Published in final edited form as:

J Infect Dis. 2022 November 01; 226(9): 1545–1550. doi:10.1093/infdis/jiac028.

Lassa Virus Replicon Particle Vaccine Protects Strain 13/N Guinea Pigs Against Challenge With Geographically and Genetically Diverse Viral Strains

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Abstract

Lassa virus (LASV) causes mild to severe hemorrhagic fever disease in humans. Strain 13/N guinea pigs are highly susceptible to infection with LASV strain Josiah (clade IV), providing a critical model system for therapeutics and vaccine development. To develop additional models of disease, we detail the clinical course in guinea pigs infected with 5 geographically and genetically diverse LASV strains. Two of the developed models (LASV clades II and III) were then used to evaluate efficacy of a virus replicon particle vaccine against heterologous LASV challenge, demonstrating complete protection against clinical disease after a single vaccination dose.

Keywords

Lassa fever; virus; hemorrhagic fever; strain 13/N guinea pigs; animal model; disease; viral replicon particle; vaccine; protection

Lassa fever, caused by the rodent-borne Lassa virus (LASV), is endemic in West Africa. Most infections in humans are mild or asymptomatic, but a subset result in severe and fatal hemorrhagic disease. LASV strains display a high level of genetic diversity; they are

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed

currently phylogenetically grouped into 6 geographically distinct West African clades, of which 3 are found in Nigeria, 1 in Sierra Leone and Liberia, 1 in Mali, and 1 in Togo [1]. To date, no vaccines are approved for LASV, and the only available treatment is off-label use of the nucleoside analog drug ribavirin [2]. Several vaccines are in development, including a LASV replicon particle (VRP) vaccine we described previously [3, 4].

Identifying vaccines effective against current and emerging strains of LASV has been limited by lack of disease models that use diverse strains. Thus, to develop models that more faithfully recapitulate the spectrum of human disease and natural LASV diversity, we performed a detailed clinical characterization of infection by strains representing clades II, III, and VI in inbred guinea pigs known to develop severe disease from non–rodent-adapted LASV infection [5]. Then, expanding on our previous VRP vaccine studies that reported protection against homologous challenge with strain Josiah [3, 4], we investigated efficacy against 2 of these newly characterized LASV strains and demonstrate complete protection of VRP vaccination against clinical disease from heterologous infection.

METHODS

Cells, Viruses, and VRP Vaccine

Vero-E6, A549 (American Type Culture Collection [ATCC] No. CCL-185), and GPC-16 (ATCC No. CCL-242) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin. LASV strains Sauerwald (clade II), Nigeria-322 (N-322; clade II), Nigeria-231 (N-231; clade III), Nigeria-383 (N-383; clade III), Togo (clade VI), and recombinant Josiah (clade IV) (Supplementary Table 1, Supplementary Table 2, and Supplementary Figure 1) were grown on Vero-E6 cells (multiplicity of infection [MOI] 0.01, harvested 5–6 days postinfection [dpi]). Viral titers were calculated as focus-forming units (FFU) in Vero-E6 cells. All viral stocks were sequenced by next-generation sequencing and confirmed as mycoplasma-free by MycoAlert Plus reagents (Lonza). LASV VRP vaccine was produced, purified, and concentrated as described earlier [4].

Growth Analysis

A549 and GPC-16 cells were infected at MOI 0.05 for 1 hour at 37°C, inocula were removed, and cells were washed 4 times with phosphate-buffered saline. Supernatants collected at designated times were titrated on Vero-E6 cells under 1.25% carboxymethylcellulose overlay. Virus foci were detected 4 days later by immunofluorescence analysis using in-house monoclonal antibody mix SPR628 as the primary detection antibody.

Interferon Induction

A549 cells were infected at MOI 3 or mock infected. RNA was isolated from cells 18 hours later and quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed as described below. Relative mRNA levels were calculated by accounting for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and interferon-β (IFN-β) amplification efficiencies and are presented as fold over mock-infected sample. This

experiment was performed only in human A549 cells, because LASV inadequately infected GPC-16 guinea pig cells.

