LETTER

Lipid nanoparticle siRNA treatment of Ebola–virus–Makona–infected nonhuman primates

Emily P. Thl1,2*, Chad E. Mire1,2, Amy C. H. Lee1, Joan B. Geisbert2,3, Joy Z. Zhou1, Krystle N. Agans2,3, Nicholas M. Snead1, Daniel J. Deer2,3, Trisha R. Barnard1, Karla A. Fenton2,3, Ian MacLachlan1 & Thomas W. Geisbert2,3

The current outbreak of Ebola virus in West Africa is unprecedented, causing more cases and fatalities than all previous outbreaks combined, and has yet to be controlled. Several post-exposure interventions have been employed under compassionate use to treat patients repatriated to Europe and the United States. However, the in vivo efficacy of these interventions against the new outbreak strain of Ebola virus is unknown. Here we show that lipid-nanoparticle-encapsulated short interfering RNAs (siRNAs) rapidly adapted to target the Makona outbreak strain of Ebola virus are able to protect 100% of rhesus monkeys against lethal challenge when treatment was initiated at 3 days after exposure while animals were viraemic and clinically ill. Although all infected animals showed evidence of advanced disease including abnormal haematology, blood chemistry and coagulopathy, siRNA-treated animals had milder clinical features and fully recovered, while the untreated control animals succumbed to the disease. These results represent the first, to our knowledge, successful demonstration of therapeutic anti-Ebola virus efficacy against the new outbreak strain in nonhuman primates and highlight the rapid development of lipid-nanoparticle-delivered siRNA as a countermeasure against this highly lethal human disease.

Historical Ebola virus (EBOV) outbreaks have previously ranged in size from a few to more than 400 cases, and were relatively well controlled by contact tracing and quarantine methods. In late 2013, an unprecedented outbreak caused by the Zaire species of EBOV began. This outbreak focused around the West African countries of Guinea, Liberia and Sierra Leone and has continued unabated for more than a year so far, with 25,213 cases and 10,460 deaths. Despite intensive containment efforts, the outbreak is still not under control and the need for medical countermeasures to both prevent and treat infections has never been greater.

While there are no approved vaccine or therapeutic treatment modalities available for preventing or managing EBOV infections, a few post-exposure approaches have demonstrated convincing efficacy against EBOV in a nonhuman primate (NHP) model that closely reproduces human infection. These include anti-EBOV monoclonal antibody administration alone (such as ZMapp) or with adenovirus-vectored interferon-α, and EBOV-targeting siRNAs encapsulated in lipid nanoparticles (LNPs) (TKM-Ebola) to potentiate cellular delivery. Several experimental treatments including ZMapp and TKM-Ebola have been employed under compassionate use protocols to treat small numbers of repatriated EBOV-infected medical staff in Europe and the United States. However, the contribution of these experimental treatments towards patient survival cannot be established, as several experimental treatments were applied in parallel alongside aggressive supportive care. Clinical trials have been initiated in West Africa to evaluate the efficacy of several experimental treatments including convalescent serum, vaccines, small molecules (brincidofovir, now halted) and recently ZMapp, although these investigations may become hampered by the dwindling number of new cases of infection. Furthermore, up to now no treatments have been tested against the current outbreak strain of EBOV under experimentally well-controlled conditions. Because much of the previous vaccine and antiviral development has been conducted in NHPs using the historical EBOV 1995 Kikwit strain from central Africa, there is a possibility that sequence changes documented in the West African strain4–8 may interfere with medical countermeasure efficacy, highlighting the need for treatments that can be rapidly adapted to mutated aetiological agents. While siRNA recognition is sequence dependent, adjustments for small viral nucleotide changes can be made rapidly. Monoclonal antibodies rely on cross-reactivity to conserved epitopes; if these are considerably changed, suitable antibodies must be identified de novo.

