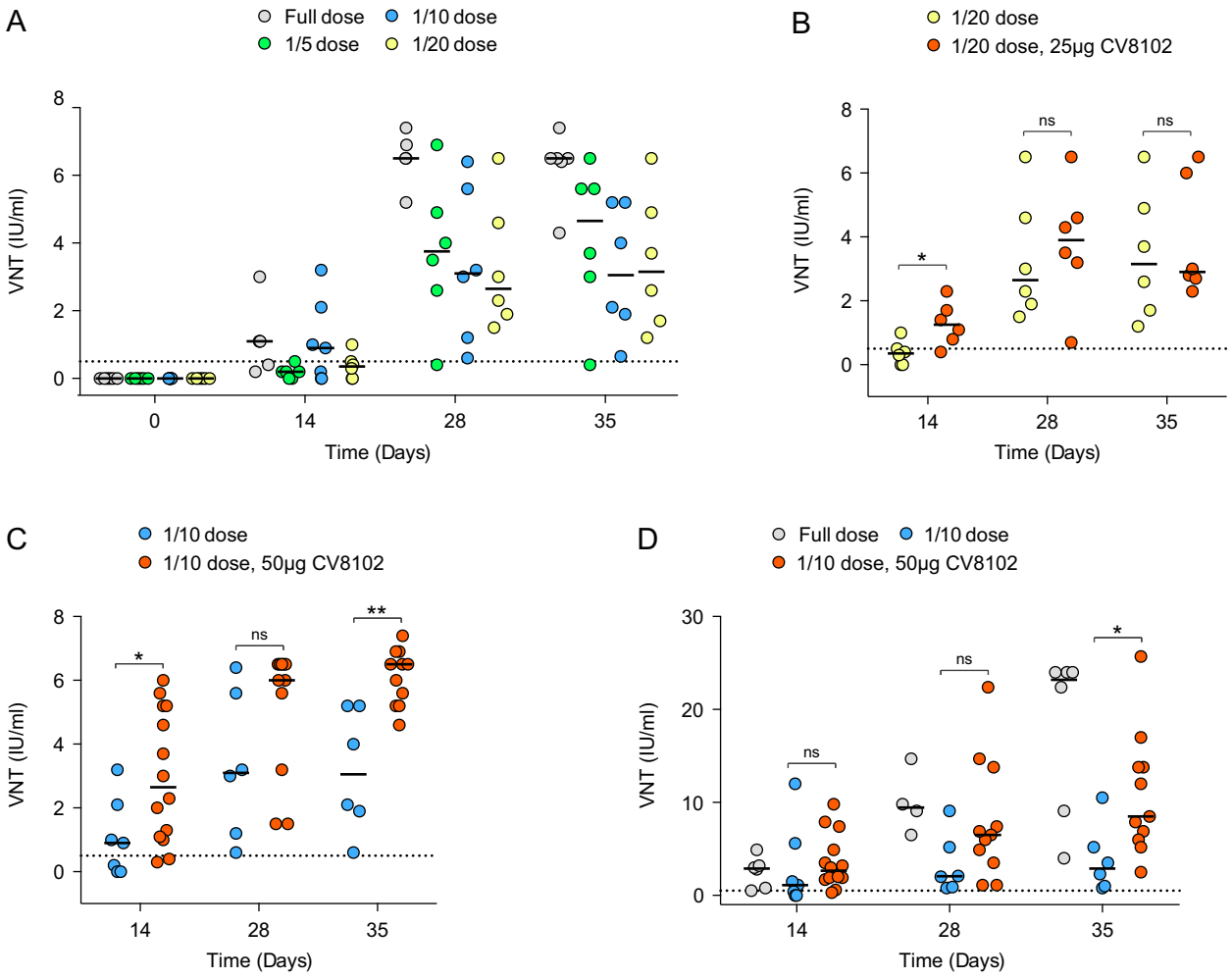

Fig. 4. Rabies virus neutralising titres (VNT) measured in part B study groups given Rabipur alone (groups B1-4, panel A), 1/20 dose of Rabipur without or with 25 µg CV8102 (groups B4 & B6, panel B), or 1/10 dose of Rabipur without or with 50 µg CV8102 (groups B3 & B5, panel C). The upper limit of quantification in panels A-C was at approximately 6.5 IU/mL. Panel D shows VNT measured post-hoc at higher dilutions in the groups which received full (B1) or 1/10 doses of Rabipur alone (B3), or 1/10 dose of Rabipur with 50 µg CV8102 (B5). Bars show medians for each group, dotted line shows protective threshold (0.5 IU/mL). Differences between 1/20 and 1/10 dose groups are shown as either non-significant (ns), or statistically significant: * p = 0.0173 in panel B; * p = 0.0211, ** p = 0.0015 in panel C; * p = 0.01112 in panel D.

