Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A randomized dose-escalation phase I clinical trial of a nucleoprotein-based genetic vaccine against Crimean-Congo Hemorrhagic Fever

Ali Mirazimi

ali.mirazimi@ki.se

Department of Laboratory Medicine (LABMED), Karolinska Institute https://orcid.org/0000-0003-2371-6055

christian nordentoft
Karolinska Inst

per ljungman
Karolinska Hospital

lara sweetapple
karolinska Inst

Jingy Yan
Karolinska Institutet

Soo Aleman
Karolinska Institutet https://orcid.org/0000-0003-0461-4870

Matteo Cadossi
IGEA SPa

SImona Salati
IGEA Spa https://orcid.org/0000-0002-8072-1128

katja tobin
Karolinska Hospital

Ola Tuvesson
Northx Biologics

Eva-karin Gidlund

olivia merinder
Legado bio

urban hoglund
Legado bio

Matti Sallberg
Karolinska Institute https://orcid.org/0000-0002-8858-5132

maria creignou

Karolinska Inst

Friedemann Weber

Justus-Liebig University https://orcid.org/0000-0001-9737-337X

Sofia Appelberg

FOhM https://orcid.org/0000-0002-7381-3606

Stéphanie Devignot

Karolinska Institute https://orcid.org/0000-0002-0618-5611

Lars Frelin

Karolinska Institutet https://orcid.org/0000-0001-7452-1667

Gustaf Ahlen

Karolinska Institute

Article

Keywords:

Posted Date: November 2nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10776782/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: There is **NO** Competing Interest.

A randomized dose-escalation phase I clinical trial of a nucleoprotein-based disease modulating genetic vaccine against Crimean-Congo Hemorrhagic Fever

Gustaf Ahlén[1, 2, 3] *, Per Ljungman[4, 5]*, Soo Aleman[5, 6, 14] *, Jingyi Yan[1, 2, 3], Christian An Binh Nordentoft[1, 2, 3], Lara Sweetapple[1, 2, 3], Sofia Appelberg[8], Matteo Cadossi[9], Simona Salati[9], Katja Tobin[10], Stephanie Devignot[1,8], Ola Tuvesson[11], Eva-Karin Gidlund[11], Friedemann Weber[12], Olivia Larsson[13], Urban Höglund[13], Maria Creignou[14], Lars Frelin[1, 2, 3, 15], Ali Mirazimi[1,8]* and Matti Sällberg[1, 2, 3]*

*) contributed equally

Affiliations:

1. Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
2. Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden
3. Karolinska ATMP Center, Stockholm, Sweden
4. Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Karolinska Comprehensive Cancer Center, Stockholm, Sweden
5. Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden
6. Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden
7. Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden
8. Public Health Agency of Sweden, Stockholm, Sweden
9. IGEA Spa, Carpi, Italy

[10.] Department of Clinical Cancer Studies, Karolinska University Hospital, Stockholm, Sweden

[11.] Northx Biologics, Matfors, Sweden

[12.] Institute for Virology, FB10-Veterinary Medicine, Justus-Liebig University Giessen, Germany

[13.] Adlego Biomedical AB, Uppsala, Sweden

[14.] Phase 1 Unit, Centre for Clinical Cancer studies, Karolinska University Hospital, Sweden

[15.] Medical Diagnostics Karolinska, Karolinska University Hospital, Stockholm, Sweden

Corresponding author

Matti Sällberg, DDS, PhD, Professor

Department of Laboratory Medicine

Alfred Nobels Allé 8

141 52 Huddinge

Sweden

Mobile: +46706082101

Email: matti.sallberg@ki.se

DISCLOSURES

MS and LF are founders of SVF Vaccines AB.

FUNDING STATEMENT

This study was supported by the CCHFVaccine consortium, funded by the European Union's Horizon 2020 research and innovation program under grant agreement 732732. The study was also supported by the Swedish Research Council (M.S. and A.M.), the Swedish Cancer Society (M.S.), Karolinska Institutet (M.S.) Stockholm County Council CIMED, ALF grants (M.S.), and private donations to M.S. and A.M

ABSTRACT

Crimean–Congo haemorrhagic fever virus (CCHFV) is an emerging tick-borne virus transmitted primarily by Hyalomma ticks, whose geographic distribution is expanding. CCHFV infection is associated with high case-fatality rates, and there are currently no widely approved vaccines available globally. CCHFV is included on the World Health Organization (WHO) R&D Blueprint list of priority pathogens, underscoring its epidemic potential and the urgent need for effective countermeasures. We were the first to demonstrate that a genetic DNA vaccine (CCHF-N-pVAX1) expressing the nucleoprotein (N) of CCHFV can confer robust protection against lethal disease in mice and attenuate clinical symptoms in non-human primates, providing a compelling proof-of-concept for a broadly applicable and previously underexplored vaccine strategy through activation of N-specific T cells and antibodies. Here, the CCHF-N-pVAX1 vaccine was tested in a phase I clinical trial with 15 healthy volunteers (HVs) divided 5 per group in three dose groups (0.45 mg, 0.90 mg, and 1.80 mg). The participants received vaccinations at days 0, 24, and 84 by intramuscular (i.m.) injection using *in vivo* electroporation (EP; IGEA Medical), followed by 3 months of follow-up. The vaccine was safe, with mostly mild grade adverse events (AE: n=113), 3 AE of moderate intensity, and 2 AE of severe intensity (diarrhea and fatigue), likely or possibly related to the vaccine/EP. No severe AE occurred. N-specific antibodies and T cells appeared after the 2nd dose, and mean levels peaked after the 3rd dose at 1212±2753 (endpoint titers) and 621±561 (IFN-γ spotforming cells), respectively. The cell responses remained high during the 3-month follow-up. In conclusion, we show for the first time that a genetic N-based vaccine against CCHF is safe and immunogenic in humans, inducing strong T- and B-cell responses in most vaccine recipients, and suggesting a potential to confer protection against severe disease.

