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► To cite this version:

Roland Tschismarov, Pierre van Damme, Clara Germain, Ilse de Coster, Mathieu Mateo, et al.. Immunogenicity, safety and tolerability of a recombinant measles-vectored Lassa vaccine: A randomised, placebo-controlled, first-in-human trial. *The Lancet*, 2023, 401 (10384), pp.1267-1276. 10.1016/S0140-6736(23)00048-X . pasteur-04122399

HAL Id: pasteur-04122399

<https://pasteur.hal.science/pasteur-04122399>

Submitted on 8 Jun 2023

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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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Key Words: Lassa virus, vaccine, live-attenuated, measles-vectored, placebo-controlled clinical trial, phase 1, first in man, safety, immunogenicity

1 **Summary**

2 *Background* Lassa fever represents a substantial health burden in West Africa. Here we report results from a phase
3 1 trial evaluating the safety, tolerability and immunogenicity of a recombinant, live-attenuated, measles-vectored
4 Lassa vaccine candidate (MV-LASV).

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

11 *Findings* Between September 26th, 2019, and January 20th, 2020, 60 participants were enrolled to receive placebo
12 (n=12) or MV-LASV (n=48). 60 participants received at least one study treatment and were included in the safety
13 population. Most adverse events (AE) were experienced during the treatment phase, with frequencies of total
14 solicited or unsolicited AEs comparable between treatment groups. The only statistically significant difference
15 observed related to local solicited AE, with higher frequencies observed in groups receiving MV-LASV (24 [96%]
16 of 25 subjects in the low dose group; 23 [100%] of 23 in the high dose group) than placebo group (6 [50%] of 12;
17 $p=0.0001$ using Fisher-Freeman-Halton test). AE were mostly of mild or moderate severity, and no serious AE
18 were observed. MV-LASV further induced substantial levels of LASV-specific IgG.

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

22 **Research in context**

23 *Evidence before this study*

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

31 *Added Value of this study*

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

37 *Implications of all the available evidence*

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

47 The current study primarily shows Lassa virus specific humoral immune responses. While there is no immune
48 correlate of protection established for Lassa fever, T cells are thought to play a significant role. This aspect will
49 be challenging to assess in large scale studies in endemic regions. A cellular endpoint requires collection and
50 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.

54 **Introduction**

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

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

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

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

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

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

88 **Study design**

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

99 **Participants**

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

106 **Randomisation and masking**

107 106 subjects were screened, and 62 eligible subjects were assigned to the sentinel (n=8) or randomised cohort
108 (n=54). The sentinel subjects were sequentially assigned (nonrandomised) to low dose group (n=4) receiving two
109 doses of MV-LASV at 2x10⁴ TCID₅₀ and high dose group (n=4), receiving two doses of MV-LASV at 1x10⁵
110 TCID₅₀. Randomised subjects were assigned to the low dose group (n=21), the high dose group (n=21), or placebo
111 (n=12). Two of 62 subjects were not included in the Safety/modified Intention to Treat (mITT) population since

112 they did not receive the first dose of study treatment, 60 subjects were enrolled and included in the Safety/mITT
113 population (low dose: n=25, high dose: n=23, placebo: n=12). Randomisation to one of the three treatment groups
114 was performed by means of sealed, opaque randomisation envelopes generated by data management (Assign Data
115 Management and Biostatistics, Innsbruck, Austria) and containing information about the allocated treatment group
116 and the allocated vaccination arm for the low dose group. Choice of vaccination arm for MV-LASV or placebo
117 injection in the low dose group followed a 1:1 randomisation ratio. Each subject was assigned a unique 3-digit
118 randomisation number, stated on the envelope in ascending order. Trained members of the unblinded study team
119 were authorised to open one envelope per subject. The envelope was signed with the date and time of
120 randomisation, and the subject number written on it.
121 The investigator and site personnel who performed study related assessments, all subjects, as well as the sponsor
122 and representatives involved in the monitoring and conduct of the study were unblinded to vaccine administration
123 during the unblinded dose escalation phase but blinded in the observer blinded treatment phase. Unblinded study
124 team members responsible for randomisation, preparation, and administration of the study treatment, were
125 otherwise not involved in the conduct of the study.

126 **Procedures**

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

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

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

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

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

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

171 **Outcomes**

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

181 **Statistical analysis**

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

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

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

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

202 **Role of the funding source**

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

205 **Results**

206 Between September 26th, 2019, and January 20th, 2020, 106 healthy adult volunteers were screened for eligibility
207 to participate in the trial, 60 of which were enrolled. Most screening failures (37 [80.4 %] of 46) did not meet
208 inclusion or exclusion criteria, the remaining were due to withdrawal of informed consent (8 [17.4%] of 46) or the
209 treatment groups being full (1 [2.2%] of 46). The first eight participants were assigned to an open label sentinel
210 phase, receiving MV-LASV at low (n=4) or high (n=4) dose, respectively. After review by an independent DSMB,
211 52 participants were randomised to receive low dose (n=25), high dose (n=23) or placebo (n=12) in an observer
212 blinded fashion. In summary, 60 participants received at least one treatment, comprising the mITT population used
213 for safety analyses. All participants were white, and demographics were similar between groups, as shown in Table
214 1. Eleven participants were excluded due to major protocol deviations (forbidden concomitant treatment (9
215 [81.8%] of 11) and deviations from treatment compliance (2 [18.2%] of 11). Thus, 49 participants were included
216 in the per protocol population (see Figure 1).

