PREVAIL IV: Double-blind, Randomized, Two-phase, Placebo-controlled, Phase II Trial of GS-5734 to Assess the Antiviral Activity, Longer-term Clearance of Ebola Virus, and Safety in Male Ebola Survivors with Evidence of Ebola Virus Persistence in Semen

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List of Abbreviations

AE	Adverse event/adverse experience
ALT	Alanine transaminase
ANR	Assay negativity rate (percentage of samples for which Ebola virus is not detected by polymerase chain reaction)
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
BMI	Body mass index
CC	Clinical Center
CFR	Code of Federal Regulations
CNERS	Comité National d'Ethique pour la Recherche en Santé (National Ethics Committee for Health Research of the Republic of Guinea)
CRF	Case report form
CSF	Cerebrospinal fluid
CSO	Clinical Safety Office
CT	Cycle threshold
DAIDS	Division of AIDS
DCR	Division of Clinical Research
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of the Congo
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EVD	Ebola virus disease
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GP	Glycoprotein
HRPP	Human Research Protections Program
IB	Investigator's brochure
ICH	International Conference on Harmonization
IM	Intramuscular
IND	Investigational new drug
INR	International normalized ratio
IRB	Institutional review board
ISM	Independent safety monitor
IV	Intravenous
LDL	Low-density lipoprotein
LFT	Liver function test
LIBR	Liberian Institute of Biomedical Research
LOD	Limit of detection

MDRD	Modification of Diet in Renal Disease
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NP	Nucleoprotein
NREB	National Research Ethics Board of Liberia
OAT	Organic anion transporter
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PREVAIL	Partnership for Research on Ebola Virus in Liberia
PT	Prothrombin time
PTM	Placebo to match
PTT	Partial thromboplastin time
RNA	Ribonucleic acid
ROS	Review of symptoms
SAE	Serious adverse event/serious adverse experience
SERF	Safety expedited report form
SBECD	Betadex sulfobutyl ether sodium
SMC	Safety monitoring committee
SMM	Study medical monitor
SOE	Schedule of events
SOP	Standard operating procedure
SRCP	Safety review and communication plan
SUSAR	Serious and unexpected suspected adverse reaction
ULN	Upper limit of normal
UP	Unanticipated problem
UPnonAE	Unanticipated problem that is not an adverse event
WHO	World Health Organization

Protocol Summary

Short Title:	PREVAIL IV
Clinical Phase:	II
Sample Size:	N=60 to 120
Accrual Ceiling:	N=120
Study Population:	Men ≥18 years of age with at least one of two semen samples demonstrating detection of Ebola virus RNA within 42 days prior to enrollment
Accrual Period:	June 2016-January 2021
Study Duration:	Start Date: 1 June 2016 End Date: 1 January 2021 Total length of individual subject participation: 6 to 7 months
Study Design:	Blinded, randomized, two-phase (treatment and longer-term follow-up), two-arm trial of GS-5734 versus placebo to evaluate the safety, tolerability, antiviral activity, and longer-term clearance of Ebola virus from male Ebola survivors with persistent Ebola virus in semen at screening.
Study Agent/ Intervention Description:	Daily GS-5734 or placebo delivered intravenously (IV) for 5 days.
Primary Objectives:	Treatment phase: To compare the antiviral activity over 28 days following the administration of 5 days of IV GS-5734 versus placebo in male Ebola Virus Disease survivors with evidence of Ebola virus RNA in their semen. Follow-up phase: To compare the effect of 5 days of IV GS-5734 versus placebo on the detection of Ebola virus RNA in semen of male survivors of Ebola Virus Disease over 24 weeks.