Introduction

Crimean Congo Hemorrhagic fever (CCHF) is a tick-borne infection present on most continents, including Africa, Asia, and Europe [1]. Hyalomma ticks are responsible for the transmission and are expanding geographically, likely driven by climate change, migratory birds, and trade with infected live-stock [1]. Given the potentially high mortality, with reported case-fatality rates of up to 40%, an effective vaccine would represent an important tool for prevention and preparedness in endemic and at-risk regions [1]. There is currently no globally available vaccine against CCHF virus (CCHFV).

We have evaluated the M and S segments from CCHFV as potential vaccine candidates [2-4]. We could show that the nucleoprotein (N) of CCHF is highly immunogenic and raises a protective immunity in interferon alpha-receptor deficient (IFNAR-/-) models as both a DNA and mRNA vaccine [2-4]. In a study in non-human primates, we could show that three vaccinations with a combination of the M and N segment as DNA vaccines controlled viral replication and protected against clinical, biochemical and hematological symptoms of CCHF [2]. We next demonstrated that DNA vaccines encoding the M and/or N proteins were able to reduce viral replication significantly and alleviate disease markers when administered individually [4]. Based on these studies, we concluded that the DNA vaccine expressing the N protein could be successfully produced in accordance with Good Manufacturing Practice (GMP) standards and could induce protective immune responses, making it a strong candidate for clinical development. A major advantage of targeting the N protein is its high degree of conservation across CCHFV strains, with very limited genetic variation, suggesting the potential for broad cross-protective immunity against diverse circulating variants [1]. A toxicological study in rabbits showed that the vaccine was safe and immunogenic at the intended human doses. We report here, for the first time, that a

genetic vaccine expressing the CCHF N protein was safe, tolerable, and induced both B and T cell responses in humans. This is a unique development of a potentially disease modifying vaccine against CCHF.

Materials and Methods

GMP vaccines production

The N-pVAX1 plasmid was produced according to good manufacturing practice (GMP) at Northx Biologics in Matfors, Sweden.

Toxicological study

The toxicological study in New Zeeland White rabbits was performed by Scantox, Stockholm, Sweden, according to good laboratory practice (GLP). A total of 18 male and 18 female rabbits received either vehicle, N-pVAX1 at the clinical dose (per/kg bodyweight (BW)) or 10 times the clinical dose. The DNA was delivered using the EPSgun and the Cliniporator for a single-step delivery of DNA and *in vivo* electroporation (EP) as described previously [5,6]. The immune responses were determined by ELISA and by ELISpot using recombinant N protein and synthetic peptides covering the complete N protein as described [4]. The toxicological report has been provided in the Supplemental Materials.

Study procedures

Healthy volunteers were solicited through advertising. All candidates received written and oral information and signed informed consent before any study procedures were performed. After informed consent was obtained, an investigator went through the candidate's medical history and performed a detailed physical examination. Blood samples were collected for hematology, including coagulation, and clinical chemistry parameters. Vital signs were measured, and an ECG was obtained. If minor deviations were noted in the laboratory samples, a candidate could be rescreened on one occasion within the screening period of 45 days. The study protocol has been provided in the Supplemental Materials.

Vaccine, dose groups, and dosing schedule

The study was conducted as a dose-escalation phase 1/first-in-human study with administration using the EPSgun for a single-step injection of DNA and *in vivo* EP as described in a recent phase I study [7]. There were three dose groups, each including five healthy subjects. The different doses were 0.45 mg diluted in 0.25 mL Tris/EDTA, 0.9 mg diluted in 0.5 mL Tris/EDTA, and 1.8 mg diluted in 1.0 mL Tris/EDTA, respectively. Each participant was to receive three doses of the vaccine on days 0, 28, and 84 (Figure 1a). The study was approved by the Swedish Medical Products Agency and the Ethical Review Board submitted using the CTIS.

Vaccination procedure

Vaccinations were performed in a phase I/first in human unit at the Karolinska Univeristy Hospital, with approval from the Swedish Medical Products Agency (EU clinical trial number 2023-508556-18-00/IN). The vaccine was administered by a

trained research nurse under the observation of either the PI or a co-investigator. The vaccine vas delivered intramuscularly using the using the Cliniporator EPS02 pulse generator and the EPS-Gun (IGEA, Italy) as described previously [7]. The first participant in each dose group was observed for four hours after the first injection. If no serious reaction occurred, the participant was observed for one hour after subsequent doses. The remaining four participants in each group could be vaccinated no earlier than 7 days after the first participant in that dose group and were observed for 1 hour after each vaccination. The day after each vaccination, a study nurse had telephone contact with the participants, and follow-up visits for blood sampling were conducted on days +7 and +14 after each vaccination. In addition, there were visits on days +28 and +90 after the last vaccination for assessments of AEs.

Safety

Immediately after each vaccination, participants were asked to rate pain intensity on the visual analogue scale (VAS) for the first hour. The participants also completed a diary for 7 days after each vaccination to collect information on reactogenicity. In addition, the study staff asked about adverse events at each study visit. The causality of all AEs in the study was determined by the principal investigator or the co-investigator, according to prespecified definitions in the study protocol.

An independent safety committee reviewed all data after each dose group had received three vaccinations and gave recommendations on whether the study could continue as planned before the following dose group could be initiated.

Laboratory procedures

All hematological and biochemical analyses were performed at the clinical laboratories within Medical Diagnostics Karolinska, Karolinska University Hospital, Sweden, according to clinical routines.