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

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

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

236 The most frequent unsolicited AE were nasopharyngitis, presyncope, injection site haemorrhage, and
237 musculoskeletal stiffness. The number of subjects with these AEs was comparable between treatment groups.
238 Severe unsolicited AEs were influenza like illness, food poisoning, influenza, concussion, lymphocyte count
239 decreased, neck pain, and syncope. Two subjects experienced severe unsolicited AEs judged as related, one subject
240 in the placebo group experienced influenza and neck pain, and one subject in the low dose group experienced
241 syncope. No statistically significant differences between treatment groups were found in the frequencies of severe
242 AEs. All severe AEs resolved before study closure.
243 While the vast majority of AEs were reported during the treatment period up to study day 56, three events of
244 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%]
245 of 23), and one event of sinusitis and deafness, respectively (placebo: 1 [8.3%] of 12) were recorded in the follow
246 up period. None of these AEs were serious. Of note, hypoacusis (in the high dose group and resolved by end of
247 study) and deafness (in the placebo group and unresolved by end of study) were the only AESIs observed during
248 the study, both assessed as unlikely related to study treatment.

249 MV-LASV induced substantial amounts of LASV-specific IgG at both dose levels, with peak GMTs observed on
250 day 42 (Figure 2A). GMTs were statistically significantly elevated in MV-LASV recipients on study days 42 (low
251 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]),
252 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,
253 175.3], 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
254 (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,
255 12.7]). Titres were higher in high dose recipients at these timepoints, with the difference reaching statistical
256 significance on day 56 ($p=0.0426$). Baseline GMTs were 6.763 for low and 6.168 for high dose. While 3
257 participants in the low dose group did not induce detectable LASV IgG, all high dose immunized subjects produced
258 LASV IgG. Assessment of the humoral immune response induced by MV-LASV at selected timepoints revealed
259 that the IgG response was dominated by IgG1 and IgG3 subtypes (Supplementary Figure S1).
260 LASV-specific IgM titres on the other hand remained low throughout the study (data not shown). No neutralising
261 antibodies specific for LASV were detected in any treatment group (data not shown).
262 Assessment of sera from selected timepoints revealed that MV-LASV induced antibodies contribute to antibody
263 dependent cellular cytotoxicity (ADCC), as demonstrated by induction of the degranulation marker CD107a and
264 the proinflammatory cytokine IFN γ on an NK reporter cell line (Figure 2). ADCC parameters were significantly
265 elevated in high dose recipients on day 56, as compared to baseline values on study 0 ($p=0.023$ for CD107a,
266 $p=0.009$ for IFN γ , using Kruskal-Wallis one way analysis of variance on ranks test) (Figure 2B).
267 MV-LASV vaccination also yielded an IgG response directed against the measles vector backbone (Figure 3A).
268 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,
269 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,
270 6210.8]) were significantly higher as compared to the placebo group (day 28 GMT 804.0 [95% C.I. 520.7,
271 1241.4], day 56 GMT 844.1 [95% C.I. 561.7, 1268.5]; $p<0.0001$ for all comparisons). A dose dependent
272 difference in the measles-specific IgG response was evident, showing higher anti-MV specific IgG levels in the
273 high dose group compared to low dose on day 28 and 56; these differences were statistically significant at day 28
274 ($p=0.0267$). To evaluate the potential impact of pre-existing immunity against measles on the LASV-specific
275 immune response, subjects of the MV-LASV treatment groups (PP population) were pooled ($n=40$) and stratified
276 by baseline anti-measles ELISA quartiles, where quartiles were calculated among all day 0 measles titres in
277 subjects in the low and high dose groups (Figure 3B). No significant differences in the induction of LASV-specific
278 IgG were detected between the baseline measles titre groups.
279 At most timepoints, no significant difference between MV-LASV and placebo induced T cell response was
280 detectable and cytokine levels were generally low. This was at least partially due to viability issues accrued during
281 the isolation procedure. Still, the induction of surface activation marker CD154 on CD4⁺ T cells specific for NP
282 and GPC indicated the presence of a functional helper T cell response partially obscured by PBMC viability.
283 Pair-wise comparisons to baseline by means of Wilcoxon signed rank test revealed a statistically significant
284 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
285 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

286 recipients. Similarly, NP-specific CD4⁺ T cells were significantly induced on study days 14 (p=0.0078), 28
287 (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
288 (p=0.0002), 56 (p=0.0001), and 365 (p=0.0474) in high dose recipients (Figure 4). CD154 expression on CD8⁺ T
289 cells was negligible after stimulation with GPC and NP at all time points (data not shown). To determine if memory
290 T cells still circulated one year after immunization, PBMCs collected on study day 365 were stimulated with
291 recombinant LASV GPC and NP and analysed for the expression of several activation markers on CD4⁺ and CD8⁺
292 T cells. We observed an increase in the percentage of CD4⁺ T cells expressing CD134 and CD137, two co-
293 stimulatory molecules expressed by activated T cells, in response to GPC and granzyme B, a cytotoxic effector
294 molecule, following NP stimulation in PBMCs from a subset of immunized subjects. Further, an increase in the
295 proportion of CD4⁺ T cells expressing CD279, an immune checkpoint molecule specifically induced upon T cell
296 receptor engagement, was detected in response to both GPC and NP stimulations (Supplementary Figure 2A). In
297 addition, CD134 expression on CD8⁺ T cells from immunized subjects was elevated (Supplementary Figure 2B).
298 No viral RNAs were detected in whole blood, urine, saliva, and nasal swab samples, indicating that the vaccine is
299 not shed to the environment.