Secondary Objectives:

Treatment phase:

- To evaluate the safety and tolerability of GS-5734 in male Ebola survivors with evidence of Ebola virus RNA in their semen.
- To evaluate the pharmacokinetics of GS-5734 (and metabolites as appropriate) in blood and PBMCs.
- To compare the antiviral activity over 28 days following the administration of 5 days of IV GS-5734 versus placebo in subjects with: a CT value of either the GP or NP targets of ≤ 39 in at least one semen sample during the 42-day pre-screening period and subjects with one versus two positive semen samples during the 42-day screening period.

Follow-up phase:

- To evaluate 24-week safety outcomes following IV GS-5734 treatment in male Ebola survivors.
- To compare individual changes from baseline to 24-week signs and symptoms related to "Post Ebola Syndrome" as identified in PREVAIL III.
- To compare the effect of GS-5734 and placebo on change from baseline to 24-week signs and symptoms related to "Post Ebola Syndrome" identified in PREVAIL III.
- To compare the effect of IV GS-5734 versus placebo on the sustained negativity rate for the detection of Ebola virus RNA in semen of male survivors of Ebola Virus Disease over 24 weeks.

Exploratory Objectives:

- To compare the sensitivity of Ebola viral RNA detection using the GeneXpert standard versus "pellet" semen preparation methods.
- To compare changes from baseline in ophthalmologic findings between the GS-5734 arm and the placebo arm.
- To compare Ebola virus RNA sequence changes from screening semen samples to day 28 and week 24 semen samples when sample volume allows.

- To assess participant satisfaction during screening and study participation.

Primary Endpoints:

Treatment phase: Assay Negativity Rate (ANR) over days 4, 8, 11, 16, 24, and 28.

Follow-up phase: Assay Negativity Rate (ANR) over weeks 8, 12, 16, 20, and 24.

Secondary Endpoints:

Treatment phase:

- Proportion of Grade 1, Grade 2, Grade 3, or Grade 4 aspartate transaminase (AST) and alanine transaminase (ALT) levels at days 1, 2, 3, 4, 5, 8, 11, 16, 24, and 28.
- New Grade 3 and Grade 4 adverse events (AEs).
- Change from baseline in safety labs, including N(%) with an increase in grade.
- Change in targeted signs and symptoms (based on GS-5734 Phase I study AEs reported as associated with GS-5734 administration) including: constipation, nausea, vomiting, decreased appetite, headache, tremor, pain at infusion site, dyspepsia, and others new signs and symptoms reported by subjects.
- Incidence of SAEs.
- Frequency of discontinuation of study medication due to AEs or intolerance.
- Post-dose concentrations of GS-5734 and metabolites in serum on days 1 and 5.
- Pre-dose concentrations of GS-5734 and metabolites in serum on day 5.
- Steady-state PBMC-associated concentrations of GS-441524 and its phosphorylated metabolites on Day 5.

Follow-up phase:

- Sustained Negativity Rate at weeks 8, 12, 16, 20, and 24.
- Proportion of ALT or AST above baseline, at Grade 1 and above at weeks 8, 12, 16, 20, and 24.

- Incidence of serious adverse events (SAEs).
- Change from baseline and 24-week signs and symptoms identified as part of the "Post Ebola Syndrome" from PREVAIL III data on completed review of symptoms and physical exam, depression, and post-traumatic stress disorder screening.

Exploratory Endpoints:

- Proportion of Ebola virus RNA detection, either the GP or the NP gene targets, at all CT levels in semen.
- Average minimum CT levels at which Ebola virus RNA is detected for GP and NP gene targets in semen.
- Proportion of Ebola virus RNA detection or either GP or NP gene targets of CT $\geq$ 40.
- Proportion of abnormal ophthalmologic exams.
- Proportion of abnormal ophthalmologic exams with an inflammatory etiology at baseline which have improved or become worse between baseline and week 24.
- Proportion of Ebola virus RNA sequence changes in the polymerase and elsewhere between screening sample day 28 and 24-week semen samples.

Précis

With the unprecedented size of the 2014-2016 West African Ebola outbreak, the scientific community is learning a great deal about the psychological and physical consequences of Ebola, Ebola viral persistence in survivors, risk of Ebola disease relapse in survivors, and the potential for survivors to transmit the virus to others.