Detection of IgG-specific antibodies

Serum IgG antibodies against N were measured by ELISA. Briefly, 96-well plates were coated overnight at 4°C with recombinant N protein (REC31639, Native Antigen) at a concentration of 2 µg/mL in 50 mM sodium carbonate buffer (pH 9.6). Plates were then blocked with 10% skimmed milk for 1 h at 37°C. Serum samples were added in serial dilutions starting at 1:60. Bound IgG antibodies were detected using horseradish peroxidase-conjugated goat anti-human IgG (A0170, Sigma) diluted 1:10,000, followed by development with TMB substrate. Optical density (OD) was measured at 450 nm with background correction at 650 nm. All samples from the same dose group were run at the same time. Endpoint antibody titers were defined as the highest serum dilution yielding an OD value higher than the mean of the pre-immunization sera at the same dilution from the same dose group tested at the same time plus three times the standard deviation.

Detection of specific IFNγ-producing T cells by ELISpot

Peripheral blood mononuclear cells (PBMCs) were isolated using the SepMate separation protocol and cryopreserved at −80°C until analysis. Antigen-specific T-cell responses were assessed by measuring IFN-γ secretion following 36 h of stimulation using the ELISpot Plus: Human IFNγ (ALP) kit (Mabtech). Briefly, 250,000 rested PBMCs were cultured in duplicate and stimulated with either pools of CCHF N peptides

or recombinant N protein (REC31639, Native Antigen). The peptide library comprised 48 overlapping 20-mer peptides spanning the N protein sequence, with an overlap of 10 amino acids between consecutive peptides and was purchased from GenScript. Results have been given by subtracting the mean of the negative control IFNγ spot forming cells (SFCs) from each analysis. The cut off was set at 50 SFCs/10^6 PBMC.

Administration

The DNA was administered using the Cliniporator EPS02 pulse generator and the EPS-Gun (IGEA Spa, Italy), as described previously [7]. In brief, the DNA was injected into the right deltoid muscle at a depth of 2 mm. The injected DNA was in the middle of four electrodes mounted in a square pattern and two electrical pulses were delivered immediately after the injection and retraction of the injection needle.

Statistics

The antibody responses were analyzed by both examining individual values and mean titers. The sample size was too small for reliable statistical comparisons, whereby results are mainly presented as descriptive statistics and grouped comparisons. In the exploratory analyses comparisons were done using Social Statistics online service (https://www.socscistatistics.com) and GraphPad Prism.

RESULTS

Participants

A total of 24 healthy volunteers were screened after providing written informed consent. Eight volunteers did not fulfill all inclusion criteria or had at least one exclusion criterion and were therefore considered screen failures. Of the 16 participants enrolled in the study, one was excluded before vaccination due to a positive pregnancy test.

Thus, 15 study participants were included in the study and vaccinated (Table 1). They had a mean ± SD age of 39 ± 12 years (range 22-57). Ten were male (67%) and five (33%) were female. The mean ± SD BMI was 25.0 ± 2.6 kg/m^2 (range 20.8-29.7); all participants therefore fulfilled the inclusion criterion of a BMI between 20 and 30 kg/m^2.

Due to personal reasons, one participant in the second dose group received only two doses and had thereafter to leave the country. No follow-up samples were collected after the 2nd dose. The participant was contacted by the study team, and no significant adverse events were reported. The participant was not replaced.

Safety and tolerability

No serious adverse events (SAEs) or suspected unexpected serious adverse-reactions (SUSARs) were observed during the study. The reported adverse events (AEs) were mainly due to pain at the time of vaccination and local and systemic reactogenicity.

Discomfort during and directly after vaccination

All 15 participants reported pain at the time of each injection (Figure 1b). The median intensity of discomfort, assessed using VAS, ranged from 5 to 6 across the three dose groups (min-max: 2-8). In all participants, discomfort decreased within five minutes

after injection. At the time of 1st injection, discomfort was significantly higher in the 0.9 mg dose group than in the other dose groups ($p<0.05$, Mann-Whitney U-test). There was no statistical increase in discomfort observed after at the 2nd or 3rd vaccination in any dose group (Figure 1b). Overall, these findings were consistent with those of a previous single-dose DNA vaccination study [7].

Reactogenicity

Thirteen of 15 participants reported a total of 69 reactogenicity events (Table 2). All events were regarded as related to the vaccine or the EP procedure. The most common events were local injection-site reactions (redness, pain, local swelling; n = 23). Pain reported only on the day of vaccination was excluded from this category, as it is described above. All local injection-site reactions were of mild intensity. Other frequently reported symptoms were fatigue (n = 17; 9 mild, 4 moderate, 4 severe), headache (n = 15; 11 mild, 3 moderate, 1 severe), and diarrhea (n = 6; 3 mild, 2 moderate, 1 severe). All reactogenicity events resolved, most within the first 7 days after vaccination. The number and severity of these reactions did not increase with repeated dosing (34 after the 1st dose, 20 after the 2nd, and 15 after the last dose). Similarly, there was no increase in the number or severity of events with increasing dose level (23 in dose group 1; 24 in dose group 2; and 22 in dose group 3).

Other Adverse events

All 15 study participants reported at least one other AE (Table 2). A total of 70 AEs were reported; all but one were assessed as being mild. The most common AEs were laboratory abnormalities and upper respiratory tract infections. Nine AEs were judged

as being likely related to either the vaccine or the EP procedure, 36 as possibly related, and 25 as non-related. There was no increase in the number of reported AEs with repeated dosing (31 after the 1st dose; 24 after the 2nd, and 15 after the last dose). There were four cardiovascular reactions reported in two participants, with two events occurring in each participant (tachycardia, hypertension, dizziness, and dyspnea) developing in direct temporal association with vaccination. All four events were mild and assessed as likely related to the procedure, resolved without sequele, and did not recur with subsequent doses.