300 Discussion

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

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

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

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

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

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

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

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

347 **Contributors**

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

355 **Declaration of interests**

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

360 **Acknowledgments**

361 We thank the trial participants for their contribution. We thank the team at the Center for the Evaluation of
362 Vaccination - Vaccine & Infectious Disease Institute, University of Antwerpen for their dedication and support to
363 make the trial possible. We thank CEPI for funding the MV-LASV project. We thank the CEPI project team for
364 their continuous support and helpful discussions. We thank L. Branco (Zalgen Labs) for providing recombinant
365 LASV Josiah proteins.

366 **Data sharing statement**

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

- 369 1. Merson L, Bourner J, Jalloh S, Erber A, Salam AP, Flahault A, et al. Clinical
370 characterization of Lassa fever: A systematic review of clinical reports and research to inform
371 clinical trial design. *PLoS Negl Trop Dis*. 2021;15(9):e0009788.
- 372 2. Yadouleton A, Picard C, Rieger T, Loko F, Cadar D, Kouthon EC, et al. Lassa fever in
373 Benin: description of the 2014 and 2016 epidemics and genetic characterization of a new
374 Lassa virus. *Emerging microbes & infections*. 2020;9(1):1761-70.
- 375 3. Gunther S, Lenz O. Lassa virus. *Crit Rev Clin Lab Sci*. 2004;41(4):339-90.
- 376 4. Fichet-Calvet E, Rogers DJ. Risk maps of Lassa fever in West Africa. *PLoS Negl*
377 *Trop Dis*. 2009;3(3):e388.
- 378 5. Murphy HL, Ly H. Pathogenicity and virulence mechanisms of Lassa virus and its
379 animal modeling, diagnostic, prophylactic, and therapeutic developments. *Virulence*.
380 2021;12(1):2989-3014.
- 381 6. Shao J, Liang Y, Ly H. Human hemorrhagic Fever causing arenaviruses: molecular
382 mechanisms contributing to virus virulence and disease pathogenesis. *Pathogens (Basel,*
383 *Switzerland)*. 2015;4(2):283-306.
- 384 7. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge
385 to Lassa Virus Vaccine Development. *Viruses*. 2020;12(4).
- 386
- 387 9. Mateo M, Reynard S, Carnec X, Journeaux A, Baillet N, Schaeffer J, et al. Vaccines
388 inducing immunity to Lassa virus glycoprotein and nucleoprotein protect macaques after a
389 single shot. *Sci Transl Med*. 2019;11(512).
- 390 10. Mateo M, Reynard S, Journeaux A, Germain C, Hortion J, Carnec X, et al. A single-
391 shot Lassa vaccine induces long-term immunity and protects cynomolgus monkeys against
392 heterologous strains. *Sci Transl Med*. 2021;13(597).
- 393 11. Russier M, Pannetier D, Baize S. Immune responses and Lassa virus infection.
394 *Viruses*. 2012;4(11):2766-85.
- 395 12. Baillet N, Reynard S, Perthame E, Hortion J, Journeaux A, Mateo M, et al. Systemic
396 viral spreading and defective host responses are associated with fatal Lassa fever in
397 macaques. *Commun Biol*. 2021;4(1):27.
- 398 13. Hastie KM, Saphire EO. Lassa virus glycoprotein: stopping a moving target. *Curr*
399 *Opin Virol*. 2018;31:52-8.
- 400 14. Carrion R, Jr., Patterson JL, Johnson C, Gonzales M, Moreira CR, Ticer A, et al. A
401 ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of
402 Lassa virus and can provide sterilizing immunity. *Vaccine*. 2007;25(20):4093-102.
- 403 15. Fisher-Hoch SP, Hutwagner L, Brown B, McCormick JB. Effective vaccine for lassa
404 fever. *J Virol*. 2000;74(15):6777-83.
- 405 16. Abreu-Mota T, Hagen KR, Cooper K, Jahrling PB, Tan G, Wirblich C, et al. Non-
406 neutralizing antibodies elicited by recombinant Lassa-Rabies vaccine are critical for
407 protection against Lassa fever. *Nat Commun*. 2018;9(1):4223.
- 408 17. . !!! INVALID CITATION !!! (17).
- 409 18. Emond RT, Bannister B, Lloyd G, Southee TJ, Bowen ET. A case of Lassa fever:
410 clinical and virological findings. *Br Med J (Clin Res Ed)*. 1982;285(6347):1001-2.
- 411 19. Leifer E, Gocke DJ, Bourne H. Lassa fever, a new virus disease of man from West
412 Africa. II. Report of a laboratory-acquired infection treated with plasma from a person
413 recently recovered from the disease. *Am J Trop Med Hyg*. 1970;19(4):677-9.
- 414 20. Frame JD, Verbrugge GP, Gill RG, Pinneo L. The use of Lassa fever convalescent
415 plasma in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*.
416 1984;78(3):319-24.
- 417 21. Lorin C, Segal L, Mols J, Morelle D, Bourguignon P, Rovira O, et al. Toxicology,
418 biodistribution and shedding profile of a recombinant measles vaccine vector expressing
419 HIV-1 antigens, in cynomolgus macaques. *Naunyn Schmiedebergs Arch Pharmacol*.
420 2012;385(12):1211-25.
- 421 22. Ramsauer K, Schwameis M, Firbas C, Mullner M, Putnak RJ, Thomas SJ, et al.
422 Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya
423 vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial.
424 *Lancet Infect Dis*. 2015;15(5):519-27.