There are no licensed therapies for the treatment of Ebola virus disease nor for the clearance of persistent Ebola virus in survivors. A safe, effective therapy that can reduce and/or eliminate persistent Ebola virus from semen would reduce the risk of transmission and enable male survivors to resume normal sexual relations without fear of harming loved ones. The mechanism underlying Post-Ebola Syndrome is as yet unknown, but improvement in Post-Ebola signs and symptoms resulting from GS-5734 treatment would be an added benefit.

This study is a double-blind, randomized, two-phase (treatment and longer-term follow-up), two-arm trial of GS-5734 versus placebo among male Ebola survivors with persistent Ebola virus RNA in their semen. Participants are randomized 1:1 to receive either 100 mg of GS-5734 or placebo once daily by intravenous catheter for 5 days. Informed by transaminase elevations in prior Phase I studies in normal healthy subjects, a risk-mitigation strategy includes a built-in dose de-escalation. Participants will be stratified by country and on the basis of one versus two positive semen samples for Ebola virus RNA using the Cepheid GeneXpert platform assessed within 42 days prior to study enrollment. The early DSMB review in August 2016 concluded there was no need for a cohort dose reduction. The protocol expanded to Guinea in October 2017, where the outbreak ended later. Currently there is an outbreak in the Democratic Republic of the Congo where the study team may evaluate conducting this study following the completion of the outbreak.

Antiviral activity, as well as safety and tolerability, will be assessed during the treatment phase. Longer-term clearance of Ebola virus will be assessed during the week 8 to week 24 follow-up phase. Primary analyses for the assessment of antiviral activity in the treatment phase will focus on the assay negativity rate (ANR; percentage of genital samples that are negative for Ebola) over the first 28 days of the study, as well as clinical and laboratory adverse events. A sample is considered negative by PCR if the result of GeneXpert analysis reads undetectable. Primary analysis for the follow-up phase will focus on the ANR collected monthly from months 2 to 6.

1 Background Information and Scientific Rationale

1.1 Background Information

The Zaire Ebola virus disease (EVD) outbreak in West Africa was first recognized in December 2013 in Guinea. Over the course of the outbreak, EVD spread to Liberia, Sierra Leone, Nigeria, Mali, Spain, Senegal, and the US, with over 28,000 cases of EVD in West Africa.[1] Although all three West African countries have been declared Ebola-free at times, regional flare-ups of EVD, twelve at the time of this writing, continue in West Africa. Molecular sequencing is able to link these Ebola flares or clusters to transmission from survivors.

Ebola virus is a negative-strand RNA virus composed of 7 genes encoding viral proteins, including a single glycoprotein (GP). Five distinct species of Ebola virus have been identified: Bundibugyo (BDBV), Reston (RESTV), Sudan (SUDV), Taï Forest (TAFV), and Zaire (ZEBOV). BDBV, ZEBOV, and SUDV have been associated with large outbreaks of EVD in Africa with reported case fatality rates of up to 90%. Transmission of Ebola virus to humans is not yet fully understood, but is likely due to incidental exposure to infected animals. EVD then spreads through human-to-human transmission, with infection resulting from direct contact with blood, secretions, organs or other bodily fluids of infected people, and indirect contact with environments contaminated by such fluids.

