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Immunogenicity, safety and tolerability of a recombinant measles-vectored Lassa vaccine: A randomised, placebo-controlled, first-in-human trial

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Summary

Background Lassa fever represents a substantial health burden in West Africa. Here we report results from a phase 1 trial evaluating the safety, tolerability and immunogenicity of a recombinant, live-attenuated, measles-vectored Lassa vaccine candidate (MV-LASV).

Methods This first-in-human phase 1 trial consisting of an open-label dose escalation stage and an observer-blinded, randomised, placebo-controlled treatment stage was conducted at a single site in Belgium. Healthy adults aged 18 to 55 received two intramuscular administrations of MV-LASV at 2×10^4 or 1×10^5 TCID₅₀, or placebo. Randomisation in the double-blinded treatment stage was performed using opaque envelopes provided by a data management service. Primary endpoint was defined as safety and tolerability up to study day 56 and was assessed in all participants. The trial is registered with ClinicalTrials.gov (NCT04055454) and EudraCT (2018-003647-40).

Findings Between September 26th, 2019, and January 20th, 2020, 60 participants were enrolled to receive placebo (n=12) or MV-LASV (n=48). 60 participants received at least one study treatment and were included in the safety population. Most adverse events (AE) were experienced during the treatment phase, with frequencies of total solicited or unsolicited AEs comparable between treatment groups. The only statistically significant difference observed related to local solicited AE, with higher frequencies observed in groups receiving MV-LASV (24 [96%] of 25 subjects in the low dose group; 23 [100%] of 23 in the high dose group) than placebo group (6 [50%] of 12; $p=0.0001$ using Fisher-Freeman-Halton test). AE were mostly of mild or moderate severity, and no serious AE were observed. MV-LASV further induced substantial levels of LASV-specific IgG.

Interpretation MV-LASV showed an acceptable safety and tolerability profile. Immunogenicity appeared unaffected by pre-existing immunity against the vector. Thus, MV-LASV remains a promising candidate for further development.

Research in context

Evidence before this study

We searched PubMed and ClinicalTrials.gov on September 27, 2022, for published research articles, with no language restrictions, using the terms “lassa”, “lassa vaccine”, and “clinical trial”. Two candidates, a recombinant vesicular stomatitis virus-based vaccine encoding for Lassa Virus GPC (NCT04794218) and a DNA-based vaccine (NCT03805984) are currently in clinical trials. No data from these two studies were published. Numerous other vaccine candidates have been evaluated in animal models, including recombinant virus vector based, virus like particle-based and subunit vaccine candidates as well as a Mopeia/Lassa virus reassortant expressing Lassa antigens.

Added Value of this study

There are no approved vaccines for prevention of Lassa fever. This study presents the first clinical data on a Lassa virus vaccine candidate. We show that the measles vector-based MV-LASV vaccine candidate was safe and well tolerated. Administration of the vaccine induced Lassa-specific humoral responses as well as moderate CD4⁺ T cell responses. Importantly, immune responses induced by the vaccine appeared unaffected were not affected by pre-existing immunity to the measles vector backbone.

Implications of all the available evidence

The recent steady increase of Lassa Fever cases in West Africa emphasizes the urgent need for rapid development of an effective and safe vaccine. Lassa virus was included in the World Health Organisation (WHO) Blueprint List to highlight the urgent public health need to tackle this emerging disease and both the Coalition for Epidemic Preparedness Innovations (CEPI) and the European and Developing Countries Clinical Trials Partnership (EDCTP) have dedicated substantial funding for Lassa vaccine development. This will allow the development of preventive and/or reactive vaccines despite their limited commercial value. The promising safety and immunogenicity outcome of the first-in-man trial presented here encourages further development of this vaccine candidate. The safe and effective measles vector backbone of MV-LASV is attractive as it will allow for the administration to a broad population, including children.

The current study primarily shows Lassa virus specific humoral immune responses. While there is no immune correlate of protection established for Lassa fever, T cells are thought to play a significant role. This aspect will be challenging to assess in large scale studies in endemic regions. A cellular endpoint requires collection and storage of peripheral blood mononuclear cells, which adds costs and complexity to the trial operations and might

51 not be feasible in the Lassa virus endemic regions in West Africa. Thus, a key focus of upcoming Lassa vaccine
52 research in general should include the investigation of biomarkers correlating with benign disease outcome to
53 facilitate clinical development and licensure.

Introduction

The development of a vaccine for the prevention of Lassa fever (LF), an acute viral haemorrhagic disease caused by Lassa virus (LASV), represents a major public health challenge. LASV is endemic to major parts of West Africa, including Nigeria, Guinea, Liberia, Sierra Leone, with recent outbreaks in Benin and Togo (1, 2). Most infections are zoonotic, with the virus spreading to humans from infected rodents, but human to human transmission has been frequently reported. LF causes 100,000 to 300,000 cases and 5,000 deaths a year (3) with an estimated 180 million people at risk of infection (4). No licensed vaccine or specific treatments are available. Ribavirin is included in several treatment guidelines, but cost and availability represent a challenge in many affected regions and efficacy is controversial (1, 5). The World Health Organization (WHO) has included LASV in its R&D Blueprint list of epidemic threats and the Coalition for Epidemic Preparedness Innovations (CEPI) has supported the development of vaccines for the prevention of LF.

LASV belongs to the genus Mammarenavirus, a group of single-stranded negative-sense RNA viruses with bi-segmented genomes, in the family Arenaviridae (6). LASV exists in remarkable genetic heterogeneity, with seven lineages defined via phylogenetic analysis (7). This diversity creates a challenge for vaccine development (8).

The measles-virus-based candidate vaccine (MV-LASV) is a live attenuated recombinant viral vectored vaccine based on the licensed measles Schwarz strain. This replication competent vector was modified to include the genes encoding for nucleoprotein (NP) and the glycoprotein precursor (GPC), based on clade IV LASV strain Josiah as described previously (9). The immunogenicity and efficacy of this candidate was evaluated in lethal challenge studies in non-human primates (NHP), demonstrating a striking protective effect after only a single dose. Protection was maintained against highly genetically diverse heterologous challenge strains. Further, protection against homologous challenge remained in effect for at least one year after vaccination (10).

While the mechanisms of virus clearance in patients are incompletely understood, T cells are widely considered important (11, 12) and acute CD8⁺ T cell responses correlated with protection in the initial NHP studies evaluating MV-LASV (9). The contribution of neutralising antibodies is not fully understood. While patients undergoing natural infection develop neutralising antibodies, which frequently occurs when the virus is no longer detectable (13). In line with these observations, several vaccine candidates protective in animal models, including MV-LASV, induce non-neutralising IgGs (9, 10, 14-16). However, neutralising antibodies can clearly mediate protection, as highlighted by the effectiveness of monoclonal antibody administration in NHPs (17) and selected reports on the use of convalescent plasma in the treatment of patients (18-20).

MV-LASV was found to be safe and well tolerated in a repeated dose toxicity study in NHP, with a biodistribution and shedding profile like MV-Schwarz (21) and the Chikungunya vaccine candidate MV-CHIK based on the same vector (22, 23).

Based on preclinical results, MV-LASV was moved to a first-in-human, clinical phase 1 study, to determine safety, tolerability, and immunogenicity at two dose levels.

Study design

The current study was a single-centre, randomised, placebo-controlled phase 1 study performed in Belgium. The safety, tolerability, and immunogenicity of two dose levels of MV-LASV was evaluated in healthy, adult volunteers over a duration of one year. This first-in-human trial was initiated with an unblinded dose escalation phase followed by external safety review by an independent data safety monitoring board (DSMB). The positive safety assessment allowed full recruitment for an observer-blinded treatment phase. The study was performed in accordance with the Good Clinical Practice Guidelines, the Helsinki Declaration, and all applicable national laws. The protocol was approved by the lead ethics committee of the university of Antwerpen (Universiteit Antwerpen Ethisch Comité). Informed written consent was obtained from all participants before entry into the study. The study was conducted at University of Antwerpen, Campus Drie Eiken - Center for the Evaluation of Vaccination - Vaccine & Infectious Disease Institute.

Participants

Healthy volunteers aged 18 to 55 years were included if they provided signed informed consent obtained before any study-related activities, were able to comprehend the full nature and purpose of the study including possible risks and were able to cooperate with the investigator to comply with study requirements. All female participants of childbearing potential had to have a negative pregnancy test at screening. Participants further needed to be willing not to become pregnant or father a child up to 182 days after the first vaccination by practicing reliable methods of contraception as specified in the protocol. Exclusion criteria are listed in the Appendix.