Antibody responses

The anti-N responses were detected in two (40%) in the 0.45 mg dose group, in one (20%) in the 0.90 mg dose group, and in four (80%) in the 1.8 mg dose group (Figures 1c and S1). A total of 7/14 (50%) subjects developed antibodies with mean titers of 1594±2919 two weeks after 3rd dose, and 2830±3770 three months after 3rd dose among the responders. With inclusion of the non-responders as zero in the calculation, the mean titers were 797±2047 and 1212±2753, respectively. N antibodies were significantly more common at two weeks after, and three months after, the 3rd vaccination (Figure 1d; $p<0.01$, respectively, Fisher's exact test), with the earliest detected two weeks after the 2nd vaccination (Figure 1c). In the 1.8 mg dose group, two developed titers $>10^3$ that were well maintained at three month follow up (Figure 1c).

T cell responses

T cell responses to N peptides or protein developed in 14/14 (100%) subjects tested after the 2nd dose (one in the 0.90 mg dose group was lost after the 2nd vaccination; Figure 1e, f and g). The peak T cell responses were recorded after the 3rd dose (Figure 1e, f and g). The strongest T cell responses were generally seen in the subjects who developed anti-N antibodies (Figure S1). All subjects in the 0.45 and 1.8 mg dose groups developed T cell responses to N, and four out of five in the 0.90 mg dose group (Figure 1; one lost to follow up). After the 3rd dose 3/5, 3/4, and 5/5 in the 0.45, 0.90, and 1.80 mg dose groups, respectively, developed cumulated N-peptide specific responses >200 SFCs/10^6 SFCs. The mean (±SD) cumulated SFCs at days 84, 99, and 169 for all groups were 334±441, 621±560, and 489±465, respectively, with all <50 SFCs as zero in the calculations (Figure 1g). The T cell response mainly targeted the N- and C-terminal thirds of the N protein in almost all subjects, suggesting that these regions are the hot spots for T cell recognition (Figures S2 and S3). The T cell responses to the N peptides mirrored to a large extent those to the recombinant N protein suggesting that a large part of the T cell response may of the CD4+ T helper type.

In conclusion, the N-pVAX1 DNA vaccine is highly effective at inducing N-specific T cell responses, a vital component of the vaccine's potentially protective and disease-modifying properties, as documented in animal challenge models.

DISCUSSION

There is currently no vaccine approved by the EMA or the FDA against CCHF. This represents an important unmet clinical need, as CCHF is associated with substantial morbidity and mortality, can cause severe hemorrhagic disease, and may lead to

nosocomial transmission, particularly among healthcare workers exposed to infected blood or body fluids [1]. An inactivated whole virus vaccine produced in mouse brain has been approved in Bulgaria since the 1970s and is used locally. The vaccine has most likely been one important component in reducing the number of annual cases in Bulgaria [8]. A study by us demonstrated that the Bulgarian inactivated CCHFV vaccine was able to induce a robust T-cell response directed against the viral nucleoprotein [9]. However, the neutralizing antibody responses were relatively low, suggesting that cellular immunity may play a major role in protection against CCHFV [9].

We recently showed both mRNA and DNA vaccines against the M and S segments of CCHF can protect mice against lethal CCHF infections [3]. Subsequently the vaccine was evaluated as a combination of the coding regions for the M and S segments in NHPs with a clear protective effect against symptoms of CCHF-related disease [2]. In the NHPs, high levels of T cells were recorded after the DNA vaccinations with the M and N genes[2], which is most likely a central part of the protection against disease as previously shown in the mouse model [10]. Furthermore, we could also show that a CCHF N-based vaccine alone had similar effects in NHPs, with a reduction of clinical signs and a significantly lower CCHF replication in several organs [4]. Interestingly, studies from United States Army Medical Research Institute of Infectious Diseases demonstrated that monoclonal antibodies targeting the CCHFV nucleoprotein can protect mice from lethal CCHFV infection [11]. Importantly, these studies showed that N-specific antibodies were protective despite the nucleoprotein being an internal viral antigen and not a classical target for virus neutralization [11,12]. The work further revealed that N can be detected on the surface of infected cells, providing a possible explanation for how N-directed antibodies mediate protection *in vivo*. More recently, studies provided mechanistic insight into this protection [13]. Using an RNA vaccine expressing

the CCHFV nucleoprotein, they demonstrated that high levels of non-neutralizing anti-N antibodies could nevertheless confer significant protection against lethal CCHFV challenge in mice [13]. Importantly, the protective effect was shown to depend on the intracellular Fc receptor TRIM21 rather than classical Fcγ receptor or complement-mediated neutralization pathways. Passive transfer experiments further confirmed that N-specific immune sera could protect naïve animals in a TRIM21-dependent manner [13]. Thus, it cannot be excluded that the N-mediated protection against severe disease in CCHF is composed of both B and T cells.

Together, these findings strongly support focusing on the nucleoprotein as a vaccine target. In addition to its demonstrated protective potential, the CCHFV N is highly conserved across geographically diverse strains and exhibits remarkably limited genetic variation compared with the viral glycoproteins. This high conservation increases the likelihood of achieving broad cross-protective immunity against multiple circulating CCHFV variants while reducing the risk of immune escape. Based on these observations, we progressed the N-based DNA vaccine to a clinical development and a phase I clinical trial.

The GMP-produced DNA vaccine was evaluated in a toxicological study in rabbits which showed that the vaccine and the delivery method was safe which supported the start of the phase I clinical trial (Supplemental Appendix I).

A total of 15 HVs were vaccinated thrice at weeks 0, 4 and 12. The injection and EP induced acute pain at the time of injection, although the delivery was found to be tolerable. Overall, the N-pVAX1 DNA vaccine induced several mild to moderate vaccine-related adverse events, but no severe adverse events were recorded. Importantly, repeated vaccination did not increase discomfort or AEs. Thus, we

conclude that the vaccine and delivery method are safe and tolerable in healthy volunteers at least up to three vaccinations.