Sequence alignments of the nucleotide target sites of the TKM-Ebola siRNA cocktail, siEbola-2, with available sequences from the West African outbreak4–8 revealed conserved mismatches at antisense position 6 for siLpol-2 and at positions 3 and 15 for siVP35-2 that are not present in virus sequences endemic to central Africa (Fig. 1a). While certain positions within the prototypical siRNA structure are considered more crucial for function, and others better able to tolerate mismatches without erosion of activity, such effects are sequence-dependent and difficult to predict. Given this uncertainty, we took advantage of the rapid adjustment capability of the siRNA–LNP platform and designed a new siRNA cocktail, siEbola-3, in which these mismatches were corrected to enable full complementarity to West African outbreak EBOV sequences. We used a virus-free dual luciferase reporter assay to model the gene-silencing ability of the adjusted siRNA components against a representative central African strain versus the West African strain. Results demonstrated that the new siEbola-3 cocktail is fully active against the West African EBOV sequence, and retains activity against the central African sequence despite an impairment of the siVP35-3 siRNA component (Fig. 1b, see also Methods).

To assess medical countermeasure antiviral efficacy against the West African EBOV strain, we utilized in vitro and rhesus macaque models using a virus isolate from a lethal case in Guinea. Deep sequencing of the challenge stock confirmed viral identity with 100% of the sequences containing the wild-type phenotype of 7 consecutive template uridines (7U) at the glycoprotein-editing site, confirming that viral virulence was not compromised during preparation of the challenge stock. It has been shown that macaques infected with 7U EBOV Kikwit succumb to infection earlier than those infected with 8U virus, and the protection afforded by some vaccine candidates decreases with EBOV 7U infection. Consistent with dual luciferase reporter predictions, both siEbola-2 and siEbola-3 LNPs were able to inhibit viral RNA levels in cultured cells infected with either EBOV Makona or EBOV Kikwit, although the siRNAs with full complementarity resulted in more activity (Extended Data Fig. 1).

siEbola-3 LNP treatment was able to protect NHPs against lethal challenge. NHPs were infected with the West African EBOV isolate and either left as untreated controls or administered siEbola-3 LNP beginning at 72h after infection when animals were viraemic and

1Telmira Pharmaceuticals, Burnaby, British Columbia V5J 5J8, Canada. 2Galveston National Laboratory, University of Texas Medical Branch, Galveston, Texas 77550, USA. 3Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas 77550, USA.

*These authors contributed equally to this work.
clinically ill. All treated animals survived to study endpoint, while untreated control animals succumbed on days 8 and 9 (Fig. 2a). The time-to-death observed in untreated animals was similar to that reported after symptom onset in patients (9.8 ± 0.7 (mean ± s.e.m.) days), suggesting that EBOV infection in NHPs closely reproduces this aspect of human infection. Untreated control animals displayed mild clinical signs up until the day of euthanasia, after which a rapid deterioration of condition necessitated euthanasia (Fig. 2b). This is different from the disease course observed in NHPs infected with the EBOV Kikwit strain, in which animals tend to show a more gradual decline over the course of 1–3 days (Extended Data Table 1). In contrast to control animals, all siEbola-3-LNP-treated animals developed only transient mild clinical symptoms (Fig. 2b). Fever was observed in all infected animals with the exception of one treated animal, beginning at day 5 or 6 and continuing for 2–3 days until temperature returned to baseline (treated animals) or animals became hypothermic (Table 1). Petechial rashes were observed in all untreated and two treated animals, and these were milder than that seen previously in animals infected with EBOV Kikwit (Extended Data Table 1). Diarrhoea was also observed in two untreated animals infected with EBOV Makona, a clinical symptom associated with a fatal outcome in patients from this outbreak. Diarrhoea was not as notable as those seen historically in rhesus macaques infected with EBOV Kikwit (Extended Data Fig. 2b–e). Treated animals also showed protection against EBOV-induced renal dysfunction as assessed by dual luciferase reporter assay (see Methods). Shown is the Renilla luciferase/firefly luciferase ratio of each sample normalized to untreated cells. Results are mean ± s.e.m. from one (negative control) or two (other data) biological replicates, conducted in technical triplicate.

In conjunction with reductions in viral load, animals treated with siEbola-3 LNP showed moderate protection against liver dysregulation, with EBOV Makona- and Kikwit-infected animals consistent with historical EBOV Kikwit-infected macaques, whereas detection of EBOV antigen in tissues of the fully recovered siEbola-3 LNP-treated animals was rare and limited to cells associated with antigen presentation (Fig. 3). No difference in viraemia levels was observed between EBOV Makona- and Kikwit-infected animals on the basis of limited available data (Extended Data Fig. 2a).