Randomisation and masking

106 subjects were screened, and 62 eligible subjects were assigned to the sentinel (n=8) or randomised cohort (n=54). The sentinel subjects were sequentially assigned (nonrandomised) to low dose group (n=4) receiving two doses of MV-LASV at 2x10⁴ TCID₅₀ and high dose group (n=4), receiving two doses of MV-LASV at 1x10⁵ TCID₅₀. Randomised subjects were assigned to the low dose group (n=21), the high dose group (n=21), or placebo (n=12). Two of 62 subjects were not included in the Safety/modified Intention to Treat (mITT) population since

they did not receive the first dose of study treatment, 60 subjects were enrolled and included in the Safety/mITT population (low dose: n=25, high dose: n=23, placebo: n=12). Randomisation to one of the three treatment groups was performed by means of sealed, opaque randomisation envelopes generated by data management (Assign Data Management and Biostatistics, Innsbruck, Austria) and containing information about the allocated treatment group and the allocated vaccination arm for the low dose group. Choice of vaccination arm for MV-LASV or placebo injection in the low dose group followed a 1:1 randomisation ratio. Each subject was assigned a unique 3-digit randomisation number, stated on the envelope in ascending order. Trained members of the unblinded study team were authorised to open one envelope per subject. The envelope was signed with the date and time of randomisation, and the subject number written on it.

The investigator and site personnel who performed study related assessments, all subjects, as well as the sponsor and representatives involved in the monitoring and conduct of the study were unblinded to vaccine administration during the unblinded dose escalation phase but blinded in the observer blinded treatment phase. Unblinded study team members responsible for randomisation, preparation, and administration of the study treatment, were otherwise not involved in the conduct of the study.

Procedures

MV-LASV encodes GPC and NP from the clade IV Lassa strain Josiah, with two mutations introduced in NP to abrogate its interferon-antagonist activity as described previously (9)(24). The recombinant virus was manufactured in Vero cells by Batavia Biosciences BV (Netherlands) and was filled by Biofabri S. L. U. (Spain), according to Good Manufacturing Practises. MV-LASV is presented as a liquid formulation in HEPES buffer and stored at -65°C or below. Placebo (0.9% saline) was sourced locally.

Dose selection was based on human clinical safety and immunogenicity data on MV-CHIK (22, 23). All subjects received intramuscular injections of MV-LASV or placebo in the deltoid region of the arm. Study treatment was administered on days 0 and 28. High dose recipients received 1×10^5 TCID₅₀ of MV-LASV on days 0 and 28 (on each occasion, participants received two administrations, one injection of 1 ml per arm). Low dose recipients received 2×10^4 TCID₅₀ of MV-LASV on days 0 and 28 (on each occasion, participants received 1 injection of 0.4 ml verum in one arm and 0.4 ml of placebo in the other). Placebo recipients received two injections of 1 ml physiological saline, one in each arm.

The first sentinel subject in the low dose group was vaccinated on day 0, followed by safety assessment the following day. Absence of related severe adverse events allowed for vaccination of three sentinel subjects in the low dose group, followed by 14 days safety monitoring and review by an external DSMB. Upon positive recommendation the same procedure was followed for the high dose group. To improve local tolerability, the vaccine was diluted two-fold prior to administration for the remainder of the study.

All participants had blood drawn on days 0, 14, 28, 42, 56, 182, and 365 for the assessment of safety and immunogenicity. Sentinel participants additionally had blood, saliva, nasal swabs, and urine collected on study days 0, 10, 14, 28, and 42 for the evaluation of potential virus shedding. All subjects enrolled in the study and who received at least one dose of study treatment were followed up for 28 days after each treatment. Solicited and unsolicited AEs were recorded at each visit until the end of the treatment phase on study day 56. Serious adverse events (SAE) were monitored until end of study on day 365. Local tolerability was inspected and evaluated by the investigator one hour after the first vaccination, prior to and one hour after the second vaccination, as well as on study days 14, 42 and 56. Subjects recorded local and systemic reactions in a subject diary for up to 14 days after each vaccination. The relationship of AEs to the investigational product was determined and graded by a medically qualified person based on temporal relationship and similarity to known reaction patterns according to the FDA guidance for industry

AEs of special interest (AESI) for this study were defined as autoimmune disease, inflammatory-mediated disease, thrombocytopenia, idiopathic thrombocytopenic purpura, hearing deficit, and neurological disorders. Hearing deficit was assessed by means of audiometry testing at screening (using a FIM Medical Audiolyser ADL20), on study day 365 or at early termination, if applicable, as well as upon reporting of hearing deficits by participants.

Immunogenicity was assessed on all study days via enzyme-linked immunosorbent assay (ELISA) to quantify LASV-specific IgG and IgM, by virus neutralisation test (VNT) to quantify LASV-specific neutralising antibodies, by intracellular cytokine staining and flow cytometry to quantify LASV-specific T cell responses in peripheral blood mononuclear cells (PBMC) stimulated with pools of peptides covering the whole LASV GPC and NP sequences. Further, the MV-specific IgG response was quantified by ELISA on days 0, 28, and 56. The potential shedding of viral RNA to blood, saliva, nasal swabs, and urine was determined by reverse transcriptase quantitative polymerase chain reaction in the sentinel subjects up to study day 42. In addition, three types of exploratory assessment were conducted to supplement the immunogenicity data prespecified in the protocol. The subtypes for LASV-specific IgG were determined via ELISA on day 56, antibody-dependent natural killer cell activation in samples collected on days 0, 28, 56, and 365 was assessed via flow cytometry and the establishment of memory T cells was assessed on samples from day 365 via flow cytometry. Further details are provided in the Appendix. An interim analysis was conducted after all participants had concluded study day 56.

Outcomes

The primary endpoint of this study was the evaluation of MV-LASV safety by rate of solicited and unsolicited AEs in the treatment period up to study day 56. Secondary endpoints were rate of SAEs during the treatment period, up to day 365 or early termination. In addition, measurement of functional CD4⁺/CD8⁺ T cells and LASV-GPC/NP specific antibodies on day 0, 14, 28, 42, and 56 were determined. Moreover, quantification of neutralising antibodies on day 0, 28, and 56, safety laboratory parameters (haematology, serum chemistry, coagulation, urinalysis), and shedding of recombinant virus up to day 42 in sentinel subjects was investigated. Exploratory endpoints consisted of cell mediated immunity on day 182 and 365, presence of LASV-specific neutralising antibodies on day 14, 42, 182, and 365, presence of LASV- binding antibodies on day 182 and 365, and presence of MV-specific binding antibodies on day 0, 28, and 56.

Statistical analysis

A formal sample size calculation was not conducted. The sample size of 60 participants was determined based on prior experience in evaluating the safety and immunogenicity of vaccines and is common for early phase clinical studies.

All statistical analyses were performed using SAS® version 9.3 or higher and exploratory analyses performed using Sigma Plot 14 (Systat Software Inc.).

The analysis for primary endpoint compared AE rates up to day 56 between treatment groups using Fisher-Freeman-Halton test. The same overall test between treatment groups was performed for all AE tables. Immunogenicity analysis compared antibody geometric mean titres (GMT) in the Per Protocol (PP) population. GMTs and GMT ratios were estimated by applying an analysis of variance including the factor treatment group. This was done using log₁₀ transformed data and taking the anti-log of the resulting point estimates for the least square means, least square means differences and the corresponding two-sided 95% CIs. P-values were also provided for pair-wise comparisons of GMTs between treatment groups adjusted for multiple comparisons according to Tukey-Kramer. Pairwise comparisons in T cell analysis were performed using Wilcoxon Signed-Rank test.

All safety analyses were based on the safety population, defined as participants who entered the study and received at least one investigational medicinal product (IMP) administration. The mITT population was defined to include all participants randomised who received at least one IMP administration. As no subjects received incorrect treatment, safety and mITT population were identical. Immunogenicity was assessed in the PP population, comprised of all participants without protocol deviations that could impact the immune response. The study was registered under ClinicalTrials.gov identifier NCT04055454 and EudraCT Number 2018-003647-40.

Role of the funding source

CEPI supported the pre-clinical activities, manufacturing, clinical trial and participated in the clinical study design but had no role in data collection, analysis, and interpretation or writing of the report.

Results

Between September 26th, 2019, and January 20th, 2020, 106 healthy adult volunteers were screened for eligibility to participate in the trial, 60 of which were enrolled. Most screening failures (37 [80.4 %] of 46) did not meet inclusion or exclusion criteria, the remaining were due to withdrawal of informed consent (8 [17.4%] of 46) or the treatment groups being full (1 [2.2%] of 46). The first eight participants were assigned to an open label sentinel phase, receiving MV-LASV at low (n=4) or high (n=4) dose, respectively. After review by an independent DSMB, 52 participants were randomised to receive low dose (n=25), high dose (n=23) or placebo (n=12) in an observer blinded fashion. In summary, 60 participants received at least one treatment, comprising the mITT population used for safety analyses. All participants were white, and demographics were similar between groups, as shown in Table 1. Eleven participants were excluded due to major protocol deviations (forbidden concomitant treatment (9 [81.8%] of 11) and deviations from treatment compliance (2 [18.2%] of 11). Thus, 49 participants were included in the per protocol population (see Figure 1).