Apoptosis Induction and Cytopathic Effect

A549 cells were infected at MOI 3 or mock infected. Induction of apoptosis was measured using Caspase Glo 3/7 assay (Promega) 18 hours postinfection, and overall cell viability was measured using CellTiter-Glo 2.0 assay (Promega) 48 hours postinfection.

Guinea Pig Studies

All work with infectious virus or infected animals was conducted in a biosafety level 4 (BSL-4) laboratory at the Centers for Disease Control and Prevention. For model development, groups of 5 strain 13/N guinea pigs (15 males and 10 females; 234–1093 days of age) were infected subcutaneously (SC) in the interscapular region with a target dose 1×10^4 FFU of LASV (Sauerwald, N-322, N-231, N-383, or Togo strains) in 0.5 mL DMEM. Challenge inocula were back-titered; delivered doses ranged $3.6–5.9 \times 10^3$ FFU. For vaccine challenge, groups of 5 strain 13/N guinea pigs (10 males and 10 females; 239–815 days of age) were vaccinated with target dose 1×10^7 FFU of purified VRPs in HBSS or with Hanks' balanced salt solution (HBSS) vehicle control, and challenged 28 days later with LASV (target dose 1×10^4 FFU; strain Sauerwald or N-231). Vaccine and challenge were both delivered SC in the interscapular region (0.5 mL total volume) and were back-titered; delivered virus dose was $0.9–1.0 \times 10^4$ FFU and VRP dose was 5.1×10^6 FFU. All animals were monitored daily for up to 42 dpi and assigned clinical scores daily (Supplementary Table 3). Animals were humanely euthanized when end-point criteria were reached (score ≥12), or at study completion.

Quantitative RT-PCR

RNA samples were obtained using the MagMAX Pathogen RNA/DNA Kit (RNA eluted in 75 μL; Thermo-Fisher Scientific). Genomic DNA was removed using BaseLine Zero DNase (Epicentre), and qRT-PCR was performed using SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen) with pan-clade primers targeting the L gene of LASV [6]. Strain-specific standard curves were used to quantify viral RNA in tissues. RNA transcripts were produced using viral RNA as template for cDNA synthesis using SuperScript IV First-Strand Synthesis System (Invitrogen), followed by amplification with Q5 DNA polymerase (NEB) and in vitro transcription and DNase treatment with MegaScript T7 kit (Ambion/ThermoFisher Scientific). Tissue-specific correction for sample preparation was applied using a commercial assay for GAPDH (Cp03755743_g1; Thermo-Fisher Scientific). Human IFN-β in A549 cells was quantified using commercial assays for IFN-β and GAPDH mRNA (Hs01077958_s1 and Hs02786624_g1, respectively; Thermo-Fisher Scientific).

Data Analysis and Graphics

All graphs were created in GraphPad Prism (version 9.2.0). Significance was calculated using a one-sample *t* test.

RESULTS

Josiah (clade IV) and Sauerwald (also known as LASV-803213; clade II) [7], 2 LASV strains previously reported to be lethal in strain 13/N guinea pigs, were compared in cell culture experiments to 4 additional strains: N-322 (clade II), N-213 and N-383 (both clade III), and Togo (clade VI). We hypothesized that strains were more likely to cause disease in guinea pigs if relative (1) viral growth was high; (2) interferon induction was low; and (3) apoptosis or other types of cell death was absent. We found that, in general, higher titers (and higher infectivity) were observed in A549 cells than in GPC-16 cells (Figure 1A). Strain Josiah grew most robustly in both cell lines; strain Sauerwald reached lowest maximal titers in both cell lines (9- and 10-fold less than Josiah in A549 and GPC-16 cells, respectively). Growth kinetics of other strains were between those of Josiah and Sauerwald. No strain induced IFN-β transcription, apoptosis, or cell death by other pathways in A549 cells (Figure 1B and 1C).