In untreated control animals, Fig. 2c), which correlated with 7.6- to 114-fold decreases in circulating viral genome detection (Fig. 2d, day 6). Peak viral RNA levels in untreated control animals were 8 and 9 log(viral copies per ml), respectively, well over the 10 million EBOV copies ml−1 threshold associated with a higher fatality rate in patients. At euthanasia, viral RNA was also widespread in tissues of untreated control animals, whereas it was only detected in the lymph nodes and spleen of treated animals at levels that were several magnitudes lower (Fig. 2e). These tissues were negative for infectious virus by plaque assay (data not shown), suggesting that the presence of viral RNA was not due to incomplete viral clearance. However, viral RNA detection at study endpoint in these sites of antigen presentation may reflect enforced viral replication in antigen presenting cells, which allows for adequate amounts of antigen to be presented to promote the adaptive immunity critical for survival after infection with a cytopathic virus. In accordance with this, immunohistochemical tissue evaluation showed positive EBOV antigen staining for the untreated control animals consistent with historical EBOV Kikwit-infected macaques, whereas detection of EBOV antigen in tissues of the fully recovered siEbola-3 LNP-treated animals was rare and limited to cells associated with antigen presentation (Fig. 3).

In untreated control animals infected with EBOV Makona, although the level of disturbance observed in infected animals was not as notable as those seen historically in rhesus macaques infected with EBOV Kikwit (Extended Data Fig. 2b–e). Treated animals also showed protection against EBOV-induced renal dysfunction as
Figure 2  | siEbola-3 LNP treatment confers survival and reduces viral load.  a, NHPs lethally challenged with EBOV Makona survive when treated with siEbola-3 LNP starting 72 h after infection. b, Clinical signs were improved in treated animals. Euth., euthanized. c–e, Treatment reduces infectious virus load (*P = 0.0450, one-sided t-test, day 6) (c), viral RNA in blood (**P = 0.0023, one-sided t-test, day 6) (d) and viral RNA in tissues (e). Lower limit of detection is 5 plaque-forming units (p.f.u.) per millilitre. d, e, Quantitative reverse transcription PCR (qRT–PCR) data shown are mean ± s.d. of two technical replicates. Geq, genome equivalents; LLOQ, lower limit of quantitation (4.8 log10(viral copies per g) or 5.1 log10(viral copies per ml)). LL, left lower lobe; LM, left middle lobe; LN, lymph nodes; LU, left upper lobe; ND, not detected; RL, right lower lobe; RM, right middle lobe; RU, right upper lobe.  n = 3 per group.

Table 1  | Clinical description and outcome of EBOV-challenged NHPs

| Subject no. | Sex | Group       | Clinical illness                                                                 | Clinical pathology                                                                 |
|-------------|-----|-------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| 0805068     | M   | Untreated control | Fever (d6); mild depression (d6–7); severe depression (d8); lethargy (d7–8); loss of appetite (d6–8); mild petechial rash (d9); rectorrhagia (d8); hunched posture (d6,7,8 morning); recumbency (d8 pm); animal euthanized in afternoon on d8 | Leukocytosis (d6,8); granulocytosis (d3,6,8); thrombocytopenia (d6,8); lymphopenia (d3,6,8); ALT >10-fold (d8); AST >4-fold (d6); AST >10-fold (d8); ALP >2-fold (d6); ALP >8-fold (d8); GGT >50-fold (d8); BUN >7-fold (d8); CRE sevenfold (d8); CRP >2-fold (d6,8); fibrinogen >2-fold (d6) |
| 1105274     | F   | Untreated control | Fever (d6–7); mild depression (d8); severe depression (d9); lethargy (d8–9); loss of appetite (d8–9); mild petechial rash (d9); diarrhoea (d9); hunched posture (d9 am); recumbency (d9 pm); animal euthanized in afternoon on d9 | Leukocytosis (d6,6); granulocytosis (d5,6); thrombocytopenia (d9); ALT >6-fold (d9); AST >10-fold (d9); BUN >2-fold (d9); CRP >10-fold (d6); fourfold (d9); APTT >2-fold (d9); fibrinogen >2-fold (d6) |
| JE60        | M   | Untreated control | Fever (d6), mild to moderate depression (d6–8); severe depression (d9); lethargy (d7–9); loss of appetite (d6–9); mild petechial rash (d6–9); severe epistaxis (d9); diarrhoea (d9); hunched posture (d6–8); recumbency (d9); animal euthanized in afternoon on d9 | Thrombocytopenia (d6,9); lymphopenia (d6,9); hypoalbuminemia (d9); hyperproteinemia (d9); AST >6-fold (d9); BUN >2-fold (d9); CRP >10-fold (d6); 4-fold (d9); APTT >2-fold (d9) |
| 0902056     | F   | 72 h delay to treat | Fever (d8–10); mild depression (d8–12); loss of appetite (d5–13); mild petechial rash (d9–15); animal survived | Leukocytosis (d10); granulocytosis (d6,10); thrombocytopenia (d6,10,14); lymphopenia (d6); ALT >2-fold (d6,10); AST >4-fold (d6); AST >10-fold (d10); GGT >2-fold (d10); CRP >10-fold (d6,10); fibrinogen >2-fold (d6) |
| 1005445     | M   | 72 h delay to treat | Mild depression (d8–12); loss of appetite (d5–14); mild petechial rash (d9–13); animal survived | Granulocytosis (d10); Thrombocytopenia (d6,10); lymphopenia (d6); ALT >10-fold (d6); ALT >5-fold (d10); AST >10-fold (d6,10); CRP >10-fold (d6,10); APTT >3-fold (d10) |
| 1006241     | M   | 72 h delay to treat | Fever (d5–7); mild depression (d8–11); loss of appetite (d7–14); animal survived | Leukocytosis (d6,14); granulocytosis (d6,14); AST >7-fold (d10); CRP >10-fold (d6,10); fibrinogen >2-fold (d6) |