MV-LASV was generally well tolerated. Adverse events in the safety population are summarized in Table 3. No death, SAE or AESIs was reported in any treatment group up to day 56. All participants experienced one or more AE, regardless of treatment group within the treatment phase up to study day 56. Overall, there were comparable percentages of subjects in all treatment groups who had solicited and unsolicited AEs with exception of a higher frequency of solicited local AEs. Differences in frequencies were found to be statistically significant by means of a Fisher-Freeman-Halton test (p=0.0001).

AEs reported in subjects were mostly of mild and moderate severity. The number of subjects with severe solicited and unsolicited AEs was comparable between treatment groups; however, comparison across groups was limited since severe AEs were reported with low frequency.

The most frequently reported solicited local AEs were injection site pain with and without touching, injection site induration and injection site erythema/redness. A statistically significant difference ($p<0.0001$) across treatment groups was observed for the number of subjects with solicited local AEs. A higher frequency of solicited local AEs was observed in the MV-LASV groups. The most frequently reported solicited systemic AEs were headache, flu-like symptoms, fatigue, myalgia and diarrhoea. While headache, flu-like symptoms and fatigue occurred with similar frequencies over treatment groups, myalgia was more frequent in the MV-LASV groups. Unexpectedly, diarrhoea was more frequent in the low dose and placebo groups as compared to the high dose group. Severe solicited AEs included injection site pain with touching, headache, flu like symptoms, fatigue, and fever. The number of subjects with these AEs was comparable between treatment groups. Solicited AEs are summarised in Table 2.

The most frequent unsolicited AE were nasopharyngitis, presyncope, injection site haemorrhage, and musculoskeletal stiffness. The number of subjects with these AEs was comparable between treatment groups. Severe unsolicited AEs were influenza like illness, food poisoning, influenza, concussion, lymphocyte count decreased, neck pain, and syncope. Two subjects experienced severe unsolicited AEs judged as related, one subject in the placebo group experienced influenza and neck pain, and one subject in the low dose group experienced syncope. No statistically significant differences between treatment groups were found in the frequencies of severe AEs. All severe AEs resolved before study closure.

While the vast majority of AEs were reported during the treatment period up to study day 56, three events of tinnitus (low dose: 2 [8.0%] of 25 and high dose: 1 [4.3%] of 23), one event of hypoacusis (high dose: 1 [4.3%] of 23), and one event of sinusitis and deafness, respectively (placebo: 1 [8.3%] of 12) were recorded in the follow up period. None of these AEs were serious. Of note, hypoacusis (in the high dose group and resolved by end of study) and deafness (in the placebo group and unresolved by end of study) were the only AESIs observed during the study, both assessed as unlikely related to study treatment.

MV-LASV induced substantial amounts of LASV-specific IgG at both dose levels, with peak GMTs observed on day 42 (Figure 2A). GMTs were statistically significantly elevated in MV-LASV recipients on study days 42 (low dose: $p<0.0001$; GMT 62.9 [95% C.I. 38.2, 103.8]; high dose: $p<0.0001$; GMT 145.9 [95% C.I. 87.4, 243.8]), day 56 (low dose: $p=0.0004$; GMT 41.6 [95% C.I. 25.1, 68.9]; high dose: $p<0.0001$, GMT 103.00 [95% C.I. 60.5, 175.3], and, for high dose, day 182 ($p=0.0005$, GMT 35.8 [95% C.I. 22.0, 58.0]) as compared to the placebo group (day 42 GMT 6.1 [95% C.I. 2.9, 12.9], day 56 GMT 6.2 [95% C.I. 2.9, 13.5], day 182 GMT 6.3 [95% C.I. 3.1, 12.7]). Titres were higher in high dose recipients at these timepoints, with the difference reaching statistical significance on day 56 ($p=0.0426$). Baseline GMTs were 6.763 for low and 6.168 for high dose. While 3 participants in the low dose group did not induce detectable LASV IgG, all high dose immunized subjects produced LASV IgG. Assessment of the humoral immune response induced by MV-LASV at selected timepoints revealed that the IgG response was dominated by IgG1 and IgG3 subtypes (Supplementary Figure S1).

LASV-specific IgM titres on the other hand remained low throughout the study (data not shown). No neutralising antibodies specific for LASV were detected in any treatment group (data not shown).

Assessment of sera from selected timepoints revealed that MV-LASV induced antibodies contribute to antibody dependent cellular cytotoxicity (ADCC), as demonstrated by induction of the degranulation marker CD107a and the proinflammatory cytokine IFN γ on an NK reporter cell line (Figure 2). ADCC parameters were significantly elevated in high dose recipients on day 56, as compared to baseline values on study 0 ($p=0.023$ for CD107a, $p=0.009$ for IFN γ , using Kruskal-Wallis one way analysis of variance on ranks test) (Figure 2B).

MV-LASV vaccination also yielded an IgG response directed against the measles vector backbone (Figure 3A). Titres in low dose (day 28 GMT 2523.5 [95% C.I. 1867.6, 3409.7], day 56 GMT 3465.0 [95% C.I. 2613.1, 4594.7]) and high dose (day 28 GMT 4530.6 [95% C.I. 3287.0, 6244.5], day 56 GMT 4627.8 [95% C.I. 3448.3, 6210.8]) were significantly higher as compared to the placebo group (day 28 GMT 804.0 [95% C.I. 520.7, 1241.4], day 56 GMT 844.1 [95% C.I. 561.7, 1268.5]; $p<0.0001$ for all comparisons). A dose dependent difference in the measles-specific IgG response was evident, showing higher anti-MV specific IgG levels in the high dose group compared to low dose on day 28 and 56; these differences were statistically significant at day 28 ($p=0.0267$). To evaluate the potential impact of pre-existing immunity against measles on the LASV-specific immune response, subjects of the MV-LASV treatment groups (PP population) were pooled ($n=40$) and stratified by baseline anti-measles ELISA quartiles, where quartiles were calculated among all day 0 measles titres in subjects in the low and high dose groups (Figure 3B). No significant differences in the induction of LASV-specific IgG were detected between the baseline measles titre groups.

At most timepoints, no significant difference between MV-LASV and placebo induced T cell response was detectable and cytokine levels were generally low. This was at least partially due to viability issues accrued during the isolation procedure. Still, the induction of surface activation marker CD154 on CD4⁺ T cells specific for NP and GPC indicated the presence of a functional helper T cell response partially obfuscated by PBMC viability. Pair-wise comparisons to baseline by means of Wilcoxon signed rank test revealed a statistically significant activation of GPC specific CD4⁺ T cells on study days 42 ($p=0.0027$), 56 ($p=0.0229$), and 365 ($p=0.0317$) in low dose recipients and on study days 28 ($p=0.0425$), 42 ($p=0.0020$), 56 ($p=0.0017$), and 365 ($p=0.0039$) in high dose

recipients. Similarly, NP-specific CD4⁺ T cells were significantly induced on study days 14 ($p=0.0078$), 28 ($p=0.0302$), 42 ($p=0.0036$), and 56 ($p=0.0022$) in low dose recipients and on study days 28 ($p=0.0156$), 42 ($p=0.0002$), 56 ($p=0.0001$), and 365 ($p=0.0474$) in high dose recipients (Figure 4). CD154 expression on CD8⁺ T cells was negligible after stimulation with GPC and NP at all time points (data not shown). To determine if memory T cells still circulated one year after immunization, PBMCs collected on study day 365 were stimulated with recombinant LASV GPC and NP and analysed for the expression of several activation markers on CD4⁺ and CD8⁺ T cells. We observed an increase in the percentage of CD4⁺ T cells expressing CD134 and CD137, two co-stimulatory molecules expressed by activated T cells, in response to GPC and granzyme B, a cytotoxic effector molecule, following NP stimulation in PBMCs from a subset of immunized subjects. Further, an increase in the proportion of CD4⁺ T cells expressing CD279, an immune checkpoint molecule specifically induced upon T cell receptor engagement, was detected in response to both GPC and NP stimulations (Supplementary Figure 2A). In addition, CD134 expression on CD8⁺ T cells from immunized subjects was elevated (Supplementary Figure 2B). No viral RNAs were detected in whole blood, urine, saliva, and nasal swab samples, indicating that the vaccine is not shed to the environment.