- 425 23. Reisinger EC, Tschismarov R, Beubler E, Wiedermann U, Firbas C, Loebermann M,
426 et al. Immunogenicity, safety, and tolerability of the measles-vectored chikungunya virus
427 vaccine MV-CHIK: a double-blind, randomised, placebo-controlled and active-controlled
428 phase 2 trial. *The Lancet*. 2018.
- 429 24. Carnec X, Mateo M, Page A, Reynard S, Hortion J, Picard C, et al. A Vaccine
430 Platform against Arenaviruses Based on a Recombinant Hyperattenuated Mopeia Virus
431 Expressing Heterologous Glycoproteins. *J Virol*. 2018;92(12).
- 432 25. Vanhoutte F, Liu W, Wiedmann RT, Haspeslagh L, Cao X, Boundy K, et al. Safety
433 and immunogenicity of the measles vector-based SARS-CoV-2 vaccine candidate, V591, in
434 adults: results from a phase 1/2 randomised, double-blind, placebo-controlled, dose-ranging
435 trial. *EBioMedicine*. 2022;75:103811.
- 436 26. Launay O, Artaud C, Lachatre M, Ait-Ahmed M, Klein J, Luong Nguyen LB, et al.
437 Safety and immunogenicity of a measles-vectored SARS-CoV-2 vaccine candidate, V591 /
438 TMV-083, in healthy adults: results of a randomized, placebo-controlled Phase I study.
439 *EBioMedicine*. 2022;75:103810.
- 440 27. Sommerstein R, Flatz L, Remy MM, Malinge P, Magistrelli G, Fischer N, et al.
441 Arenavirus Glycan Shield Promotes Neutralizing Antibody Evasion and Protracted Infection.
442 *PLoS Pathog*. 2015;11(11):e1005276.
- 443 28. Zhu X, Liu Y, Guo J, Cao J, Wang Z, Xiao G, et al. Effects of N-Linked Glycan on
444 Lassa Virus Envelope Glycoprotein Cleavage, Infectivity, and Immune Response. *Virology*.
445 2021;36(4):774-83.
- 446 29. Cross RW, Hastie KM, Mire CE, Robinson JE, Geisbert TW, Branco LM, et al.
447 Antibody therapy for Lassa fever. *Curr Opin Virol*. 2019;37:97-104.
- 448 30. Liu Q, Fan C, Li Q, Zhou S, Huang W, Wang L, et al. Antibody-dependent-cellular-
449 cytotoxicity-inducing antibodies significantly affect the post-exposure treatment of Ebola virus
450 infection. *Scientific reports*. 2017;7:45552.
- 451 31. He W, Tan GS, Mullarkey CE, Lee AJ, Lam MM, Krammer F, et al. Epitope specificity
452 plays a critical role in regulating antibody-dependent cell-mediated cytotoxicity against
453 influenza A virus. *Proc Natl Acad Sci U S A*. 2016;113(42):11931-6.