Having found no major differences between LASV strains in vitro, we chose to determine disease course of the 4 additional strains in guinea pigs, along with Sauerwald. Groups of 5 guinea pigs were inoculated SC with 1 of 5 LASV strains representing 3 clades, and followed up to 42 dpi (Figure 1 and Supplementary Table 4). Of the 20 animals, 2 met euthanasia criteria (1 from Togo at 24 dpi and 1 from N-231 at 21 dpi). A period of weight loss was seen in either a subset (N-322) or all animals (remaining strains) within each experimental group. The most substantial mean weight loss was seen in animals infected with N-231 and N-383. With the exception of the N-322 group, all groups had characteristic elevated temperatures that peaked 9–15 dpi (11 of 15 [73.3%] at 12 dpi). IgG against Josiah nucleoprotein (NP) was detected by immunofluorescence in plasma of all animals except 1 N-231–infected animal euthanized due to disease at 21 dpi (Supplementary Table 4). Blood and tissues collected at euthanasia were evaluated by qRT-PCR. In the N-322 group, no viral RNA was detected at study end point (42 dpi). In all other experimental groups, viral RNA was detected in at least 1 tissue in all animals: in all spleens, and in a variety of other tissues depending on the individual (Figure 1 and Supplementary Table 4). As expected, highest viral loads and tissue RNA distribution were seen in animals that met euthanasia criteria; in these animals, RNA was found in all blood and 15 of 16 (93.8%) tissue samples.

While only a small number of animals exhibited fatal disease, clinical signs were pronounced enough to use this model for therapeutic screening. Two of the 5 strains characterized above, representing virus clades responsible for several recent Lassa fever outbreaks in Nigeria, were selected to evaluate VRP vaccine efficacy against nonhomologous virus infection. Animals were vaccinated SC with purified VRPs based on LASV Josiah (clade IV) in HBSS, or mock-vaccinated with HBSS vehicle control. Twenty-eight days later, VRP and HBSS control groups were challenged SC either with Sauerwald (clade II) or N-231 (clade III). Prechallenge, modest Josiah NP seroreactivity was detected in approximately half of the VRP-vaccinated animals, as previously described (Supplementary Table 5) [4, 5]. Vaccination provided complete protection against clinical signs following heterologous challenge, and no viral RNA was detected in any blood or tissue samples at study completion. In contrast, mock-vaccinated groups exhibited signs of moderate to severe disease (Figure 2). Timing and degree of weight loss and temperature

elevation mirrored findings in model development studies, and 1 animal in the N-231 group again succumbed to fatal disease. In addition, viral RNA was detectable in at least 1 tissue from all mock-vaccinated survivors at study completion (42 dpi; Supplementary Table 5). At study end point, all animals were seroreactive to Josiah NP, and reactivity was increased in vaccinated animals with detectable responses prechallenge.

DISCUSSION

Heterologous protection is particularly important in LASV vaccine development due to substantial virus diversity; overall strain variation in partial NP gene sequences is up to 27% at the nucleotide level and 15% at the amino acid level [8]. Most therapeutic screening to date uses LASV strain Josiah, but inbred guinea pigs have been infected with other strains [9], including in our work characterizing LBR-USA-2015 (NJ2015; clade IV) [10]. In general, studies have focused on a few viral strains and reported binary outcome data (lethal/nonlethal) with limited descriptions of other clinical manifestations. We aimed to develop new infection models that recapitulate the spectrum of human disease, including mild to moderate clinical presentations, and address the need for models representing the genetic diversity of LASV.

All candidate LASV strains we examined were able to replicate in human cells without inducing apoptosis or expression of antiviral IFN-β. Interestingly, growth analysis showed that strain N-322, the least pathogenic in this study, grew as robustly as strain Josiah in A549 cells and GPC-16 cells. Conversely, strain Sauerwald resulted in reproducible clinical signs in the model despite reduced viral titers produced in cell culture. Mouse models have linked T cells to LASV pathology [11, 12], and strain-dependent disease in guinea pigs may be influenced by mechanisms not readily measured in cell culture.