Days (d) after EBOV challenge are in parentheses. Fever is defined as a temperature more than 1.4 °C over baseline or at least 0.8 °C over baseline and ≥39.72 °C. Mild rash: focal areas of petechiae covering less than 10% of the skin. Lymphopenia and thrombocytopenia are defined by a ≥40% drop in numbers of lymphocytes and platelets, respectively. Leukocytosis and granulocytosis are defined by ≥40% increase in numbers of white blood cells. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; APTT, activated partial thromboplastin time; BUN, blood urea nitrogen; CRE, creatinine; CRP, C-reactive protein; F, female; GGT, gamma glutamyltransferase; M, male.
assessed by creatinine and blood urea nitrogen levels (Extended Data Fig. 2f, g). Smaller differences in coagulopathy, lymphopenia and thrombocytopenia were observed between treated and untreated animals (Table 1 and Extended Data Fig. 3). No differences in these parameters were apparent in untreated animals after infection with either EBOV Makona or EBOV Kikwit. Overall, these results indicate that siEbola-3 LNP treatment may confer additional protective benefits against clinical symptoms of EBOV-induced disease in addition to survival advantage and effective control of viral load. Some clinical pathology characteristics such as liver dysfunction were found to be not as profound in EBOV-Makona-infected NHPs when compared to that observed previously for EBOV Kikwit infection.

The current EBOV outbreak in West Africa highlights the need for antiviral therapeutics and prophylactics that can be readily and rapidly adapted to address the changing viral strain landscape. The use of a cocktail format (as opposed to a single siRNA) increases the likelihood of activity retention against newly emergent viral strains, as evidenced by the activity of siEbola-2 against EBOV Makona despite the presence of several nucleotide mismatches (Fig. 1b and Extended Data Fig. 1). Furthermore, the bipartite structure of TKM-Ebola, comprising both siRNA and LNP, allows for adjustments to the siRNA component to capitalize on emerging strain sequence data while maintaining the delivery functionality of the LNP component. Once viral sequence data are available, clinical grade drug product can be produced in as little as 8 weeks. Although TKM-Ebola (containing siEbola-2 and designed for central African EBOV) is currently under a US Food and Drug Administration (FDA) partial clinical hold regarding administration to healthy uninfected subjects, this product has been allowed by the FDA for use in cases of confirmed or suspected EBOV infection as the risk/benefit profile is quite different for patients facing a prospective high mortality rate compared to normal healthy individuals. The new siEbola-3 siRNA cocktail, shown here to possess robust activity against the latest EBOV Makona outbreak strain, is now being evaluated for efficacy in EBOV-infected patients in Sierra Leone, West Africa.