Discussion

The primary objective of this trial was to evaluate the safety and tolerability of MV-LASV at two different dose levels. The vaccine candidate was generally well tolerated. AE frequencies were largely similar between MV-LASV and placebo treatment groups, except for local injection site reactions occurring more frequently in verum recipients. Most reported AEs were either mild or moderate. No SAEs or AESIs occurred during the treatment phase up to study day 56, with two AESI unlikely related to treatment recorded during the follow up period. Further, the vaccine was not shed to whole blood, saliva, or urine. Taken together the data indicate that MV-LASV has a favourable safety profile reminiscent of the parental measles strain as well as other vaccines based on the same backbone (23, 25, 26).

The candidate induced LASV-specific IgG, with peak titres reached on day 42. While the kinetics of the response seem to suggest the need for a second administration, recently published data comparing single and two dose regimens of MV-LASV in NHP showed that single administration yielded detectable IgG titres only from day 35 onwards (10). Thus, IgG induction by single administration could be masked by the timing of the second vaccination in our data set. Of note, single administration was repeatedly found to be sufficient for protection from lethal challenge in NHP (9, 10). We did not observe the induction of neutralising antibodies by MV-LASV. Importantly, neutralising antibodies were also absent in NHPs after MV-LASV immunization, but rapidly appeared after challenge (10).

LASV GPC appears to be a difficult target for neutralisation. The protein is heavily glycosylated, and the presence of *N*-linked glycans potentially shields from neutralising antibodies (27, 28). In addition, most known neutralising antibodies specific for GPC target quaternary epitopes only displayed in the pre-fusion state, which is structurally labile and may presents a difficult target for neutralisation (13, 29).

To gain additional insight on the non-neutralising effector functionality of MV-LASV induced antibodies we investigated ADCC, an important contributor to the clearance of viral infections (30, 31). ADCC by non-neutralising antibodies was previously found crucial for the efficacy of another LASV vaccine in animal experiments (16). We found that MV-LASV-induced antibodies indeed contribute to ADCC, highlighting a functional humoral immune response. In addition, LASV-specific antibodies were predominantly of IgG1 or IgG3 subtype, as expected for an antiviral response.

The potential effect of pre-existing immunity is an important concern for any live attenuated vaccine. Similar to previous findings with MV-CHIK, pre-existing immunity to the vector did not impact the insert-specific immune response (22, 23). In contrast, a measles-based vaccine for the prevention of COVID-19 seemed to be sensitive to anti-vector immunity. This discrepancy could be related to the limited immunogenicity of that candidate (26).

While the analysis of LASV-specific T cell responses induced by MV-LASV was negatively impacted by PBMC viability, we observed induction of CD154 on GPC- and NP-specific CD4⁺ T cells. Since we recorded robust IgG induction this is expected, as CD154-CD40 engagement is central to CD4⁺ T cell help. We further found indications for memory T cell populations circulating one year after immunization, particularly in high dose recipients, suggesting that long-term immunity is achieved after MV-LASV administration, similar to our results in NHP (10). In general, the immune response induced by MV-LASV in humans is reminiscent of our findings in NHP, with robust non-neutralizing IgG and moderate T cell responses (10).

Our study has limitations. The loss of T cell data, while not interfering with the primary endpoint of this first-in-human trial, has negatively impacted immunogenicity analysis and future trials with MV-LASV will have to focus on characterising the primary T cell response to the vaccine. Inherent limitations of phase 1 trials evaluating vaccines that also applies to this study relate to the size and homogeneity of the study population. It will be important to test the candidate in an endemic setting moving forward, particularly to evaluate safety and immunogenicity in individuals pre-exposed to arenaviruses.

This work is the first publication providing clinical data for a vaccine candidate for the prevention of LF. MV-LASV was safe, well tolerated, and immunogenic. Given the outcome of this study, further development should focus on the higher dose of 1×10^5 TCID₅₀.

Contributors

PVD was the coordinating principal investigator and, with IDC as chief study MD and KW as back-up MD, led the clinical conduct at the site. RT, YT, SS, MMU, ET and KR designed the study. RT, YT and KR were involved in the management of the study and led the regulatory-relevant activities. SB and MMA developed the vaccine candidate. KT and MMU were responsible for manufacturing. CG, MMA, SR, AJ and SB developed assays and conducted and coordinated experimental work. CG, RT, AD, and NE contributed to the immunological plan and assessment. RT, KR, DH, and SB interpreted the data. RT did the primary writing and editing of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

RT, YT, DH, SS, KT, MMU, ET and KR are employees of Themis Bioscience GmbH, a subsidiary of Merck & Co., Inc., Rahway, NJ USA. SB and MMA are inventors of the U.S. patent no. 20200308555 protecting the MV-LASV vaccine candidate. RT, MMU, ET, SS, YT and KR possess stock options of Merck & Co., Inc., Rahway, NJ, USA.

Acknowledgments

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Data sharing statement

Participants of this study were not asked for their data to be shared publicly, so supporting data is not available. The clinical study protocol is provided the Appendix.

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454 **Figure legends**

455 **Figure 1 Trial profile.**

456 mITT=modified intention to treat. PP=per protocol.

457 **Figure 2 LASV-specific antibodies induced by MV-LASV.**

458 (A) IgG titres specific for LASV GP and NP as determined by ELISA are shown for each treatment group as
459 indicated. Line represents geometric mean titres at indicated timepoints, error bars represent 95% confidence
460 interval, p values indicate difference to placebo group (shown in grey). (B) ADCC as determined by flow
461 cytometry at indicated timepoints. Percentages of NK cells expressing CD107 α (left panel) or IFN γ (right panel)
462 are shown, p values indicate differences between study day 56 and baseline values on study day 0 in the high dose
463 group. Data from the per protocol population was used for the assessment of immunogenicity. The low dose
464 (shown in orange) was 2×10^4 TCID₅₀ and the high dose (shown in red) was 1×10^5 TCID₅₀.

465 **Figure 3 Vector-specific immune response and pre-existing immunity.**

466 (A) IgG titres specific for measles virus as determined by ELISA are shown for each treatment group indicated.
467 Line represents geometric mean titres at indicated timepoints, error bars represent 95% confidence interval. P
468 values indicate difference to placebo group (shown in grey). (B) IgG titres specific for LASV GP and NP as
469 determined by ELISA on study day 56 are shown for participants receiving MV-LASV (either low dose shown in
470 orange or high dose shown in grey). Lines indicate geometric mean titres, error bars represent 95% confidence
471 interval. Participants were stratified according to measles-specific IgG titres on study day 0, X axis label indicates
472 quartiles.

473 **Figure 4 LASV-specific CD4+ T cells induced by MV-LASV.**

474 Percentage of CD4+ T cells expressing CD154 upon stimulation with peptide pools covering GPC (upper panels)
475 or NP (lower panels) at indicated timepoints are shown. Orange indicates low dose, red indicates high dose, and
476 grey indicates placebo recipients. Lines show mean, whiskers indicated standard error of the mean, p values
477 indicate comparison to respective baseline value on study day 0.

478 **Supplementary Figure S1 IgG isotypes induced by MV-LASV**

479 Endpoint titres of IgG1, IgG2, IgG3, and IgG4 specific for LASV GPC/NP on study day 56 are shown.

480 **Supplementary Figure S2 LASV-specific T cell memory induced by MV-LASV**

481 (A) The expression of CD134, CD137, and CD279 by day 365 CD4+ T cells stimulated with recombinant LASV
482 GP and of CD279 and Granzyme B (GrzB) by CD4+ T cells stimulated with recombinant LASV NP are presented
483 as individual data and mean \pm SEM for placebo (group C, grey symbols), low dose (group A, orange symbols),
484 and high dose (group B, red symbols) groups. The values represent the percentage of T cells expressing the marker
485 after stimulation with LASV protein minus the percentage of expression in unstimulated T cells. The p value
486 indicate comparison between the different groups for a given molecule. (B) The expression of GrzB or of CD134
487 by day 365 CD8+ T cells stimulated with GP and NP or only GP, respectively, are presented as in (A).