Guinea pigs infected with the strains herein exhibited measurable alterations in clinical parameters useful for assessing cross-protective vaccine efficacy. Potential for cross-protective vaccine development is supported by reports of T-cell and antibody cross-reactivity induced by a ChAdOx1-Lassa-GPC vaccine [13], and cross-protection with a recombinant vesicular stomatitis virus expressing strain Josiah glycoproteins [14]. In addition, an ML29 reassortant virus that carries NP and glycoprotein (GP) dominant antigens of LASV and the L polymerase and Z matrix protein of the nonpathogenic genetically related Mopeia virus protected against Sauerwald challenge [7]. VRPs are promising vaccine candidates due to their safety and efficacy; they replicate in the first cells they encounter but do not spread, thus retaining a high safety profile, and show uniform protection against disease [3]. Previously, we reported LASV VRP safety, singledose prophylactic efficacy [3], and postexposure prevention of fatal outcomes [4]. Here we also report single-dose heterologous protection against geographically and genetically diverse LASV strains. Emerging strains do not differ greatly from previously detected strains within a region [15]; genetic variation correlates with geographic distance rather than time [8]. The viruses evaluated represent distinct endemic geographic regions and thus serve as proxy for current and future strains. These data further support the promise of cross-protective vaccine development for LASV and demonstrate utility of the VRP vaccine platform against both endemic and emerging virus strains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment.

We thank Tatyana Klimova for assistance with editing the manuscript.

Financial support.

This work was supported in part by an appointment to the Research Participation Program at the CDC administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and CDC (S. R. W.); and by CDC Emerging Infectious Disease Research Core Funds.

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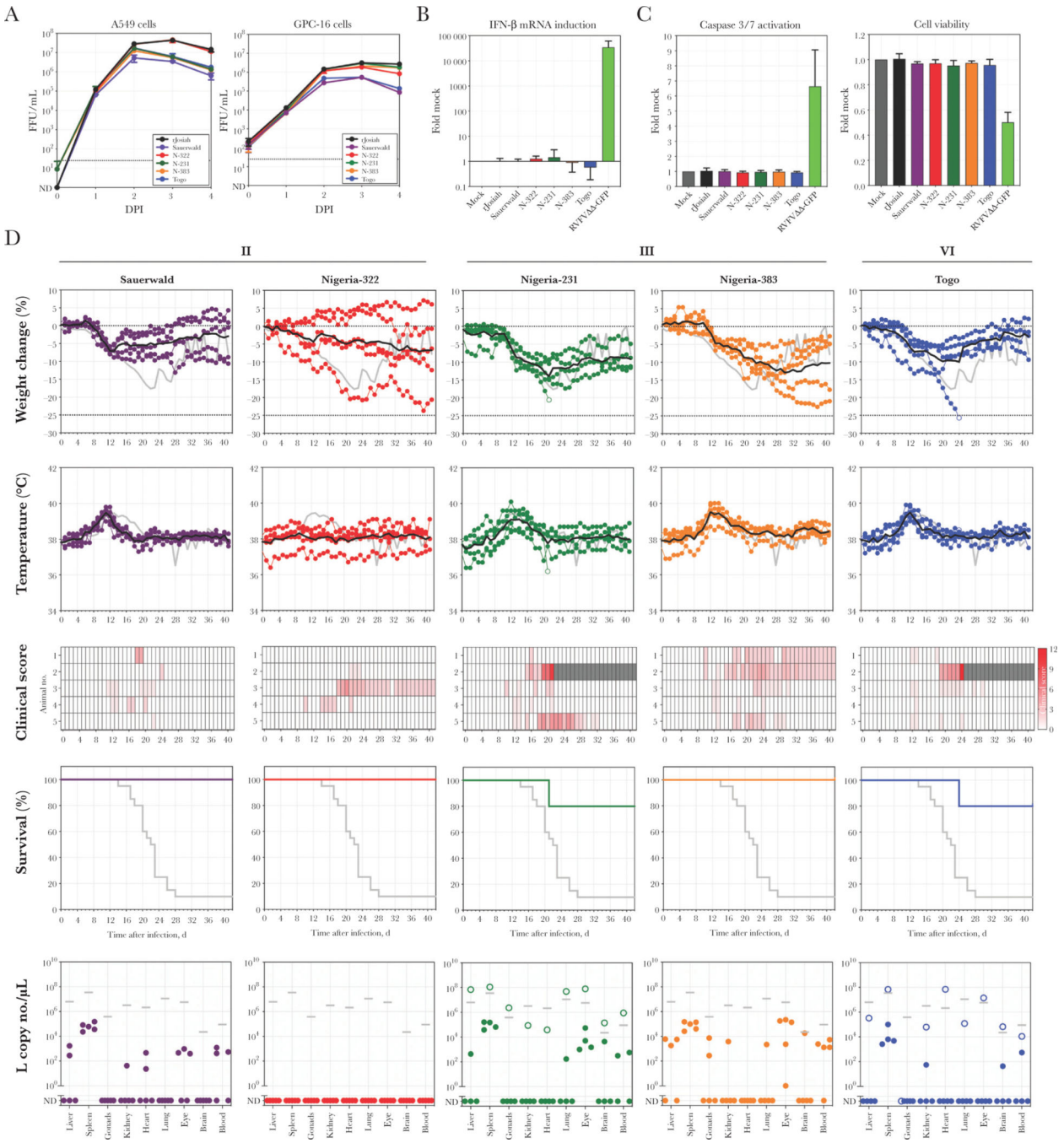