Table 2
Overview of seroconversion rates in part B study groups, and in Groups B1, B3 and B5 after dilution. Due to the high inter-assay variance of the RFFIT assay some rates with low values at Day 14 differ in the same cohorts for undiluted (original analyses) vs. diluted samples.

Group	Rabipur® dose	CV8102	Median VNTs (IU/mL)			Seroconversion rate, n/N (%)		
			Day 14	Day 28	Day 35	Day 14	Day 28	Day 35
Original analyses								
B1	Full	0	1.1	>6.5*	>6.5*	4/6 (66.7)	5/5 (100)	6/6 (100)
B2	1/5	0	0.2	3.8	4.7	1/6 (16.7)	5/6 (83.3)	5/6 (83.3)
B3	1/10	0	0.9	3.1	3.1	4/7 (57.1)	6/6 (100)	6/6 (100)
B5	1/10	50 µg	2.7	>6.0*	>6.5*	12/14 (85.7)	11/11 (100)	11/11 (100)
B4	1/20	0	0.4	2.7	3.2	2/6 (33.3)	6/6 (100)	6/6 (100)
B6	1/20	25 µg	1.3	3.9	2.9	5/6 (83.3)	6/6 (100)	6/6 (100)
Further diluted samples								
B1	Full	0	2.9	9.8	23.2	6/6 (100)	5/5 (100)	6/6 (100)
B3	1/10	0	1.1	2.1	2.9	4/7 (57.1)	6/6 (100)	6/6 (100)
B5	1/10	50 µg	2.7	6.5	8.5	13/14 (92.9)	11/11 (100)	11/11 (100)

* Since VNTs from several subjects were above the ULOQ VNT medians could not be calculated for these cohorts and time points.

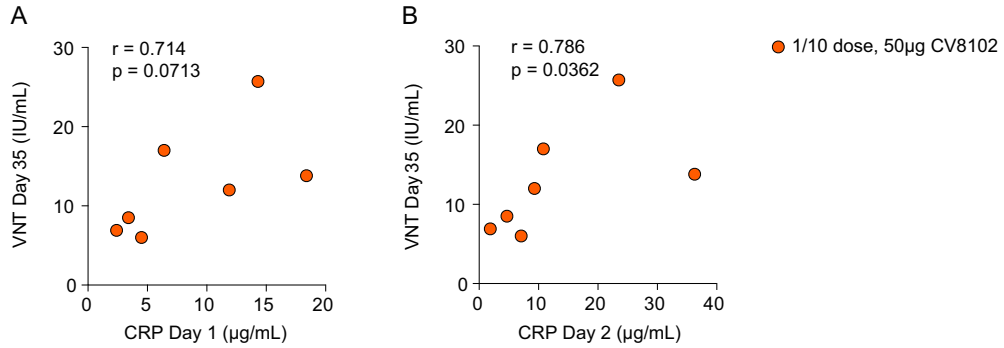

Fig. 5. Early CRP values correlate with late VNTs. Subjects were vaccinated intramuscularly (Days 0 and 21) with a combination of Rabipur® 1/10 dose and 50 μg of CV8102 (Group B5). Spearman rank correlation analysis were performed between VNTs of B5 (n = 7 subjects from Day 35 and baseline-subtracted CRP values from Days 1 (panel A) and 2 (panel B). CRP = C-reactive protein; VNT = virus neutralising titre.

4. Discussion

Highly purified or recombinant subunit vaccines generally have superior safety and tolerability but low intrinsic immunogenicity, so require adjuvantation to augment and shape immune responses, allowing for antigen- or dose-sparing, which may also be applicable to whole virus vaccines in resource-constrained situations. Furthermore, there is a important medical need for effective adjuvants for therapeutic vaccines, e.g. tumor immunotherapy, which will require novel adjuvants. We describe the first-in-human study of CV8102, an RNA-based adjuvant, using a rabies vaccine as model antigen. There was acceptable safety and tolerability with a 50 μg CV8102 given alone, but marked CRP increases in some subjects with higher dosages. The reactogenicity profile of 25 μg CV8102 with 1/20 Rabipur® was similar to that of full-dose Rabipur®, but reactogenicity to 50 μg CV8102 with 1/10 dose Rabipur® was higher.