EVD has an incubation period of 2 to 21 days (mean 4 to 10 days). The clinical manifestations of acute EVD are generally heralded by an abrupt onset of non-specific symptoms such as fever, chills, malaise, and myalgia.[2,3] The subsequent signs and symptoms indicate multisystem involvement and include systemic (prostration), musculoskeletal (arthralgia, myalgia) and gastrointestinal (anorexia, nausea, vomiting, abdominal pain, diarrhea). This may be followed by respiratory failure (chest pain, shortness of breath, cough), vascular complications (conjunctival injection, postural hypotension, edema), acute renal failure and neurological disease suggestive of a meningoencephalitis. Hemorrhagic manifestations consistent with disseminated intravascular coagulation may arise during the peak of the illness. In later stages of EVD, shock, convulsions, encephalopathy, severe metabolic disturbances, and diffuse coagulopathy may develop. Laboratory findings include leukopenia, thrombocytopenia, increased blood urea nitrogen, creatinine, creatinine kinase, and elevated liver enzymes. In general, symptoms last for about 7 to 14 days, after which either death or recovery occurs. The clinical manifestations of patients with EVD in the current epidemic in West Africa include hemorrhage in < 5% of patients.[4,5]

Due to the sporadic nature of prior outbreaks, the remote locations in which they occurred, high case-fatality rates, and few survivors, little was known about any long-term sequelae of EVD in survivors. In a study of 29 EVD survivors from the 1995 outbreak in Kikwit, DRC, the most common symptoms persisting during convalescence were arthralgia, myalgia, abdominal pain, and fatigue.[6] In a separate report from the Kikwit outbreak, 4 EVD survivors developed uveitis during convalescence.[7] Through PREVAIL III and other studies of survivors from the 2013-16 West African Ebola outbreak, the “Post Ebola Syndrome” is being further characterized.

Prior to the 2014-2016 Ebola outbreak, data on Ebola virus isolation and/or Ebola viral RNA detection from seminal fluid were limited. Ebola virus was isolated from semen at 61 days after onset of Ebola illness in a laboratory-acquired infection,[8] and Ebola RNA persistence was documented out to 91 days in one of five convalescent survivors in the Kikwit DRC outbreak.[6] Recent data from prospective studies from the West African 2014-2016 outbreak demonstrate that persistence of seminal Ebola viral RNA is common and can occur at high viral loads.[9,10] For example, a study in Sierra Leone recruited 93 male survivors between two and nine months after initial Ebola symptoms; Ebola virus RNA was detected in 65% of specimens 4 to 6 months after onset and in 26% 7 to 9 months after onset[11] but longer term longitudinal studies were needed to characterize the length and patterns of persistence.