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

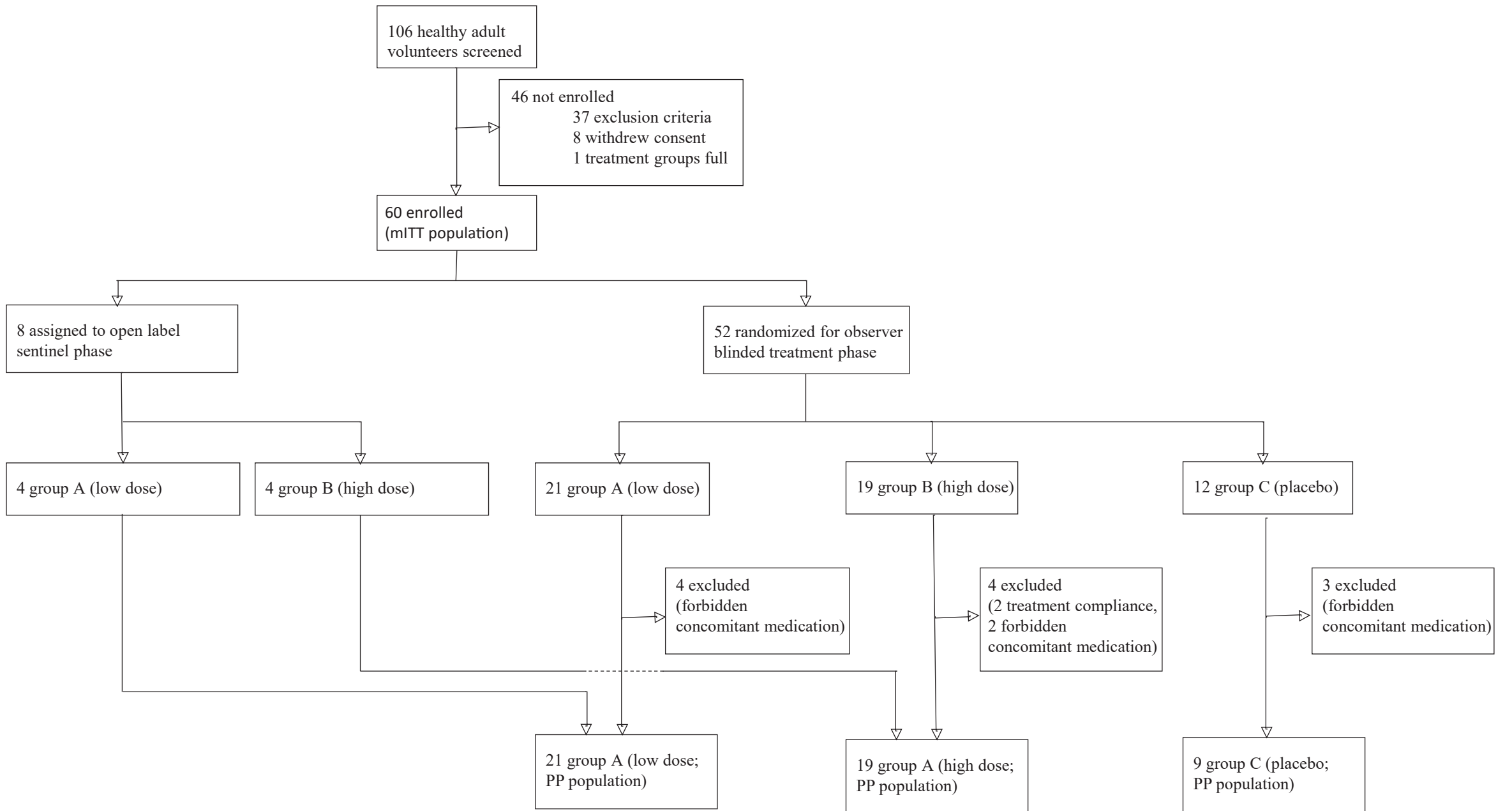
Moderate	0 (0.0) 0	1 (4.3) 1	0 (0.0) 0
Mild	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Diarrhea			
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Moderate	1 (4.0) 1	0 (0.0) 0	2 (16.7) 2
Mild	5 (20.0) 15	3 (13.0) 4	3 (25.0) 11
Headache			
Severe	1 (4.0) 1	1 (4.3) 1	2 (16.7) 2
Moderate	6 (24.0) 8	6 (26.1) 7	4 (33.3) 7
Mild	7 (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