Figure 3 | EBOV Makona tissue pathology and antigen in NHPs untreated or treated with siEbola-3 LNP. a, Immunolabelling of sinusoidal lining and Kupffer cells in untreated animal. b–d, No immunolabelling in treated animals. e, Immunolabelling of dendriform mononuclear cells in red and white pulp of untreated animal. f–h, No immunolabelling in treated animals. i, Immunolabelling of cortical and interstitial cells in untreated animal. j–l, No immunolabelling in treated animals. m, Immunolabelling, dendriform mononuclear cells within subcapsular and medullary sinuses in untreated animal. n–p, No immunolabelling in treated animals. Original magnifications ×20.
METHODS

Dual luciferase reporter assay. The psiCHECK2 (Promega) vector was used to construct the EBOV Makona and EBOV Kikwit strain reporter plasmids used in this study (GenScript). In brief, to construct the EBOV Makona or EBOV Kikwit reporter plasmids, two 201-base-pair (bp) regions of either the EBOV Makona strain or EBOV Kikwit strain genomes containing the VP35 and Lpol target sites (nucleotide positions 17287–17488, and 3817–4018 of GenBank accession number JF660347.2 or AY354458) were fused together and cloned into the 3′ untranslated region (UTR) of the Renilla luciferase gene between the Xhol and NotI restriction sites to allow for the detection of siRNA activity as represented by decreased Renilla luciferase activity. si.pol-3 and siVP35-3 were synthesized at ST Pharm, and si.pol-2 and siVP35-2 were synthesized at Integrated DNA Technologies. Individual duplexes and the si.Ebola-3 or si.Ebola-2 cocktail (1:1 molar mixture of si.pol-3 and siVP35-3 or si.pol-2 and siVP35-2, respectively) were encapsulated into LNP by the process of spontaneous vesicle formation as previously reported. The resulting LNPs were dialysed against PBS and sterilized through a 0.2-μm filter before use. siRNAs targeting Renilla luciferase and MARV NP (synthesized by Integrated DNA Technologies) were also encapsulated in LNP and were included as positive and negative controls, respectively.

Authenticated HepG2 cells were obtained from ATCC (ATCC HB-8065). Cells were tested for mycoplasma before experimentation. HepG2 cells were transfected with the EBOV Makona or EBOV Kikwit psiCHECK2 plasmid construct using Lipofectamine 2000 (Life Technologies) and treated with siRNA-LNP at 5, 50, 125, 250, 500 and 750 ng ml⁻¹. Transfected cells were incubated for 24 h, followed by measurement of Renilla and firefly luciferase activities using a luminometer. Results were expressed as a percentage of the Renilla/firefly luciferase activity in cells transfected with the reporter plasmid only (no siRNA treatment).

EBOV Makona virus and sequence analysis. The EBOV Makona strain seed stock originated from serum of a fatal case during the 2014 outbreak in Guéckédou, Guinea (Zaire ebolavirus isolate Homo sapiens-vetG1N/2014). The cells were not tested for mycoplasma. The EBOV Makona strain passage 2 seed stock was extracted in Trizol LS (Invitrogen) then purified using Zymo Research Direct-zol RNA mini-prep (Zymo Research) per manufacturer’s instructions. Complementary DNA was generated from purified RNA using the Ovation RNA-seq 2 kit, which was subsequently used for the preparation of the double-stranded DNA library using the Encore Ion Torrent library prep kit (NuGen). Sequencing was performed by the UTMB Molecular Core on the Ion Torrent using 318-v2 deep sequencing chips. Sequence analysis was performed using Seqman NGEN software (DNA Star) based on paired-end analysis of 100-bp overlaps.

In vitro infections. HepG2 cells (ATCC HB-8065) were seeded at 1E05 cells/well in 24-well culture plates and incubated at 37°C/5% CO₂ overnight before infection with 0.1 multiplicity of infection (MOI) of either EBOV Makona or Kikwit. Cells were infected with virus for 1 h, then washed four times with PBS and treated with siRNA-LNP at 51.2, 6.4 and 0.8 ng ml⁻¹. Cells were incubated for 48 h after treatment before collecting cell supernatants for RNA extraction by Trizol and qRT–PCR assessment.