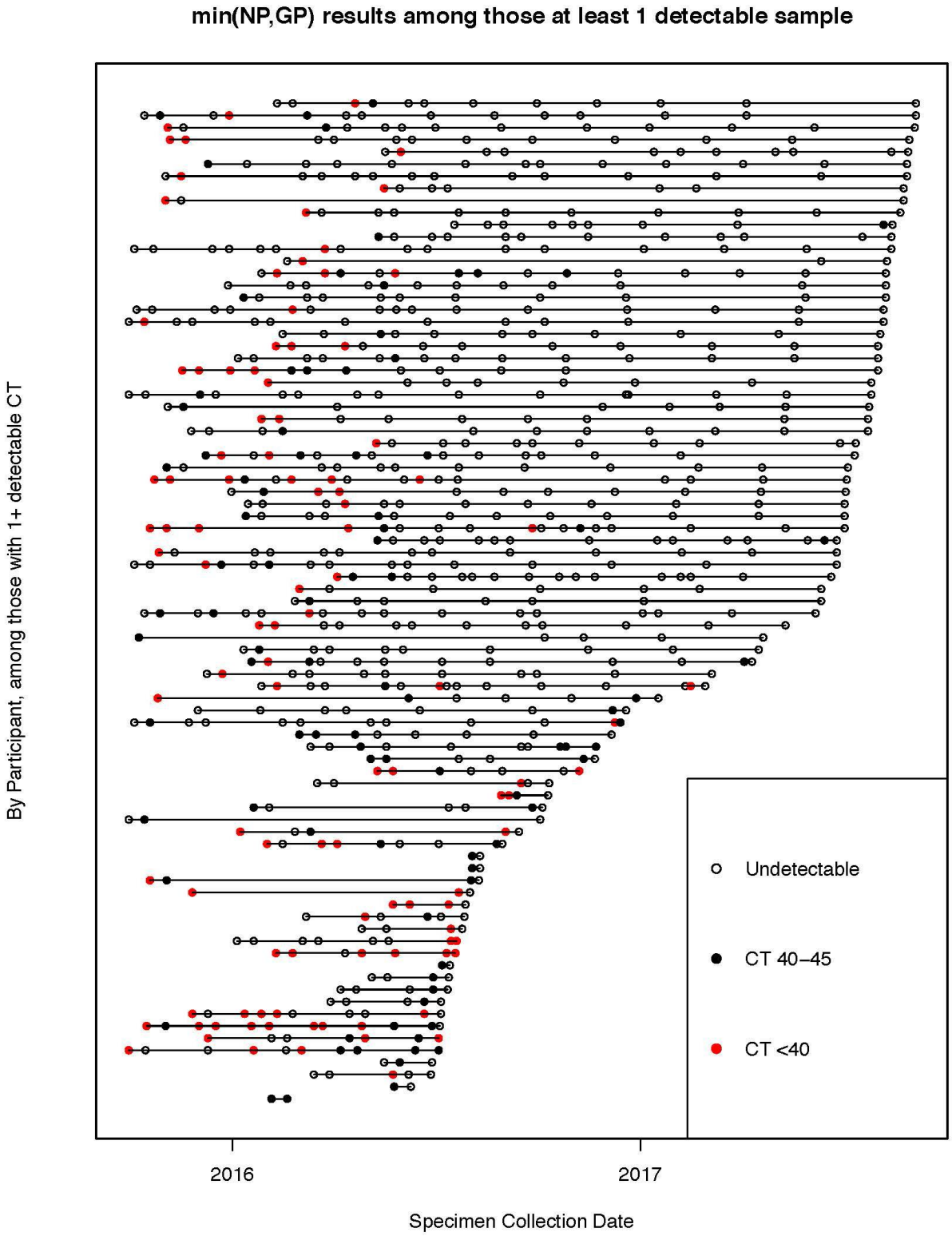
The Partnership for Research on Ebola Virus in Liberia (PREVAIL) is undertaking a 5-year study entitled Ebola Natural History Study in Liberia (also known as PREVAIL III), which began in Liberia in June 2015. As of September 2017, there were a total of 4036 subjects enrolled in PREVAIL III: 1145 survivors, 2785 close contacts, and 106 birth cohort participants. PREVAIL III plans and has enrolled of their household and sexual contacts; subjects are seen at baseline and every six months thereafter. The overall PREVAIL III goals are to characterize in Ebola survivors of all ages: the clinical sequelae, the immunology, viral persistence, and the potential for transmission of Ebola virus from EVD survivors to close contacts. Within PREVAIL III, sub-studies focus on the assessment of persistent virus in body fluids such as semen, vaginal fluid, and breast milk, a birth cohort (which involves testing of placenta, cord blood, maternal blood at birth, vaginal swabs, and breast milk), and sub-specialty areas such as neurology and ophthalmology.

To assess and characterize the persistence of Ebola viral RNA in semen, men enrolled in PREVAIL III are invited at their 6-month visit and beyond to undergo counseling and donate semen for testing of the presence of Ebola virus RNA. Semen testing is conducted at the Liberian Institute of Biomedical Research (LIBR) and the Guinean Viral Hemorrhagic Fever labs using the glycoprotein (GP) and nucleoprotein (NP) target genes on the Cepheid GeneXpert platform. The GeneXpert platform has shown superior sensitivity for detection of Ebola viral RNA, particularly in semen, at low levels of Ebola viral RNA, over other Taqman based assays in both published and unpublished studies.[12-14]