Table 1. Baseline characteristics

N		Low Dose	High Dose	Placebo
		25	23	12
Age				
	Mean	27.3	33.9	33.6
	SD	8.56	9.88	12.12
Sex				
Female n (%)		11 (44.0)	16 (69.6)	8 (66.7)
Male n (%)		14 (56.0)	7 (30.4)	4 (33.3)
Race				
White n (%)		25 (100)	23 (100)	12 (100)
Other n (%)		0 (0.0)	0 (0.0)	0 (0.0)

N=number of subjects in full cohort; n (%); n=number of subjects, %=percentage of N

Table 2. Solicited adverse events

	Low Dose	High Dose	Placebo
N	25	23	12
Any solicited event	24 (96.0)	23 (100)	11 (91.7)
Injection site pain without touching			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	4 (16.0) 4	7 (30.4) 14	0 (0.0) 0
Mild	14 (56.0) 27	14 (60.9) 61	4 (33.3) 10
Injection site pain at touching			
Severe	1 (4.0) 1	0 (0.0) 0	0 (0.0) 0
Moderate	2 (8.0) 2	3 (13.0) 5	0 (0.0) 0
Mild	18 (72.0) 38	20 (87.0) 74	5 (41.7) 11
Injection site erythema/redness			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	0 (0.0) 0	1 (4.3) 1	0 (0.0) 0
Mild	7 (28.0) 12	7 (30.4) 10	1 (8.3) 2
Injection site swelling			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	0 (0.0) 0	1 (4.3) 2	0 (0.0) 0
Mild	5 (20.0) 8	5 (21.7) 6	0 (0.0) 0
Injection site itching			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Mild	3 (12.0) 4	6 (26.1) 8	0 (0.0) 0
Injection site induration			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	0 (0.0) 0	2 (8.7) 3	0 (0.0) 0
Mild	9 (36.0) 16	7 (30.4) 11	2 (16.7) 3
Rash			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Mild	1 (4.0) 1	0 (0.0) 0	1 (8.3) 1
Nausea			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Mild	5 (20.0) 10	3 (13.0) 3	2 (16.7) 2
Vomiting			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0

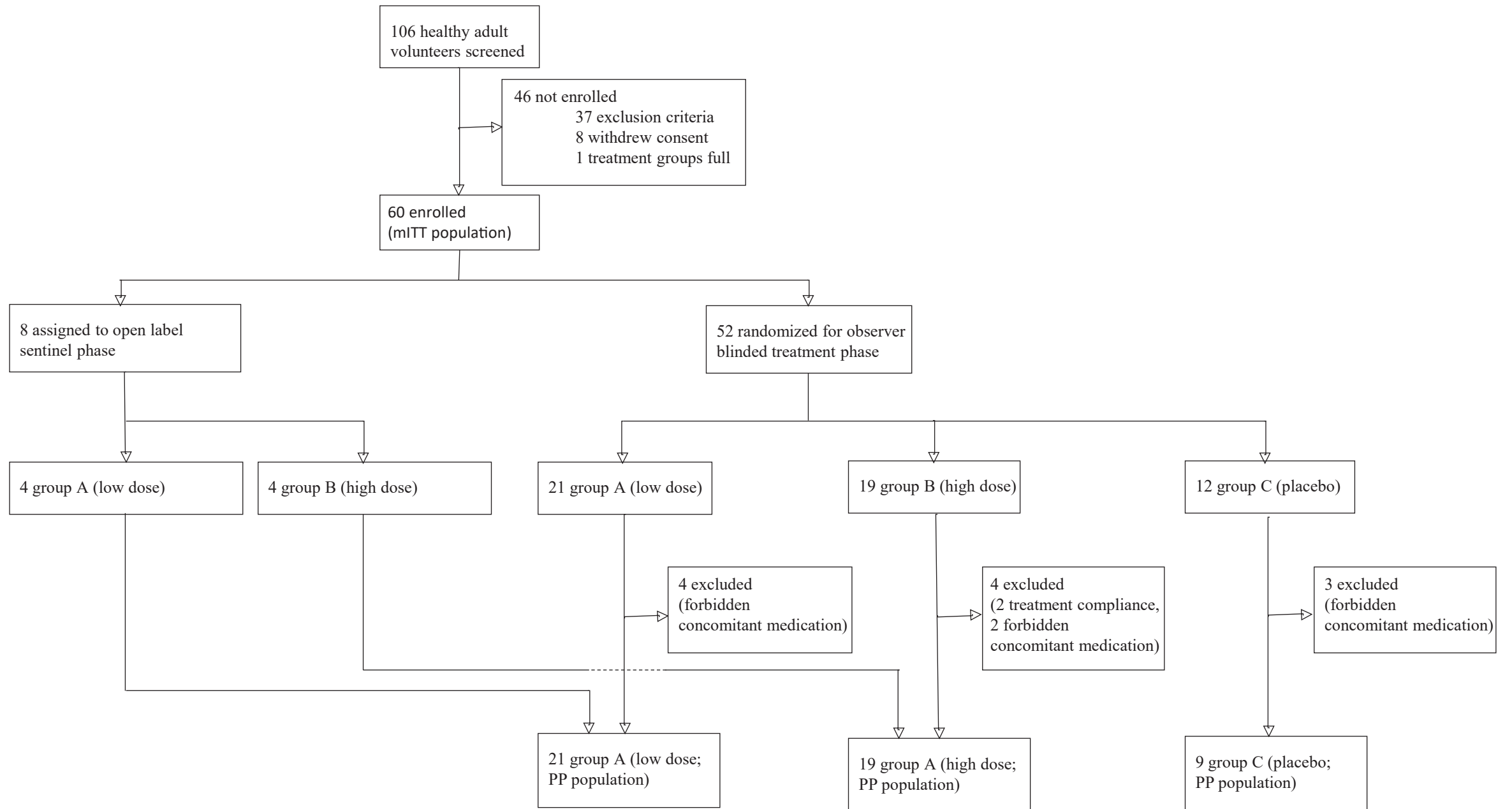
Moderate	0 (0.0) 0	1 (4.3) 1	0 (0.0) 0
Mild	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Diarrhea			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	1 (4.0) 1	0 (0.0) 0	2 (16.7) 2
Mild	5 (20.0) 15	3 (13.0) 4	3 (25.0) 11
Headache			
Severe	1 (4.0) 1	1 (4.3) 1	2 (16.7) 2
Moderate	6 (24.0) 8	6 (26.1) 7	4 (33.3) 7
Mild	7 (28.0) 17	4 (17.4) 12	3 (25.0) 14
Fatigue			
Severe	1 (4.0) 1	1 (4.3) 1	0 (0.0) 0
Moderate	0 (0.0) 1	1 (4.3) 1	2 (16.7) 4
Mild	4 (16.0) 6	4 (17.4) 8	2 (16.7) 4
Myalgia			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	2 (8.0) 2	1 (4.3) 1	1 (8.3) 1
Mild	4 (16.0) 4	5 (21.7) 9	1 (8.3) 1
Fever			
Severe	0 (0.0) 0	0 (0.0) 0	2 (16.7) 2
Moderate	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Mild	1 (4.0) 1	1 (4.3) 2	0 (0.0) 0
Flu-like symptoms			
Severe	1 (4.0) 1	1 (4.3) 1	2 (16.7) 2
Moderate	2 (8.0) 2	0 (0.0) 0	0 (0.0) 0
Mild	4 (16.0) 5	5 (21.7) 7	1 (8.3) 7

N=number of subjects in full cohort; AEs are shown as n (%) Obs, where n=number of subjects, %=percentage of N, Obs=number of events

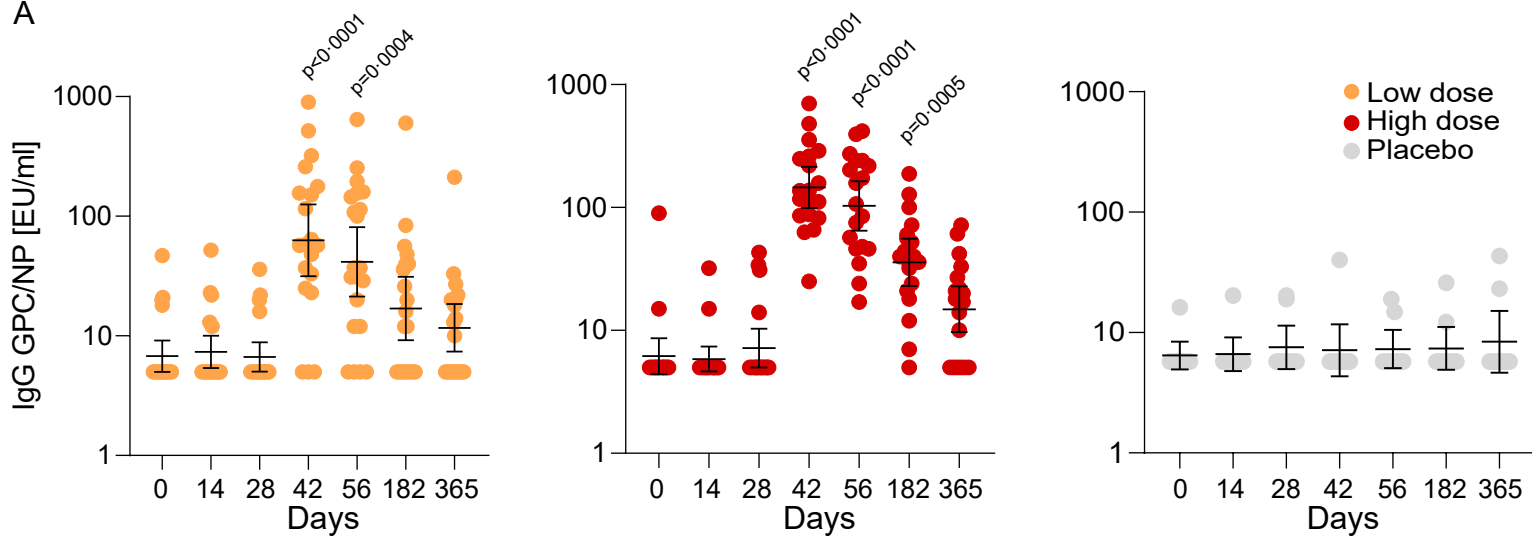
Table 3: Adverse events in the safety population