It has been shown in the NHP studies that the vaccine induces both antibody and T cell responses, and that possibly both responses help to protect against CCHF caused disease [1,2,11,13]. We found that the best overall responses were noted in the highest dose group with 80% developing antibodies, and 100% developing T cell responses, were maintained at least over a three-month follow-up period. Thus, the vaccine induces similar responses in humans as seen in animal challenge models suggesting that these responses may also protect humans from severe disease. Overall, this suggests that this vaccine could be taken to further clinical trials.

The limitations of the study are that it is small, and no firm conclusions can be drawn regarding its overall safety and immunogenicity, these needs to be confirmed in larger phase II trials. The use of *in vivo* electroporation is certainly a limiting factor as it requires the use of expensive machinery, and trained hospital staff. However, the Cliniporator is already present in over 250 hospitals across Europe for cancer therapies. Thus, the use of these devices for DNA vaccination makes this approach realistic. In addition, the discomfort of the EP itself is a limiting factor. However, in the near future, DNA vaccines will most likely also be delivered formulated in lipid nanoparticles as has been shown to be successful in recent publications [14]. A clear advantage of DNA is the stability and the ease of production, with synthetic DNA further reducing production costs.

In conclusion, we have shown that the N-pVAX1 DNA vaccine against CCHF is safe, tolerable and immunogenic in humans. This supports the concept of genetic vaccines against CCHF containing the N protein for further clinical development.

REFERENCES

1 Hawman, D. W. & Feldmann, H. Crimean-Congo haemorrhagic fever virus. *Nat Rev Microbiol* **21**, 463–477 (2023). https://doi.org/10.1038/s41579-023-00871-9

2 Hawman, D. W. *et al*. A DNA-based vaccine protects against Crimean-Congo haemorrhagic fever virus disease in a Cynomolgus macaque model. *Nat Microbiol* **6**, 187–195 (2021). https://doi.org/10.1038/s41564-020-00815-6

3 Appelberg, S. *et al*. Nucleoside-Modified mRNA Vaccines Protect IFNAR. *J Virol* **96**, e0156821 (2022). https://doi.org/10.1128/JVI.01568-21

4 Hawman, D. W. *et al*. Accelerated DNA vaccine regimen provides protection against Crimean-Congo hemorrhagic fever virus challenge in a macaque model. *Mol Ther* **31**, 387–397 (2023). https://doi.org/10.1016/j.ymthe.2022.09.016

5 Appelberg, S. *et al*. A universal SARS-CoV DNA vaccine inducing highly cross-reactive neutralizing antibodies and T cells. *EMBO Mol Med* **14**, e15821 (2022). https://doi.org/10.15252/emmm.202215821

6 Yan, J. *et al*. Distinct roles of vaccine-induced SARS-CoV-2-specific neutralizing antibodies and T cells in protection and disease. *Mol Ther* **32**, 540–555 (2024). https://doi.org/10.1016/j.ymthe.2024.01.007

7 Aleman, S. *et al*. A phase 1 clinical trial of a multi-antigen SARS-CoV DNA vaccine as a booster after dose three spike-based mRNA vaccinations. *Mol Ther Adv* **34**, 201710 (2026). https://doi.org/10.1016/j.omta.2026.201710

8 Papa, A., Papadimitriou, E. & Christova, I. The Bulgarian vaccine Crimean-Congo haemorrhagic fever virus strain. *Scand J Infect Dis* **43**, 225–229 (2011). https://doi.org/10.3109/00365548.2010.540036

9 Mousavi-Jazi, M., Karlberg, H., Papa, A., Christova, I. & Mirazimi, A. Healthy individuals' immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine. *Vaccine* **30**, 6225–6229 (2012). https://doi.org/10.1016/j.vaccine.2012.08.003

10 Rao, D. *et al.* CD8(+) T-cells target the Crimean-Congo haemorrhagic fever virus Gc protein to control the infection in wild-type mice. *EBioMedicine* **97**, 104839 (2023). https://doi.org/10.1016/j.ebiom.2023.104839

11 Garrison, A. R. *et al.* Nucleocapsid protein-specific monoclonal antibodies protect mice against Crimean-Congo hemorrhagic fever virus. *Nat Commun* **15**, 1722 (2024). https://doi.org/10.1038/s41467-024-46110-4

12 Sorvillo, T. E. *et al.* Replicon particle vaccination induces non-neutralizing anti-nucleoprotein antibody-mediated control of Crimean-Congo hemorrhagic fever virus. *NPJ Vaccines* **9**, 88 (2024). https://doi.org/10.1038/s41541-024-00877-1

13 Leventhal, S. S. *et al.* Antibodies targeting the Crimean-Congo Hemorrhagic Fever Virus nucleoprotein protect via TRIM21. *Nat Commun* **15**, 9236 (2024). https://doi.org/10.1038/s41467-024-53362-7

14 Tursi, N. J. *et al.* Modulation of lipid nanoparticle-formulated plasmid DNA drives innate immune activation promoting adaptive immunity. *Cell Rep Med* **6**, 102035 (2025). https://doi.org/10.1016/j.xcrm.2025.102035

FIGURE LEGENDS

Figure 1. The design of the randomized phase 1 clinical trial has been given in a). The pain following the DNA immunization and in vivo EP was recorded using the visual analogue pain scale (VAS) and the results have been shown as mean levels in b). The individual anti-N antibody kinetics have been given (c). The mean endpoint titers of antibodies to the CCHF nucleoprotein (NP) has been given in d) at the indicated time points. The T cell responses determined by IFNγ ELISpot to CCHF recombinant NP (e), NP peptide pools (f), and the cumulated T cell responses to the NP peptide pools (g). Data has been given as the sprot forming cells (SFCs) per million PBMC.