In the Liberian PREVAIL III study men with initial Ebola virus RNA detection are asked to continue to donate every two weeks, whereas men with an initial lack of detection, or a negative test, return to donate semen for testing every 4-6 weeks. Each time a subject donates semen, he returns for the test result and counseling on condom use within two weeks. At that time, he is advised on the frequency of follow-up. Upon repeat testing, approximately 30.7% of the 279 seropositive male survivors in PREVAIL III have had at least one semen sample with detection of Ebola virus RNA. Figure 1 summarizes the CT values for the 83 men who have had at least one semen sample with detection of Ebola virus, sorted by date of most recent specimen collection. Each individual participant is a row, with samples from the same individual connected by horizontal lines. White dots indicate samples where Ebola virus RNA was not detected; black dots indicate samples for which Ebola virus RNA was detected with a CT between 40 and 45; red dots indicate an observed CT value below 40. The displayed CT value is based on the minimum CT of NP or GP from the same sample. Of note, once a male has enrolled

in PREVAIL IV his data is no longer included in this plot, so it is slightly biased towards less positivity. As of August 2017, 257 male survivors enrolled in PREVAIL III had donated at least two semen samples for Ebola virus RNA testing. Seminal Ebola virus RNA shedding appears to be intermittent over time and quantitatively variable. The longest reported duration for detection of Ebola viral RNA in semen was 40 months following EVD.[15]

Figure 1. Minimum CT(NP,GP) results among those with at least 1 detectable sample from PREVAIL III (n=83)



The possibility of sexual transmission of filoviruses was first suggested by two cases in Marburg, Germany in 1968.[16] Based on data from the 2014-2016 outbreak,[17] it is now known that Ebola virus RNA in seminal fluid can be infectious, supporting previous molecular epidemiologic analysis of Ebola flares and cluster outbreaks suggesting that sexual transmission is taking place, though the frequency appears low. The first documented case of probable sexual transmission occurred in March 2015 in Liberia, from a male survivor to a female via unprotected vaginal intercourse, as evidenced by molecular sequencing.[18,19] Though the World Health Organization (WHO) declared Guinea Ebola-free on December 29th, 2015, after almost two challenging years of attempting to control the outbreak, a new EVD cluster began on March 17th, 2016, in NZerekore Prefectures, the Koropara sub-prefecture. This outbreak originated from a male survivor with low CT value in his semen who sexually transmitted the Ebola virus 470 days after his illness.[17] The outbreak included 10 persons in Guinea with a 90% mortality in confirmed cases and one death and two cases in Liberia.

Safely eradicating Ebola virus RNA from the semen of male survivors might reduce the risk of Ebola flares and another large-scale Ebola outbreak in Central and West Africa. The mechanism of persistence and location of ongoing replication of Ebola virus leading to detection of Ebola virus RNA in seminal fluid is not known. Finally, in addition to Ebola virus persistence, Ebola relapse causing meningoencephalitic clinical disease has been well documented.[20]

Since initiating the study on July 4, 2016, there have been 34 subjects enrolled in Liberia and no serious adverse events nor recommended changes to the protocol by the DSMB. The expansion to Guinea, where the outbreak ended later, is intended to complete study enrollment.

A focal group discussion about the PREVAIL IV study was held with male survivors from PREVAIL III with Ebola virus RNA detected in their semen. These men shared fears and concerns around transmitting Ebola virus to their sexual partners, frustrations of needing to use condoms when wanting to conceive children, and a desire to “be free” from Ebola virus.

1.2 Description of the Study Agent (GS-5734)

1.2.1 General Information

GS-5734 is a diastereomerically pure monophosphoroamidate prodrug of a modified adenine nucleoside analog GS-441524. GS-5734 exhibits a potential for in vivo clinical efficacy against Ebola virus infection in the treatment of EVD based on the following available data:

1) Potent in vitro activity against Ebola virus in multiple relevant cell types, including the Ebola virus variants isolated during the ongoing outbreak in West Africa.
2) Preclinical in vitro and in vivo pharmacokinetic profile in non-human primates and other relevant animal species indicating high and persistent levels of pharmacologically active nucleoside triphosphate metabolite in peripheral blood mononuclear cells (PBMCs); this measurement is used as a surrogate for levels in cells relevant for Ebola virus infection, supporting once daily IV or intramuscular (IM) administration.
3) Preliminary safety profile supporting safe administration to non-human primates at doses potentially active against Ebola virus infection.