Consistent observations of transient increases in CRP with CV8102 with or without Rabipur® were only major in a few individuals and these all resolved rapidly. Although not directly associated with clinical symptoms or severe AEs the DSMB considered marked CRP elevations to be undesirable for a prophylactic vaccine adjuvant and recommended cessation of further dosage escalation past 50 μg. Early CRP increases have also been reported in infants after routine vaccinations [14,15] and adults after MF59 or TLR9 agonist adjuvanted vaccines [16,17]. Influenza vaccine containing a TLR5 motif dose-dependently increased CRP in healthy adults associated with more severe reactogenicity in some subjects [18].

Fractional doses of Rabipur® dose-dependently increased rabies immunity as shown by neutralising antibodies. Bedside-mixing lower doses of Rabipur® with CV8102 partially mitigated against lower immunogenicity, manifested as faster kinetics and increased magnitude of immune responses. With Rabipur, an inactivated viral vaccine that can induce innate immune activation by itself, the adjuvant effect was more pronounced with the 50 μg dose but associated with increased reactogenicity. In cohorts given Rabipur®/CV8102 mixes 85% (17 of 20) of subjects achieved seroconversion two weeks after one dose compared with 46% (6 of 13) of those given fractional doses of Rabipur® alone demonstrating the adjuvant role of CV8102. We used Rabipur as a model antigen with no plan to develop CV8102 as rabies vaccine adjuvant. Given the reactogenicity in combination with rabies vaccine CV8102 may be more suitable for combination with less reactogenic subunit or protein vaccines that are required for high medical need prophylaxis situations (epidemic outbreaks) or therapeutic vaccines where higher reactogenicity is acceptable.

One limitation of this study is that we did not assess CRP responses to Rabipur® alone as the magnitude of these responses to CV8102 mixed with Rabipur® appeared higher than with CV8102 alone. This may be due to the TLR-activating motifs in viral vaccines like Rabipur® increasing inflammatory responses. Although excessively high levels of post-vaccination CRP are undesirable, we observed a modest correlation between transient CRP increases at Days 1 and 2 and VNTs at Day 35. Given the complexity of how the innate immune system activates and regulates the adaptive immune system there is a strong rationale for more in-depth analyses of early immune events [7–11,19]. In light of this, another limitation was that samples were not taken in part B to examine the immediate effects of CV8102 and Rabipur® on the transient changes in cytokines and chemokines observed in part A. We cannot therefore fully conclude on the contribution of Rabipur® to the early inflammatory response. Another missing element was the inability to test 25 μg CV8102 mixed with a 1/10 dose of Rabipur® due to limitations in bedside-mixing with the available concentrations, but this combination may provide an optimal balance between tolerability and adjuvant enhancement of the response against the rabies antigen.

Preclinical data supports the potential of CV8102 as an adjuvant for cancer vaccines [20]. As transient severe reactogenicity and CRP increases are clinically more acceptable in therapeutic settings investigation of CV8102 including doses exceeding 50 μg is being pursued in combination with a peptide vaccine in patients with hepatocellular carcinoma in an EU-funded phase I trial by the HEPAVAC Consortium [21]. Early observations in an ongoing phase I trial of CV8102 in patients with advanced solid tumours [NCT03291002] indicate that repeated intra-tumour injections $\geq$ 100 μg are tolerated without causing severe side effects [22].

In conclusion, this first-in-human trial demonstrates that despite some transient severe systemic reactions, at dosages with acceptable reactogenicity the RNA-based adjuvant CV8102 is a potent immune stimulator of fractional doses of licensed rabies vaccine. At dosages up to 50 μg CV8102 is a promising adjuvant candidate and may have lower reactogenicity with inherently less reactogenic whole protein or subunit vaccines than the rabies vaccine we used.

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Role of the contributors

All authors were involved in the design, performance and analysis of the study, and subsequently assisted in the drafting, review and approval of the manuscript.

Conflict of interest statement

At the time of this study Fatma Doener, Henoch S. Hong, Angelika Daehling, Regina Heidenreich, Sven D. Koch, Mariola Fotin-Mleczek and Ulrike Gnad-Vogt were full-time salaried employees of the study sponsor, and Keyvan Tadjalli-Mehr was a contractor working for the study sponsor. Ingo Meyer has no conflicts of interest to declare.

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