SUPPLEMENTAL FIGURES

Figure S1. The NP antibody (red line) and T cell responses (bars) to peptides pools have been given for the individual healthy volunteers in the 0.45 mg dose group (101-106), the 0.90 mg dose group (201-205), and the 1.8 mg dose group (301-305). The antibodies levels (red lines) have been given as mean endpoint titers ± SD. The T cell data has been given as the SFUs per million PBMC (bars) as determined by ELISpot. The black, light gray, and dark gray indicates the NP pools 1-4, 5-8, and 9-12, respectively.

Figure S2. The NP antibody (a) and T cell responses to recombinant NP and peptides pools (b) have been given for the individual dose groups. The antibodies levels have been given as mean endpoint titers ± SD. The T cell data has been given as the SFUs per million PBMC as determined by ELISpot.

TABLES

Table 1. Characteristics of participants split by dose groups.

Characteristic	All	Dose group 1	Dose group 2	Dose group 3
Number	15	5	5	5
Sex male, n (%)	10 (66.7)	2 (40.0)	5 (100.0)	3 (60.0)
Mean age ± SD, years	38.7 ± 11.5	40.8 ± 16.1	37.8 ± 11.9	37.4 ± 7.4
Mean BMI ± SD, kg/m²	25.0 ± 2.6	23.8 ± 2.7	25.6 ± 1.6	25.5 ± 3.4

Abbreviations: SD, standard deviation; BMI, body mass index.

493 Table 2. Non-reactogenicity adverse events split by dose group and relationship to
494 vaccine

495

Adverse events	Total no.	Dose group 1			Dose group 2			Dose group 3		
General or local reactions	32	Likely related	Possibly related	Unrelated	Likely related	Possibly related	Unrelated	Likely related	Possibly related	Unrelated
Upper respiratory infection	12	0	0	5	0	0	3	0	0	4
Muscular or skin reactions	8	1	2	1	1	3	0	0	0	0
Cardiovascular	4	2	0	0	2	0	0	0	0	0
GI-symptoms	3	1	1	1	0	0	0	0	0	0
Oral symptoms	3	0	0	0	0	0	0	0	1	2
Migraine	1	0	0	1	0	0	0	0	0	0
Insomnia	1	0	0	1	0	0	0	0	0	0
Laboratory abnormalities	38									
Increased D-dimer	8	0	2	1	0	1	0	0	4	0
Decreased Hemoglobin	6	0	2	1	0	1	0	0	2	0
Leukocytosis*	5	2	2	1	0	0	0	0	0	0
Decreased fibrinogen	4	0	1	1	0	2	0	0	0	0
Increased LDH	3	0	2	0	0	1	0	0	0	0
Increased liver function tests*	2	0	0	1	0	1	0	0	0	0
Increased fibrinogen	1	0	0	0	0	1	0	0	0	0
Increased platelet count	1	0	1	0	0	0	0	0	0	0

Increased C-reactive protein	1	0	0	0	0	0	1	0	0	0
Increase lipase	1	0	1	0	0	0	0	0	0	0
Incresed CK/CK MB*	1	0	0	1	0	0	0	0	0	0
Decreased potassium	1	0	1	0	0	0	0	0	0	0
Increased haptoglobin	1	0	0	0	0	1	0	0	0	0
Increased ferritin	1	0	0	0	0	1	0	0	0	0
Decreased ferritin	1	0	0	0	0	1	0	0	0	0
Decreased albumine	1	0	1	0	0	0	0	0	0	0

* Including combinations of more than one abnormal value from the same sample.

Abbreviations: LDH, lactate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase-MB isoenzyme