Solicited Adverse Events n (%) Obs	Low Dose	High Dose	Placebo	p-value
Any	24 (96.0) 188	23 (100) 253	11 (91.7) 86	0.6751
Related	24 (96.0) 173	23 (100) 244	11 (91.7) 75	0.6751
Serious	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	NC
Medically Attended	1 (4.0) 4	1 (4.3) 3	1 (8.3) 5	1.0000
AESI	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	NC
Mild	14 (56.0) 164	9 (39.1) 215	4 (33.3) 66	-
Moderate	7 (28.0) 20	13 (56.5) 35	4 (33.3) 14	-
Severe	3 (12.0) 4	1 (4.3) 3	3 (25.0) 6	-
Related severe AE	3 (12.0) 4	1 (4.3) 3	3 (25.0) 6	0.1869
Any solicited local AE	24 (96.0) 112	23 (100) 195	6 (50.0) 26	0.0001
Any solicited systemic AE	18 (72.0) 76	17 (73.9) 58	10 (83.3) 60	0.8592
Unsolicited Adverse Events n (%) Obs	Low Dose	High Dose	Placebo	p-value
Any	19 (76.0) 57	16 (69.6) 39	12 (100) 33	0.1047

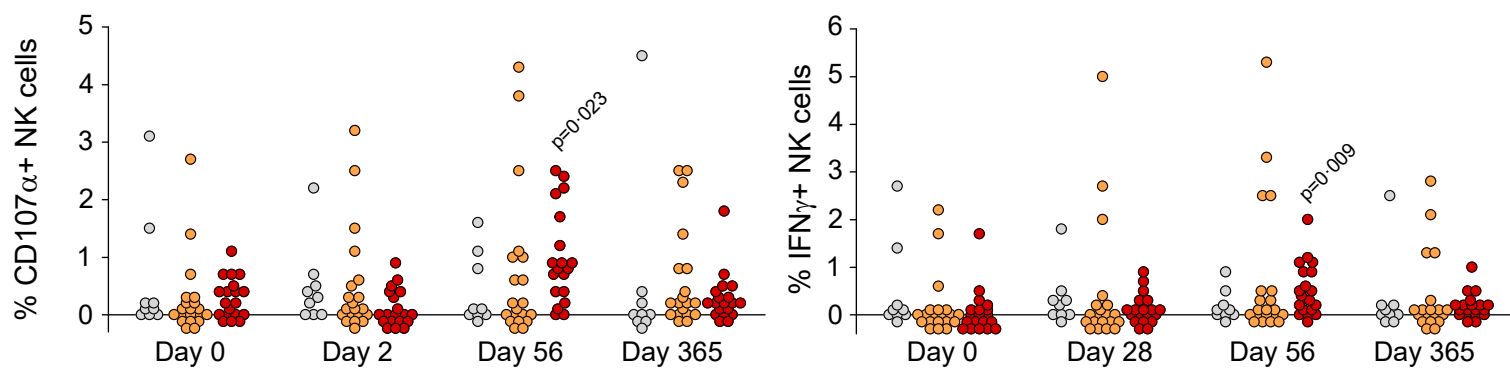
Related AE	15 (60.0) 30	12 (52.2) 23	7 (58.3) 18	0.9422
Serious AE	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	NC
Medically Attended AE	5 (20.0) 6	1 (4.3) 1	5 (41.7) 9	0.0205
AESI	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	NC
Mild AE	4 (16.0) 31	7 (30.4) 26	4 (33.3) 21	-
Moderate AE	10 (40.0) 20	8 (34.8) 12	7 (58.3) 10	-
Severe AE	5 (20.0) 6	1 (4.3) 1	1 (8.3) 2	-
Related Severe AE				