Animal challenge. Six healthy adult rhesus macaques (Macaca mulatta) of Chinese origin (4–8 kg, three males and three females, 4–8 years old) were inoculated intramuscularly with 1,000 p.f.u. of EBOV Makona strain. The historical EBOV Kikwit data was obtained from six healthy rhesus macaques (six females, 4–8 years old) inoculated intramuscularly with 1,000 p.f.u. of EBOV Kikwit strain. Sample sizes were based on the availability of rhesus macaques. Animals were randomized with Microsoft Excel into treatment or control groups. si.Ebola-3 LNP (0.5 mg kg⁻¹) was administered to three of the EBOV-Makona-infected macaques by bolus intravenous infusion 72 h after EBOV challenge while the control animals were not treated. The three treated animals received additional treatments of si.pol-3 LNP on days 4, 5, 6, 7, 8 and 9 after EBOV challenge. All animals (six infected with EBOV Makona and six infected with EBOV Kikwit) were given physical examinations and blood was collected at the time of challenge and on days 3, 6, 10, 14, 22 and 28 after EBOV challenge or at time of euthanasia. In addition, all animals were monitored daily and scored for disease progression with an internal filovirus scoring protocol approved by the UTMB Institutional Animal Care and Use Committee. The scoring changes measured from baseline included posture/activity level, attitude/behaviour, food and water intake, weight, respiration and disease manifestations such as visible rash, haemorrhage, ecchymosis or flushed skin. A score of ≥9 indicated that an animal met criteria for euthanasia. This study was not blinded.

Detection of viraemia and viral RNA. RNA was isolated from whole blood or tissues using the Viral RNA Mini Kit or RNeasy Kit (Qiagen) using 100 μl of blood into 600 μl of buffer AVL, or 100 μg of tissue per manufacturer’s instructions, respectively. Primers/probe targeting the VP30 gene of EBOV were used for qRT–PCR with the probe used here being 6-carboxytetramethylrhodamine (6FAM)-5′-CCGT CAACTAAGGAGCGCCTC3′-6 carboxytetramethylrhodamine (TAMRA) for the EBOV Makona NHP and EBOV Makona and Kikwit in vitro studies (Life Technologies). EBOV RNA was detected using the CFX96 detection system (BioRad Laboratories) in One-step probe qRT–PCR kits (Qiagen) with the following cycle conditions: 50°C for 10 min, 95°C for 10 s, and 40 cycles of 95°C for 10 s and 59°C for 30 s. Threshold cycle (Ct) values representing EBOV genomes were analysed with CFX Manager Software, and data are shown as mean ± s.d. of technical replicates. To create the genome equivalent standard, RNA from EBOV stocks was extracted and the number of EBOV genomes calculated using Avogadro’s number and the molecular mass of the EBOV genome.

Virus titration was performed by plaque assay with Vero E6 cells from all serum samples as previously described. In brief, increasing tenfold dilutions of the samples were adsorbed to Vero E6 monolayers in duplicate wells (200 μl); the limit of detection was 5 p.f.u. ml⁻¹.

Haematology, serum biochemistry and blood coagulation. Total white blood cell counts, white blood cell differentials, red blood cell counts, platelet counts, haemocrit values, total haemoglobin concentrations, mean cell volumes, mean corpuscular volumes and mean corpuscular hemoglobin concentrations were analysed from blood collected in tubes containing EDTA using a laser-based haematological analyser (Beckman Coulter). Serum samples were tested for concentrations of albumin, amylase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, glucose, cholesterol, total protein, total bilirubin, blood urea nitrogen, creatinine and C-reactive protein by using a Piccolo point-of-care analyser and Biochemistry Panel Plus analyser discs (Abaxis). Citrated plasma samples were analysed for coagulation parameters prothrombin time, activated partial thromboplastin time, and fibrinogen on the STA4t instrument using the PTT Automatic, kits, respectively (Diagnostica Stago).