4) Potent therapeutic efficacy in Ebola virus-infected rhesus monkeys, the most relevant in vivo preclinical model of EVD, at doses well tolerated in non-human primates. The in vivo therapeutic efficacy in non-human primates has been demonstrated against multiple Ebola virus variants including Makona/2014 isolated during the West African outbreak.
5) Tissue distribution studies in non-human primates indicate effective penetration and distribution of GS-5734 into immune privileged sites (genital tract, eye, and to some extent brain) that may represent a persistent reservoir of Ebola virus.

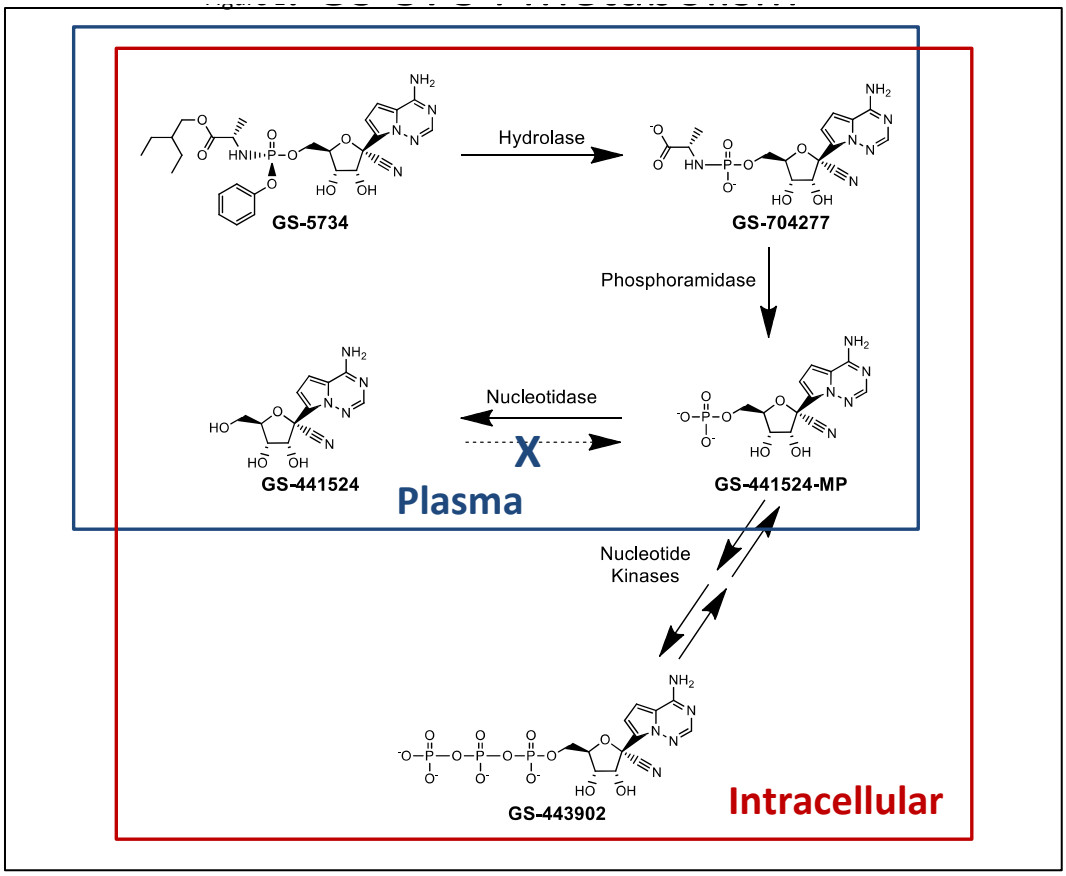
For further information on GS-5734 and the vehicle, refer to the current Investigator's Brochure (IB) for GS-5734.