Figure 1.

A, Growth comparison of 6 LASV strains in human (A549) and guinea pig (GPC-16) cells infected at MOI 0.05 for 1 hour. *B*, Relative IFN-β mRNA levels in A549 cells infected at MOI 3 or mock infected, presented as fold over mock-infected sample. *C*, Induction of apoptosis in infected A549 cells (MOI 3) measured by Caspase Glo 3/7 assay 18 hours after infection, and overall cell viability 48 hours after infection. This experiment was performed only in human cells because LASV inadequately infected GPC-16 guinea pig cells. *D*, Weight change (% from baseline at −1 dpi), body temperature, daily clinical

scores (range, 0–12), survival, and viral RNA (L gene copy No.) in tissues of strain 13/N guinea pigs infected subcutaneously with 1 of 5 strains of LASV (experimental group, n = 5; target dose: 1×10^4 FFU). In weight and temperature graphs, symbols represent individual animals and solid lines represent the group mean. Clinal signs were assessed and scored daily (Supplementary Methods and Supplementary Table 3). Score ≥12 indicated end-point criteria. Grey boxes in clinical scores indicate animals removed from study due to virus-associated fatal disease. Tissues collected at the time of euthanasia were analyzed for viral RNA (L gene copy No./μL of RNA) and included liver, spleen, gonad (ovary or testicle), kidney, heart, lung, eye, brain, and blood. Open circles denote samples from animals that succumbed to infection (fatal) and closed circles denotes animals that survived to end of study (42 dpi; survivors). Light grey lines in weight change, temperature, survival, and viral RNA graphs represent our historical data from guinea pigs infected with strain Josiah (clade IV; mean, n = 20). Abbreviations: dpi, days postinfection; FFU, focus-forming units; IFN-β, interferon-β; LASV, Lassa virus; MOI, multiplicity of infection; ND, not detected; rJosiah, recombinant strain Josiah; RVFVΔΔ-GFP, Rift Valley fever virus lacking NSm and NSs genes.