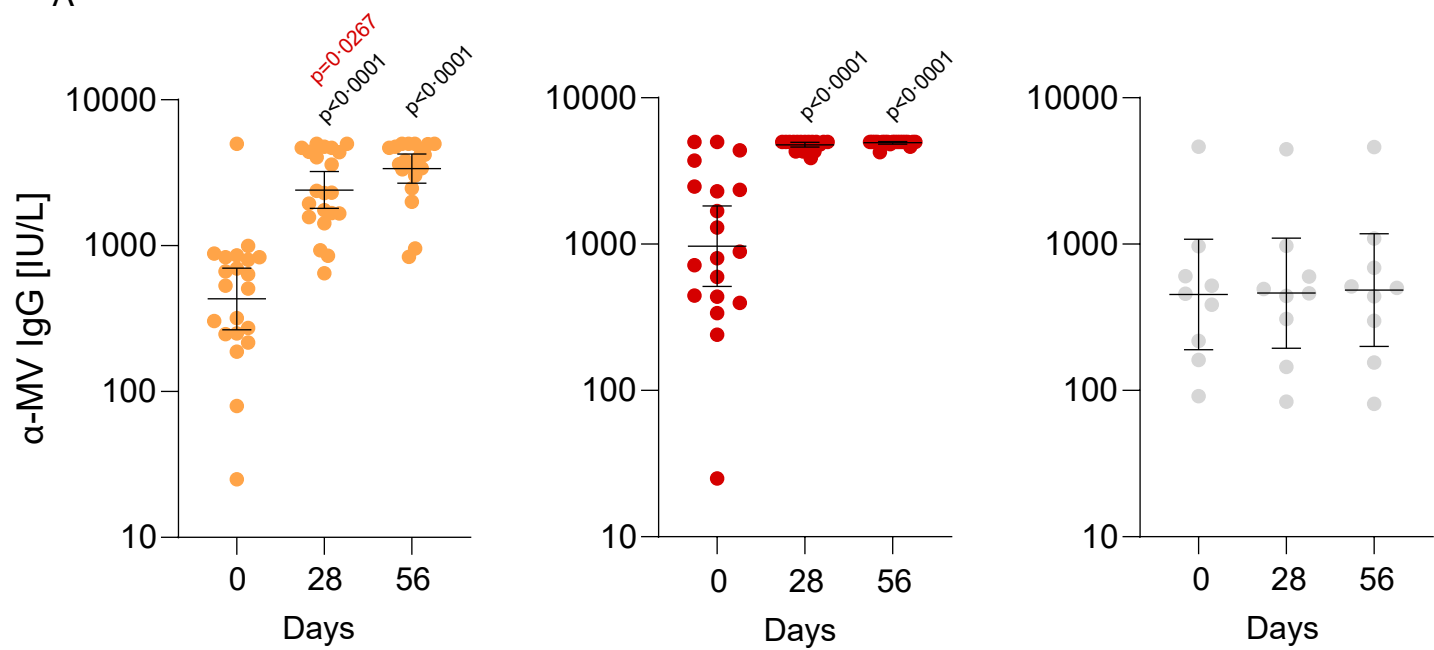
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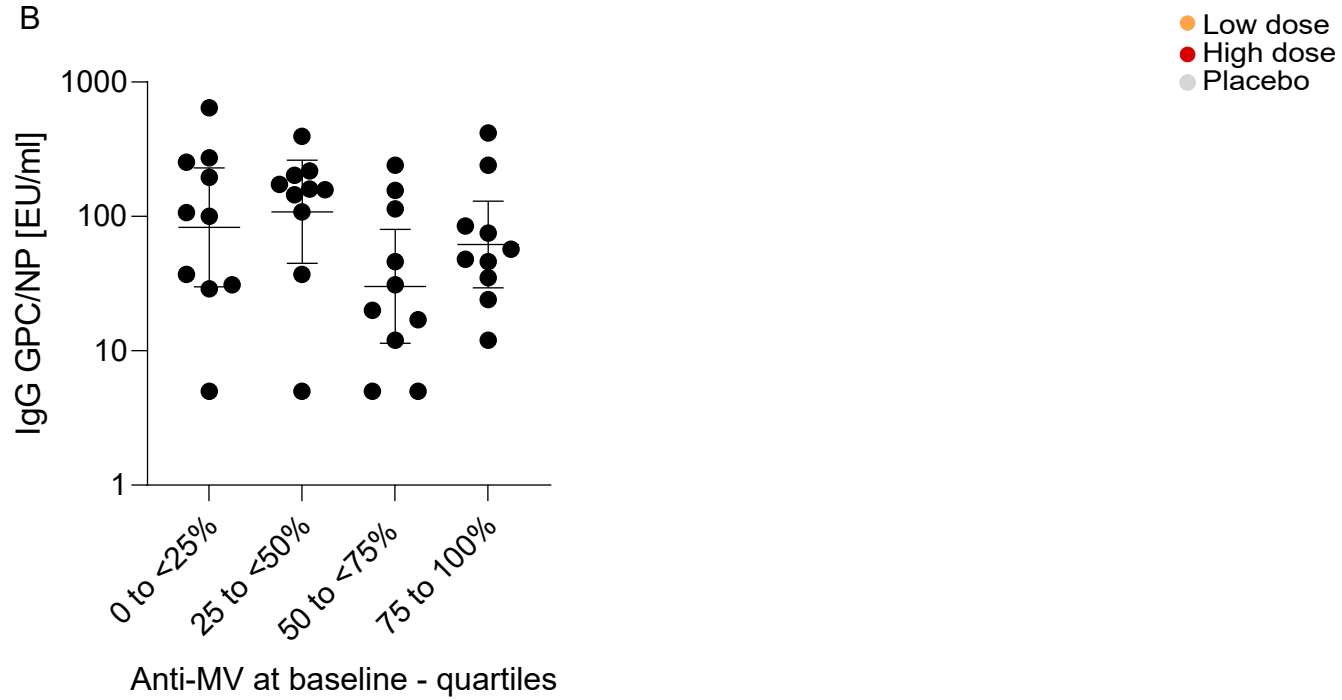
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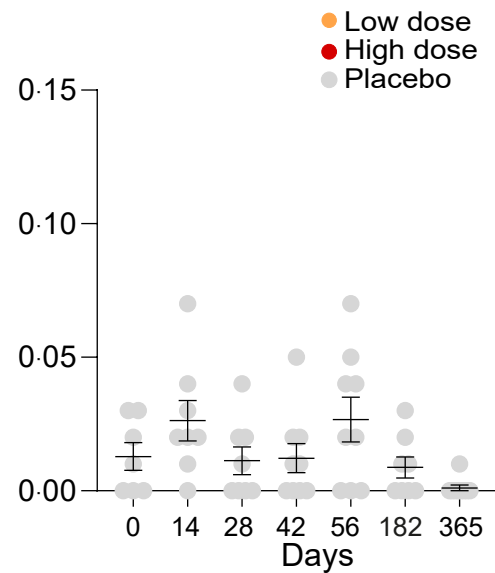
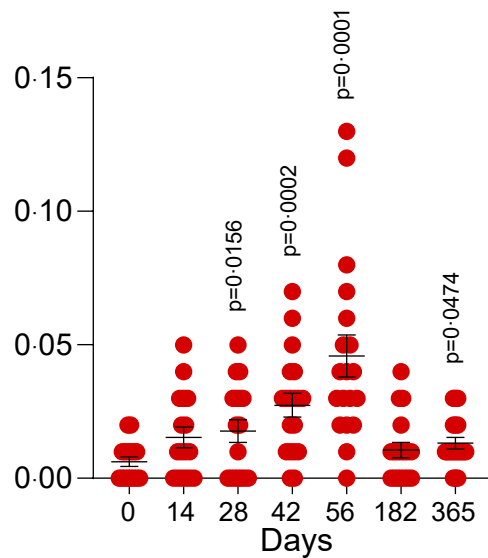
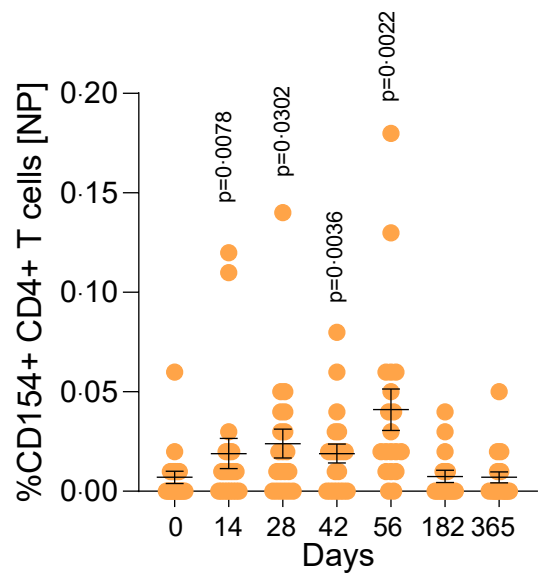
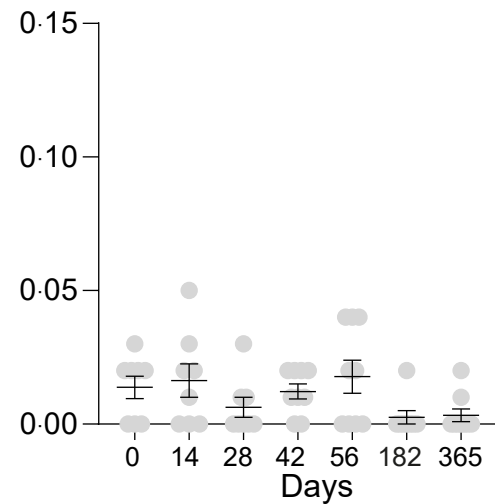
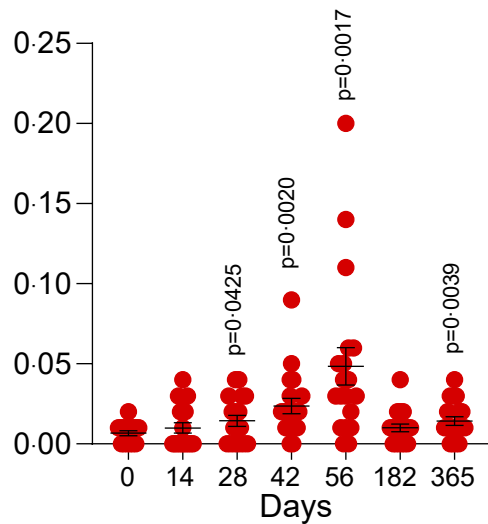
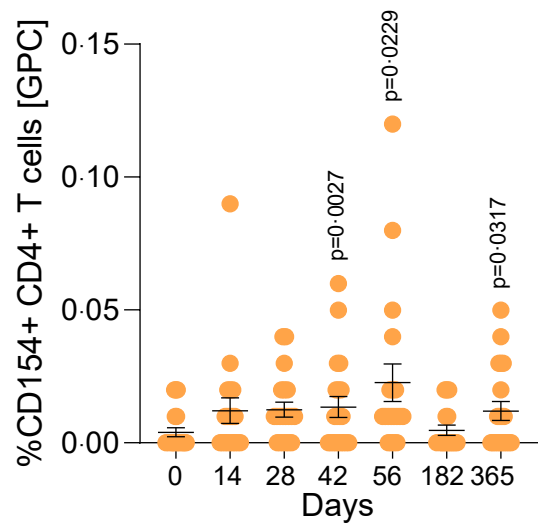


A



B





● Low dose
● High dose
● Placebo

Supplementary Appendix

1. Exclusion criteria:

Exclusion criteria consisted of: Participation in another investigational clinical study (including exposure to an investigational medicinal product or device) within four weeks before the screening visit or planned concurrent participation in another clinical study before study completion at day 365; history of immunodeficiency, known human immunodeficiency virus infection or current infection with hepatitis B or C virus; history of drug addiction including alcohol dependence within the last 2 years; inability or unwillingness to avoid intake of more than around 20 g alcohol per day for 48 hours following each treatment; any vaccination within four weeks prior to first treatment or plans to receive any non-study vaccine within 365 days after first treatment; prior receipt of any Lassa vaccine candidate; any infectious disease within one week prior to screening visit; blood or plasma donation 90 days prior to screening visit and/or anticipated blood, plasma, tissue, sperm, or organ donation until end of treatment period (i.e., day 56); clinically relevant history of renal, hepatic, cardiovascular, gastrointestinal, respiratory, endocrine, dermatological, haematological, inflammatory, autoimmune, or neurological diseases or clinically relevant abnormal laboratory values, that could interfere with the study as judged by the investigator; history of neoplastic disease in the previous five years (excluding successfully treated non-melanoma skin cancer) or a history of any haematological malignancy; behavioural, cognitive, or psychiatric conditions that in the opinion of the investigator affect the ability of the subject to understand and/or comply with the study protocol; history of severe adverse reactions to vaccine administration, including anaphylaxis, urticaria, respiratory difficulty, angioedema, and abdominal pain, or history of allergic reaction likely to be exacerbated by any component of the vaccine; history of or present hearing deficit; present thrombocytopenia and/or history of thrombocytopenia and/or bleeding disorders; history of anaphylactic reaction to drugs or other allergic reactions considered as compromising the safety of the volunteer by the investigator; use of medication considered as affecting study validity by the investigator within two weeks before the first treatment and throughout the study, with the exception of hormonal contraception or hormonal replacement therapy in female subjects; use of immunosuppressive drugs (including non-topical corticosteroids) within 30 days prior to the first treatment or anticipated use before study completion (day 365); receipt of blood products or immunoglobulins within 120 days prior to screening visit or anticipated receipt of any blood product or immunoglobulin before study completion (day 365); pregnancy (positive pregnancy test at screening or before day 182) or lactation at screening or plans to become pregnant before completion of day 182; unreliable contraception methods; persons in a direct relationship with the sponsor, investigator or other study team members, including close relatives (i.e., children, parents, partner/spouse, siblings) as well as employees of the clinical study site or the sponsor; individuals living and/or working with severely immunocompromised individuals, children under 15 months or pregnant women; subjects who travelled to a Lassa endemic country within one year prior to the first treatment or plan to travel to affected regions during the study; any rash, dermatological condition or tattoos that would, in the opinion of the investigator, interfere with injection site reaction rating.