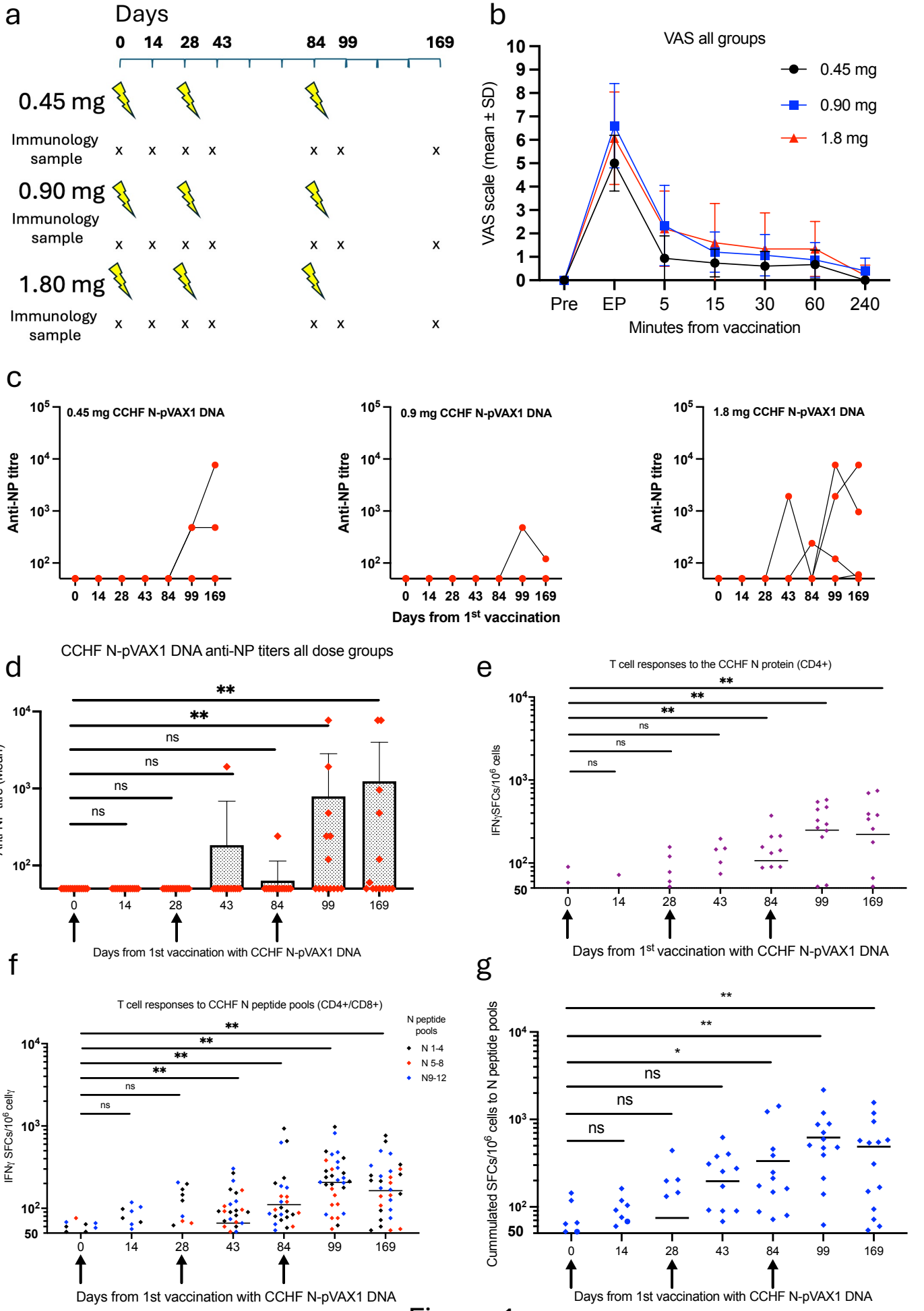

a
Days
0 14 28 43 84 99 169
0.45 mg
Immunology sample
0.90 mg
Immunology sample
1.80 mg
Immunology sample
b
VAS all groups
0.45 mg
0.90 mg
1.8 mg
VAS scale (mean ± SD)
Pre EP 5 15 30 60 240
Minutes from vaccination
c
0.45 mg CCHF N-pVAX1 DNA
0.9 mg CCHF N-pVAX1 DNA
1.8 mg CCHF N-pVAX1 DNA
Anti-NP titre
Days from 1st vaccination
d
CCHF N-pVAX1 DNA anti-NP titers all dose groups
Anti-NP titre (Mean)
Days from 1st vaccination with CCHF N-pVAX1 DNA
e
T cell responses to the CCHF N protein (CD4+)
IFNγSFCs/10^6 cells
Days from 1st vaccination with CCHF N-pVAX1 DNA
f
T cell responses to CCHF N peptide pools (CD4+/CD8+)
N peptide pools
N 1-4
N 5-8
N9-12
IFNγ SFCs/10^6 cellγ
Days from 1st vaccination with CCHF N-pVAX1 DNA
g
Cummulated SFCs/10^6 cells to N peptide pools
Days from 1st vaccination with CCHF N-pVAX1 DNA