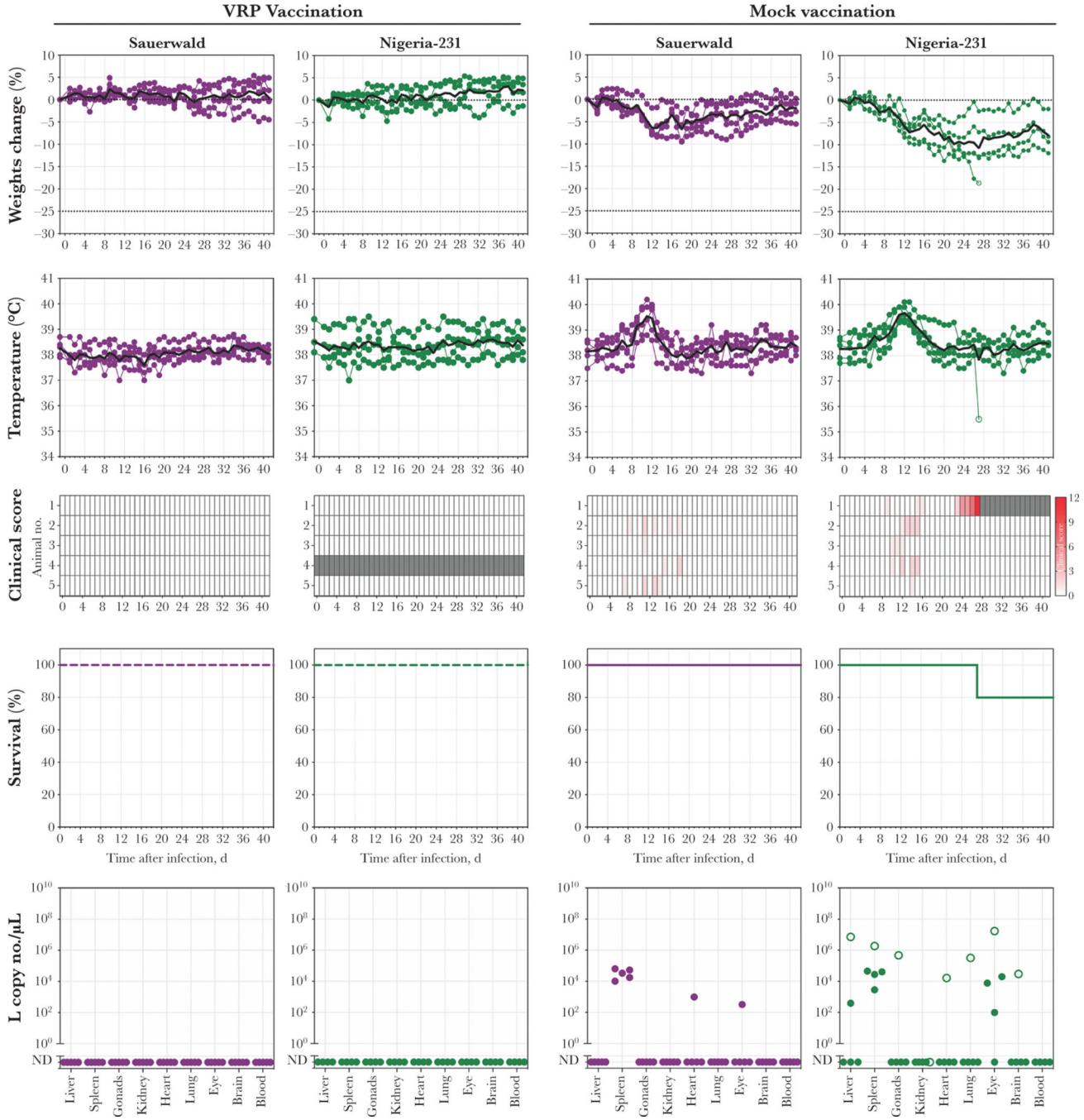

Figure 2.

Clinical outcomes of VRP- or mock-vaccinated strain 13/N guinea pigs challenged subcutaneously with LASV. Groups of guinea pigs (n = 5) were vaccinated with VRP (target dose, 1×10^7 FFU) or mock vaccinated (HBSS only). At 28 days postvaccination, animals were subcutaneously infected with 1×10^4 FFU of strain Sauerwald or Nigeria-231. Weight change (% from baseline at −1 dpi), temperature, and survival were assessed. In weight and temperature graphs, symbols represent individual animals and solid lines represent the group mean. Clinal signs were assessed and scored daily (Supplementary Methods and

Supplementary Table 3). Score ≥12 indicated end-point criteria. Grey boxes in clinical scores indicate animals removed from study due to unrelated health reasons (1 animal in N-231) or virus-associated fatal disease. Tissues collected at the time of euthanasia were analyzed for viral RNA (L gene copy No./μL of RNA) and included liver, spleen, gonad (ovary or testicle), kidney, heart, lung, eye, brain, and blood. Open circles denote samples from animals that succumbed to infection (fatal) and closed circles denotes animals that survived to end of study (42 dpi; survivors). Abbreviations: dpi, days postinfection; FFU, focus-forming units; HBSS, Hanks' balanced salt solution; LASV, Lassa virus; ND, not detected; VRP, virus replicon particle.