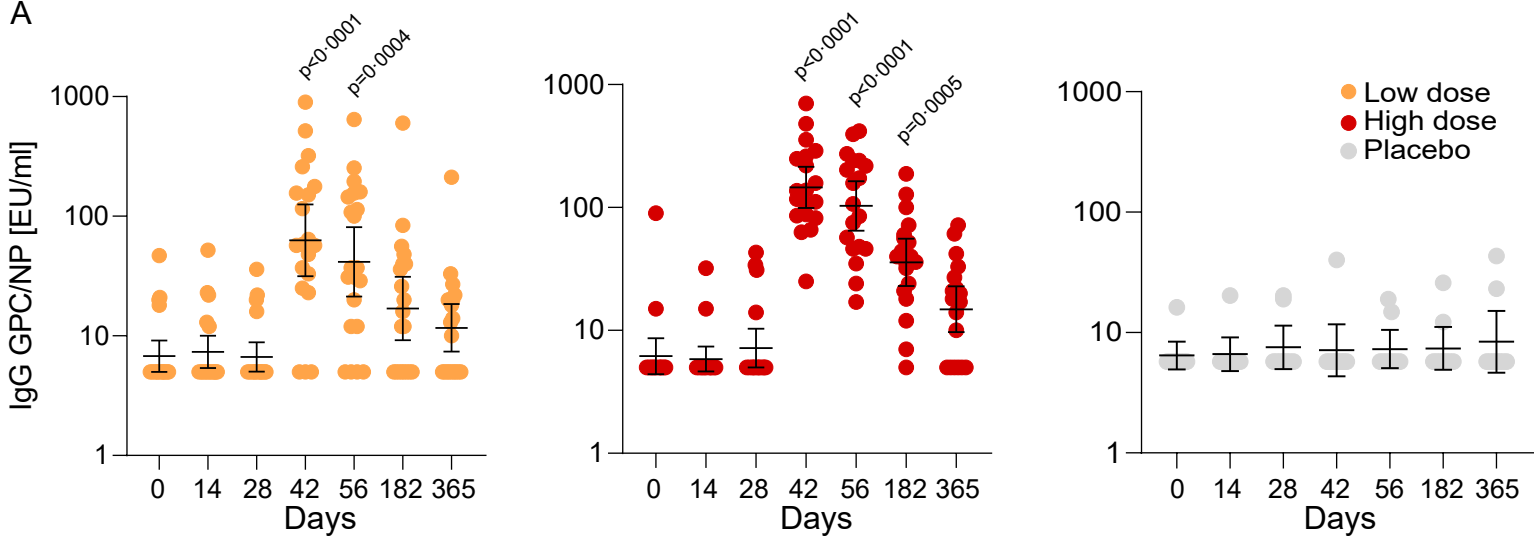
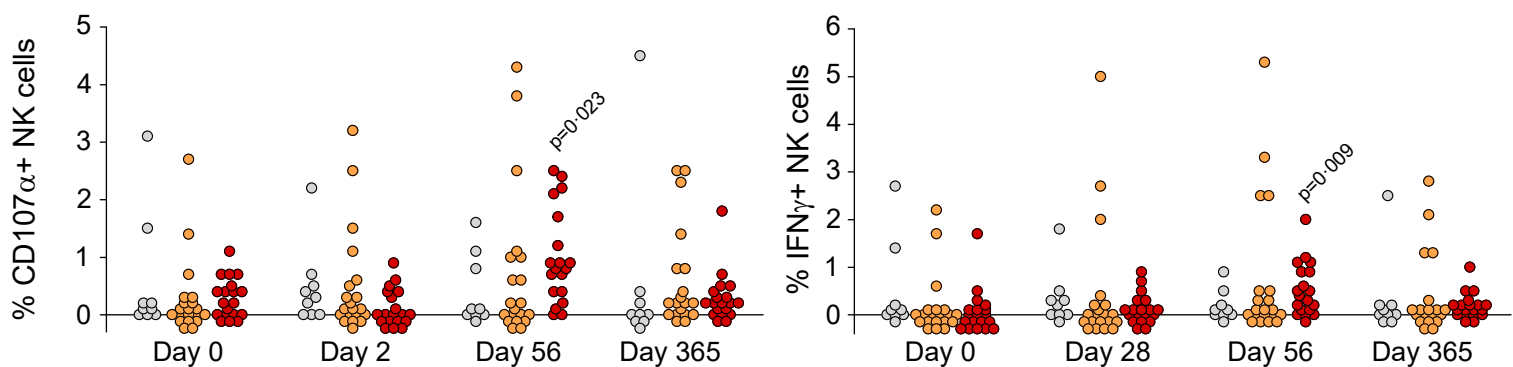
N=number of subjects in full cohort; AEs are shown as n (%) Obs, where n=number of subjects, %=percentage of N, Obs=number of events

Table 3: Adverse events in the safety population

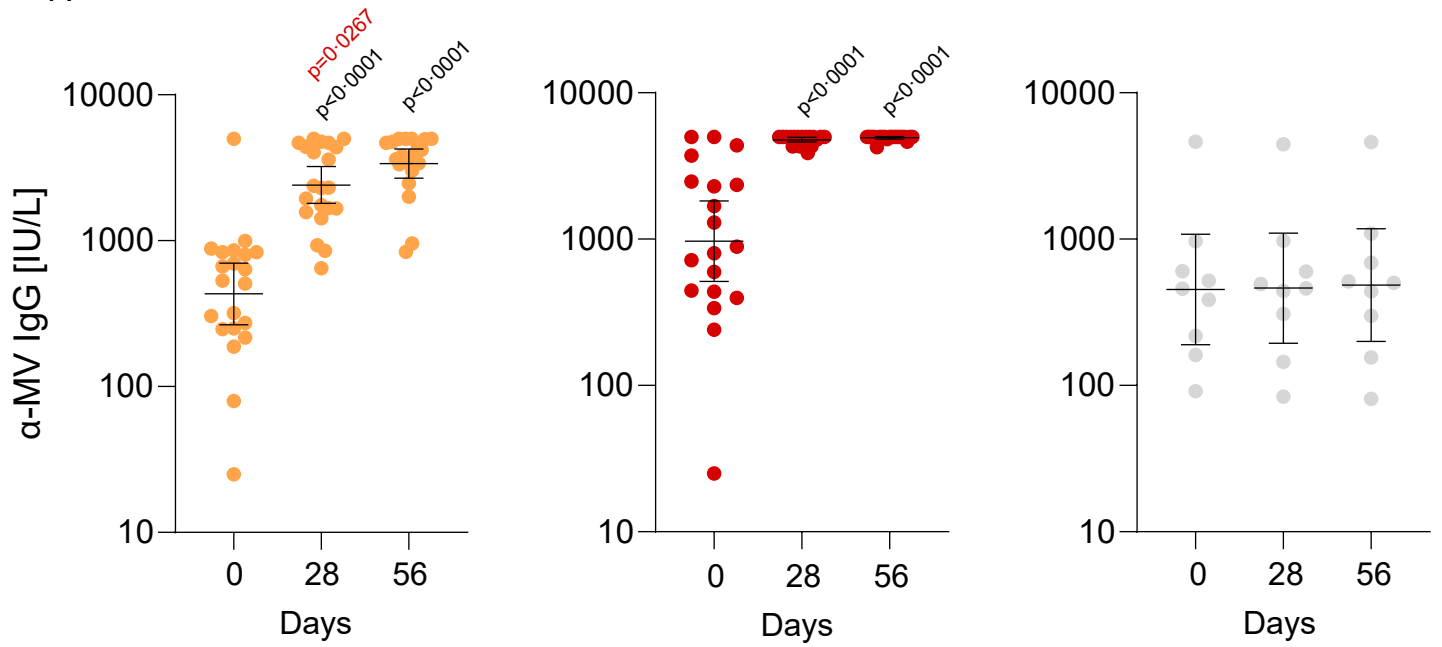
Solicited Adverse Events n (%) Obs	Low Dose	High Dose	Placebo	p-value
Any	24 (96.0) 188	23 (100) 253	11 (91.7) 86	0.6751
Related	24 (96.0) 173	23 (100) 244	11 (91.7) 75	0.6751
Serious	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	NC
Medically Attended	1 (4.0) 4	1 (4.3) 3	1 (8.3) 5	1.0000
AESI	0 (0.0) 0	0 (0.0) 0	0 (0.0) 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				