Histopathology and immunohistochemistry. Necropsy was performed on all subjects. Tissue samples of all major organs were collected for histopathological and immunohistochemical examination, immersion-fixed in 10% neutral buffered formalin, and processed for histopathology as previously described. For immunohistochemistry, specific anti-EBOV immunoreactivity was detected using an anti-EBOV VP40 protein rabbit primary antibody (Integrated Biotherapeutics) at a 1:4,000 dilution. In brief, tissue sections were processed for immunohistochemistry using the Dako Autostainer (Dako). Secondary antibody used was biotinylated goat anti-rabbit IgG (Vector Laboratories) at 1:200 followed by Dako LSAB streptavidin-HRP (Dako). Slides were developed with Dako DAB chromagen (Dako) and counterstained with haematoxylin. Non-immune rabbit IgG was used as a negative control. Liver, adrenal gland and inguinal lymph node representative images were taken at ×40 magnification, and spleen taken at ×20 magnification from control animal 0805086 (Fig. 3a, e, h, m) or treated animals 0902056 (Fig. 3b, f, j, n), 1005445 (Fig. 3c, g, k, o), and 1006421 (Fig. 3d, h, l, p).

Statistical analyses. Analysis was conducted with Graphpad Prism software (version 6.04). A paired t-test (one-sided) was used to compare untreated and treated groups on days 6 for qRT–PCR (untreated group mean ± s.d. was 8.51 log GEq ml⁻¹ ± 0.74; si.Ebola-3 LNP treated group was 6.36 log (GEq ml⁻¹) ± 0.62) and viraemia (untreated group mean ± s.d. was 5.94 log(p.f.u. ml⁻¹) ± 0.67; si.Ebola-3 LNP-treated group was 3.02 log(p.f.u. ml⁻¹) ± 1.03). No statistical methods were used to predetermine sample size.

21. Ma, H. et al. Formulated minimal-length synthetic small hairpin RNAs are potent inhibitors of hepatitis C virus in mice with humanized livers. Gastroenterology 146, 63–66 (2014).
Extended Data Figure 1 | Antiviral activity of siEbola-3 in cells infected with EBOV Makona. For comparison, siEbola-3 activity was also assessed against the central African EBOV Kikwit strain and siEbola-2 activity was evaluated against both EBOV strains. Data are viral RNA copies per millilitre of each sample normalized to untreated infected cells. Results are mean ± s.e.m. from one biological replicate, conducted in technical triplicate.
Extended Data Figure 2 | siEbola-3 LNP treatment provides partial protection against EBOV Makona clinical pathologies, and infection with EBOV Makona infection induces a lesser degree of liver dysfunction compared to EBOV Kikwit infection. a, No differences in viraemia levels were observed in untreated animals infected with EBOV Makona or Kikwit.

b–e. Liver dysfunction markers. Normal values for uninfected NHPs ranges are GGT (40–115 U l$^{-1}$), AST (20–45 U l$^{-1}$), ALT (20–165 U l$^{-1}$), ALP (130–500 U l$^{-1}$). f–g. Protection against EBOV-Makona-induced CRE and BUN elevation was observed. Normal values for uninfected NHPs range from BUN (10–25 mg dl$^{-1}$) and CRE (0.8–1.2 mg dl$^{-1}$).
Extended Data Figure 3 | Comparison of coagulation and haematology characteristics between untreated control animals infected with EBOV Makona or Kikwit. a, b, Coagulopathies are not as marked in EBOV Makona infection when compared to historical EBOV Kikwit data. c, Lymphopenia is observed in all infected animals. d, Thrombocytopenia levels are similar between EBOV-Makona and EBOV-Kikwit-infected control animals.
### Extended Data Table 1 | Comparison of clinical signs progression between untreated rhesus macaques infected with EBOV Makona or EBOV Kikwit

| Infection  | Animal ID | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |
|------------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| EBOV Makona| 1105274   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 16    |
|            | 0905068   | 0     | 0     | 0     | 0     | 0     | 1     | 1     |       | 15    |
|            | JE60      | 0     | 0     | 0     | 0     | 0     | 1     | 3     | 3     | 14    |
| EBOV Kikvit| 809066    | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 11    |
|            | 809120    | 0     | 0     | 0     | 0     | 0     | 1     | 2     |       | 17    |
|            | 809198    | 0     | 0     | 0     | 1     | 1     | 1     | 1     |       | 3     |
|            | 810158    | 0     | 0     | 0     | 0     | 1     | 1     |       | 10    |
|            | 805238    | 0     | 0     | 0     | 0     | 1     | 1     |       | 14    |
|            | 803056    | 0     | 0     | 0     | 0     | 0     | 1     | 1     |       | 10    |