2. Supplementary methods:

Detection of LASV IgG in plasma by ELISA

Maxisorp ELISA plates (Nunc) were coated overnight at 4°C with 5 µg/ml of GPC, 2.5 µg/ml of NP Cter, and 2.5 µg/ml of NP Nter (kindly provided by Zalgen, USA) diluted in PBS or negative antigen. Plates were then blocked 1h at 37°C with PBS 2.5% BSA. Plasma samples were incubated in Ag-positive and Ag-negative wells at dilutions of 1:100, 1:400, 1:1,600, and 1:6,400 in PBS 2.5% BSA 0.5% Tween20 for 1h at 37°C. A standard curve was also tested with 1:3 dilutions of a standard human plasma defined as 1,000 EU/ml (kindly provided by the CEPI) from 1:150 to 1:109,350. A peroxidase-conjugated anti-human IgG (γ-specific) goat polyclonal antibody was then incubated 1h at 37°C. TMB substrate was added and OD measured. The cut-off for positivity was calculated as 2 x mean of negative plasma + 1 standard deviation. A standard curve was drawn according to the standard, allowing to attribute quantitative values in EU/ml for positive unknown samples. The final value for each positive sample was calculated as the mean of values measured at each positive dilution. For detection of LASV IgG isotypes, the same protocol was used, except that mouse anti-human IgG1, IgG2, IgG3, or IgG4 Fc-HRP (Clinisciences) were used at a dilution of 1:8,000. The titre of positivity represented the highest dilution which gave a positive signal.

Antibody-dependent cell cytotoxicity assay

Maxisorp ELISA plates (Nunc) were coated overnight at 4°C with 500 ng per well of LASV GP linked or Ebola GP as a negative control. Plates were then incubated 1h with PBS 2.5% bovine serum albumin (Sigma Aldrich). Hundred µl of heat-inactivated (30 min. at 56°C) plasmas were incubated 4h at 37°C in LASV GP- and Ebola GP-coated wells. 500,000 PBMC purified from healthy donor blood (Etablissement Français du Sang) were then added in each well with an anti-CD107a-FITC antibody (clone H4A3, BD Biosciences) and Brefeldin A diluted 1:20. Plates were then incubated overnight at 37°C with 5% CO₂. PBMC were harvested and stained with antibodies anti-CD20-BV421 (clone 2H7), CD14-APC (clone M5E2), CD3-Alexa700 (clone UCHT1), CD8-APCH7 (clone HIT8a) (all from BD Biosciences) and Live-Dead aqua (ThermoFisher). After fixation and permeabilization using Fix-Perm buffer (Miltenyi Biotech), FcR blocking reagent (BD Biosciences) was incubated for 5 min. at 4°C then anti-KI67-PE-Cy7 (clone B56) and IFN γ -PE (clone B27) antibodies were incubated (all from BD Biosciences). The expression of CD107a, KI-67, and IFN γ by CD3-CD20- CD8⁺ cells was then measured with a LSR Fortessa cytometer (BD Biosciences).

Virus neutralisation test

For the VNT using infectious LASV (strain Josiah, clade IV), several dilutions of the samples were incubated for 1h at 37°C with 200 PFU of LASV. The suspension was then applied on confluent Vero E6 cells (in 12-well plates) and incubated for 1h at 37°C. Cells were then cover with 3.2% carboxy-methyl-cellulose (CMC) diluted in DMEM 2% FCS and incubated at 37°C with 5% CO₂ for one week. CMC was then discarded, cells were washed in PBS, fixed and permeabilized (Triton 0.5%), and incubated with a pool of mouse monoclonal antibodies specific for LASV nucleoprotein. An HRP-conjugated polyclonal antibody directed against mouse IgG (γ -chain specific) was then incubated in order to reveal and numbered the infectious focus. A dilution was considered positive for neutralisation if at least 50% of the added PFU had been neutralised. The neutralising titre of the sample was defined as the higher dilution for which a 50% neutralisation has been observed. A positive control (mouse monoclonal antibody able to neutralise LASV) was used in each plate, as well as a negative human serum diluted 1:20. Samples were used without de complementation in this assay.

The VNT using LASV GPC pseudotyped Mopeia virus was conducted in an identical fashion, except that a MOPEVAC vector expressing the LASV GPC (Josiah, clade IV; described in Carnec et al. 2018, J Virol) was used instead of infectious LASV and that FFU were revealed using a polyclonal antibody specific for MOPEVAC.

Intracellular cytokine staining in CD8⁺ and CD4⁺ T cells

Human PBMC were thawed, washed and diluted in c-RPMI (1% sodium pyruvate, 1% HEPES, 1% AANE, 0.5% ATB and 10% SVF). 200µL of cell suspension (2,5.10⁶cell/mL) were incubated at 37°C between 6 and 18h. Brefeldin A and monoclonal anti-human CD28 and anti-human CD49d (clones CD28.2 and 9F10, BD Biosciences) are then added, together with 50µg/mL SEA (positive control), LASV GPC or NP peptides (described in Mateo et al 2019, Science Transl Med), or PBS (negative control). Cells were incubated 6 h at 37°C, 5% CO₂ before staining. 20 µL PBS-EDTA 20mM + Live dead near IR (ThermoFisher) were added in each tube and incubated 15min RT in the dark. CD8-BV510 (clone RPA-T8), CD4-BV421 (clone RPA-T4), and CD3-FITC (clone UCHT1) antibodies (all from BD Biosciences) were then incubated in each tube for 30 minutes at 4°C. Cells were washed and resuspended in 1mL of Fixation / Permeabilization Solution (Miltenyi Biotech) for 30' at 4°C. Cells were resuspended in 2mL of Permeabilization Buffer 1X, centrifugated and resuspended in 20µL of FcR blocking reagent (BD Biosciences). TNF α -APC (clone MAb11), IL2-PE (clone MQ1-17H12), IFN γ -PerCP (clone B27) (all from BD Biosciences), and CD154-Vio700 (clone REA238, Miltenyi Biotech) antibodies are then incubated for 30 minutes at 4°C. Cells were analysed by flow cytometry using a Facs Canto cytometer (BD Biosciences) and data analysed with Kaluza v2.1 (Beckman Coulter).

Stimulation of PBMC and short-term culture

Thawed PBMCs were plated in 96-well plates at 1 x 10⁶ cells per well in RPMI 1640 with GlutaMAX (Life Technologies) supplemented with 10% FCS, 0.5% penicillin-streptomycin, 1% nonessential amino acids, 25 mM HEPES, and 1 mM sodium pyruvate and incubated overnight at 37°C and 5% CO₂. PBMCs were then mock-stimulated or stimulated with recombinant prefusion LASV GPC or NP (kindly provided by Zalgen) and anti-human CD28 and anti-human CD49d antibodies (BD Biosciences) and cultured at 37°C and 5% CO₂. Cells were stained with CD3-V450 (clone SP34.2), CD4-APC (clone L200), CD8-APC-H7 (clone SK1) or CD3-V450, CD4-Alexa700 (clone L200), CD8-APC-H7, CD134-PE (clone L106), CD279-PE-Dazzle 594 (clone EH12-2H7), and CD137-PE-Cy7 (clone 4B4-1) (all from BD Biosciences) for 30 min. at 4°C. Cells were permeabilized using the FoxP3 staining buffer set (Miltenyi Biotech) as per the manufacturer's instructions and stained with Ki67-PE (clone B56, BD Biosciences), GrzB-PE (clone GB11, BD Biosciences), and perforin-FITC (clone Pf-344, Mabtech) antibodies. Cells were analysed by flow cytometry using an LSR Fortessa cytometer (BD Biosciences). Data were analysed using Kaluza v2.1 software (Beckman Coulter).