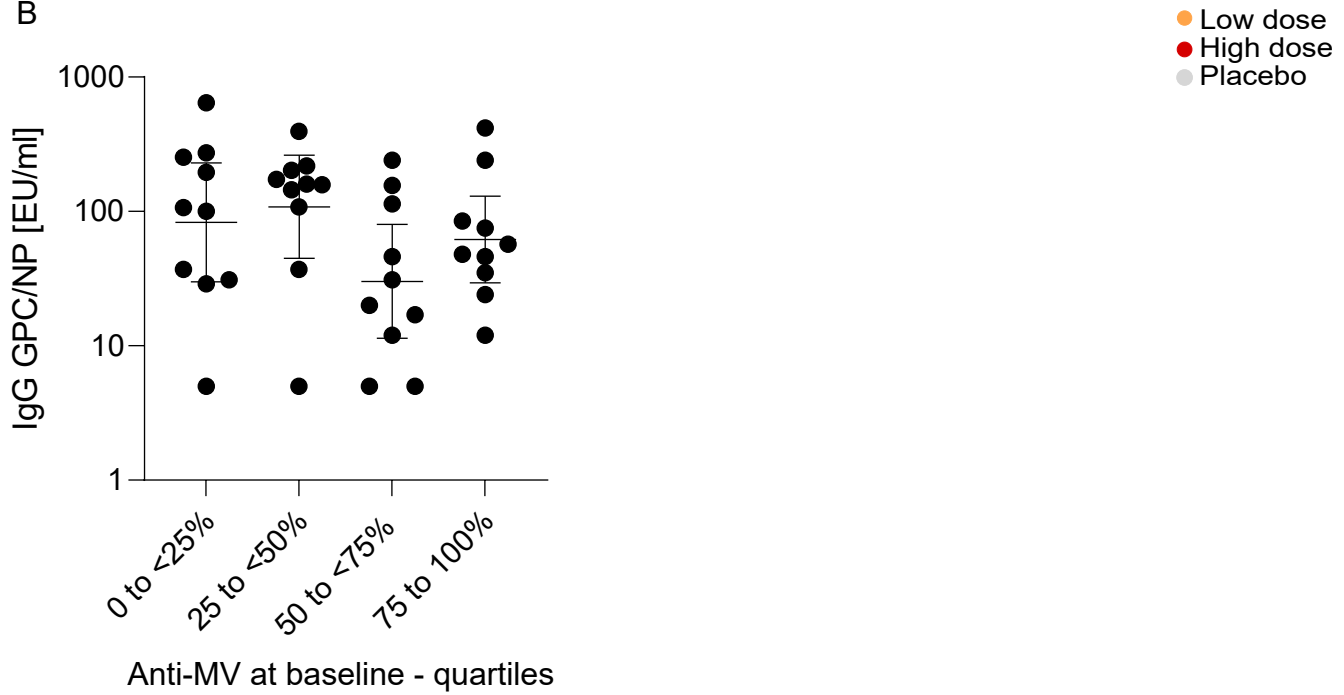


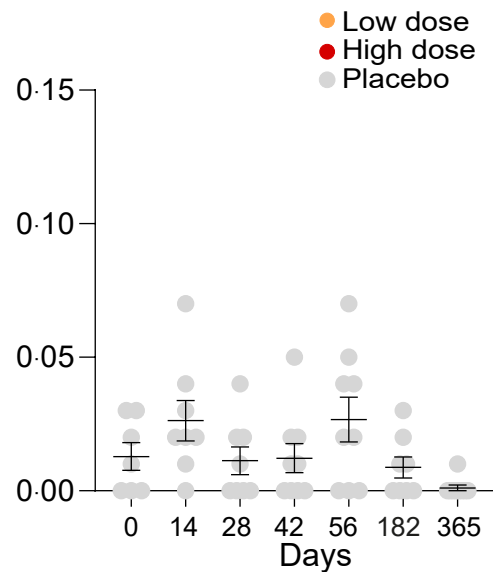
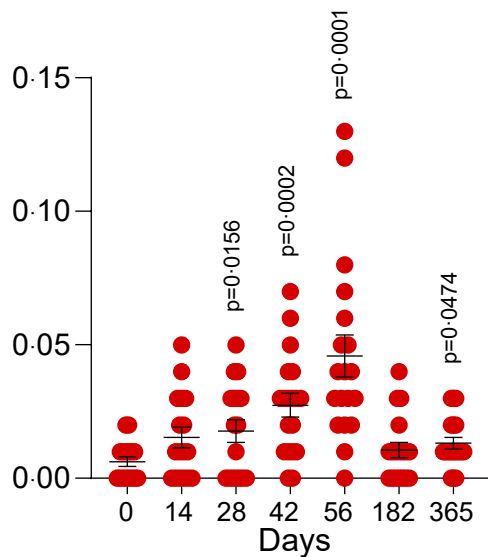
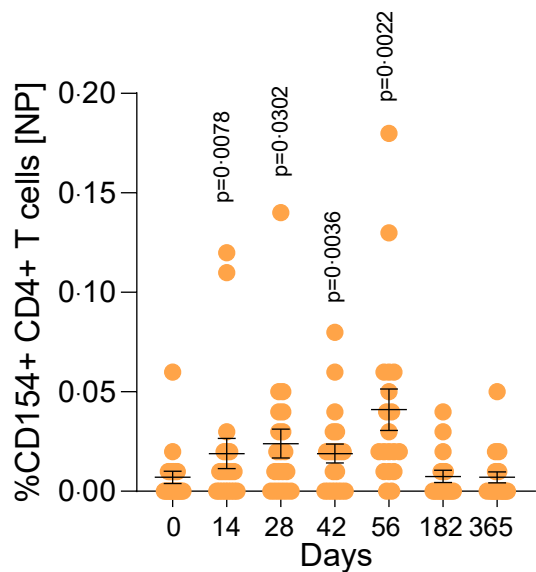
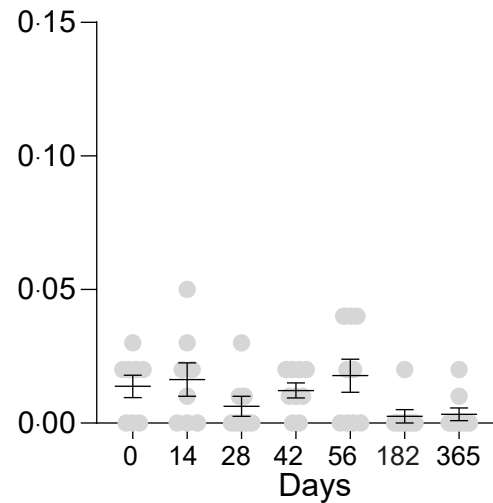
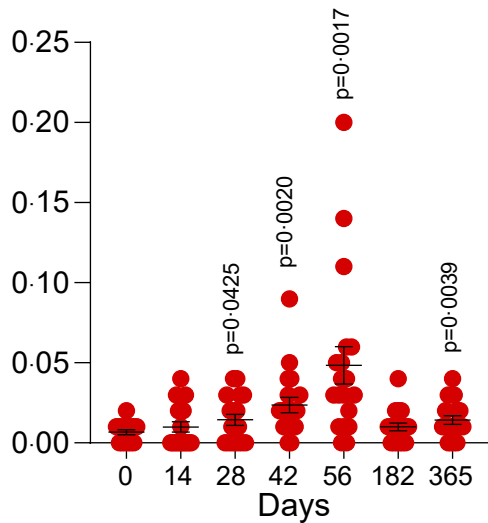
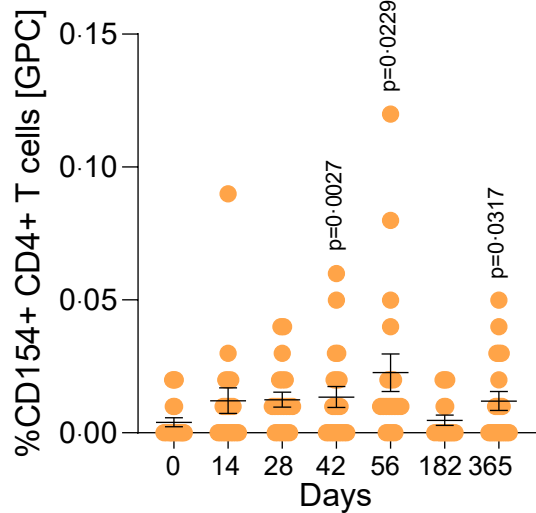
A**B**

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 (cloneM5E2), 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 (cloneB56) 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 decomplexation in this assay.

The VNT using LASV GPC pseudotyped Mopeia virus was conducted in an identical fashion, except that a MOEVAC 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 MOEVAC.

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 ($2,5 \cdot 10^6$ cell/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 1×10^6 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).