Figure 1

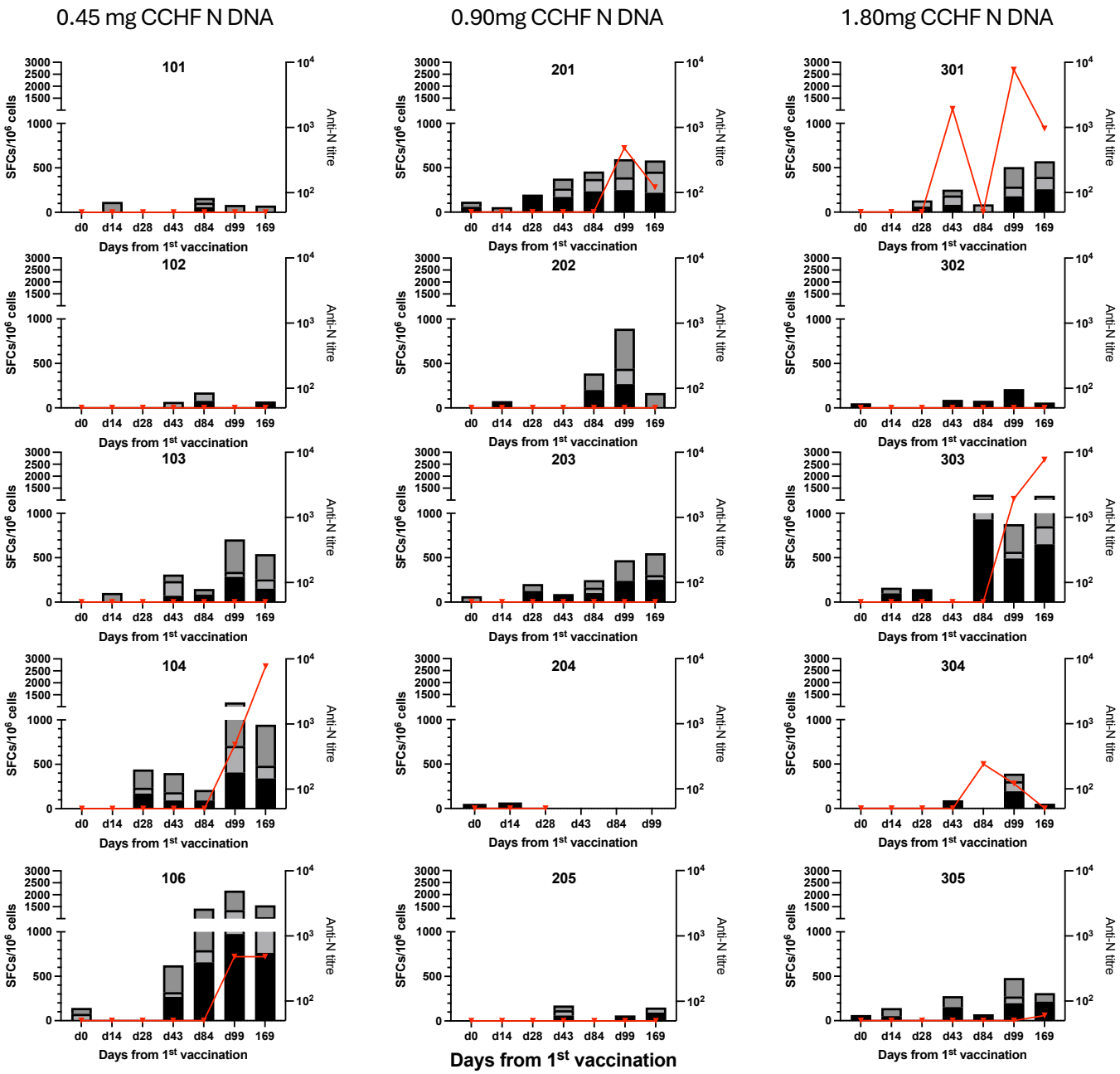

0.45 mg CCHF N DNA
0.90mg CCHF N DNA
1.80mg CCHF N DNA
101
102
103
104
106
201
202
203
204
205
301
302
303
304
305
SFCs/10^6 cells
Anti-N titre
d0 d14 d28 d43 d84 d99 169
Days from 1st vaccination
CCHF nucleoprotein (N) peptide pool numbers
N 1-4
N 5-8
N 9-12

Figure S1

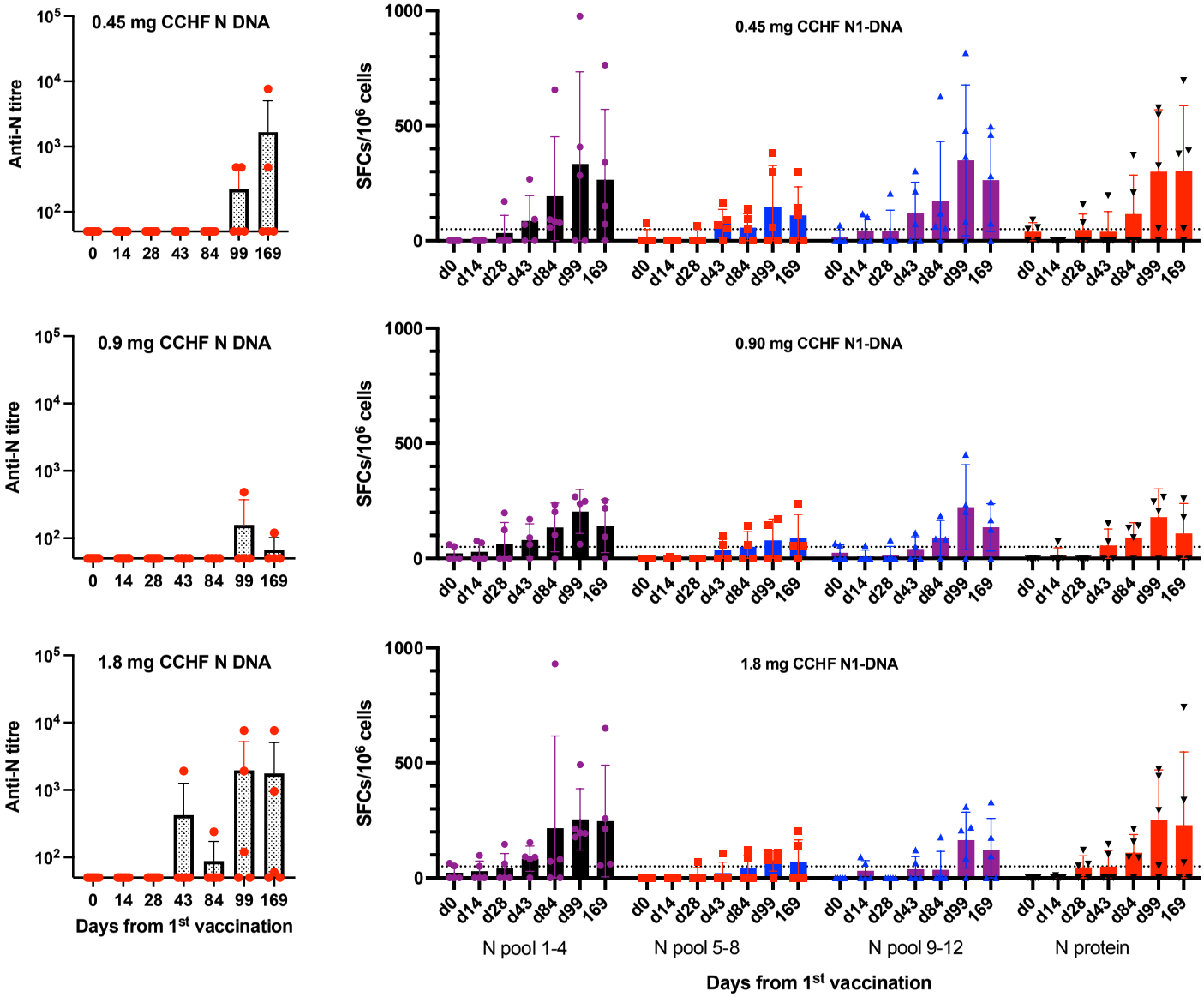

0.45 mg CCHF N DNA
0.9 mg CCHF N DNA
1.8 mg CCHF N DNA
Anti-N titre
10^5
10^4
10^3
10^2
0 14 28 43 84 99 169
Days from 1st vaccination
0.45 mg CCHF N1-DNA
0.90 mg CCHF N1-DNA
1.8 mg CCHF N1-DNA
SFCs/10^6 cells
1000
500
0
d0 d14 d28 d43 d84 d99 169
N pool 1-4
N pool 5-8
N pool 9-12
N protein
Days from 1st vaccination

Figure S2