1.2.2 Summary of Previous Preclinical Studies

1.2.2.1 Preclinical Pharmacology

GS-5734 has been designed as a prodrug for intracellular delivery of the monophosphate of GS-441524. Inside cells, the GS-441524 monophosphate undergoes fast conversion to the pharmacologically active triphosphate (GS-443902) see Figure 2.

Figure 2. GS-5734 Metabolism.



Efficient metabolism of GS-5734 to triphosphate GS-443902 has been demonstrated in multiple cell types relevant for Ebola virus replication. GS-443902 shows good intracellular persistence in primary human macrophage (T1/2 = 11 hours) supporting once daily administration. While there is no direct biochemical evidence for the inhibition of Ebola virus RNA-dependent RNA polymerase (RNA pol) by GS-443902, the compound selectively inhibits respiratory syncytial virus (RSV) RNA pol. The primary mechanism of inhibition is the incorporation of GS-443902 into nascent RNA chain by RSV RNA-dependent RNA-polymerases, causing premature viral RNA chain termination. RSV and Ebola virus share a similar mechanism of RNA transcription/replication and exhibit a relatively high degree of homology within the active sites of their respective RNA pols, suggesting that GS-443902 is likely to directly inhibit Ebola virus RNA pol. In contrast, GS-443902 inhibits neither human mitochondrial RNA polymerase nor human DNA polymerases α and γ.

GS-5734 is not suitable for oral delivery due to an extensive first-pass hepatic clearance. As a result, GS-5734 is being developed for parenteral administration. Multiple studies have been conducted to thoroughly characterize the pharmacokinetic profile of GS-5734 itself or the diastereomeric mixture GS-466547 administered IM or IV to rats, hamsters, and non-human primates. Across all species, the administered prodrug is rapidly cleared from plasma followed by the appearance of parent nucleoside GS-441524, which represents the major circulating metabolite (Figure 2). Dose-dependent rates of intact prodrug released into plasma were observed after IM administration in multiple species. In rhesus macaques, the preferred in vivo model for Ebola virus infection, once-daily IM administration of 5 mg/kg GS-466457 for 7 days resulted in trough levels of the nucleoside triphosphate GS-443902 in PBMCs above those required for 90% inhibition of Ebola virus replication in vitro. Relevant inhibitory concentrations of GS-443902 in PBMCs have also been observed in other species used as alternative models of Ebola virus infection (cynomolgus macaques and hamsters) following IM or IV administration of GS-466547.

Results obtained from a balanced excretion and tissue distribution studies in cynomolgus monkeys administered a single 10 mg/kg dose of [^{14}C] GS-5734 via the IV route indicate that GS-5734 penetrates into sanctuary sites including the brain, eye, and testes. Levels of radioactive material recovered from the testes, epididymis, and seminal vesicles were higher than plasma at 4 hours post administration, suggesting effective uptake of the drug. In addition, both GS-441524 and its phosphorylated metabolites were detected in semen of male monkeys following the single IV dose administration of 10 mg/kg GS-5734. Levels in the eye were approximately half of those in plasma at 4 hours. The amount of radioactive material recovered from plasma was approximately ten-fold higher than that in the brain at 4 hours. Levels in the brain were persistent with similar concentrations maintained through 168 hours, suggesting the potential for marked accumulation. Together, these results indicate effective penetration and distribution of GS-5734 into immune privileged sites that may represent a persistent reservoir of Ebola virus.

While GS-5734 is a substrate for CYP2C8, 2D6, 3A4 in vitro, co-administration with inhibitors of these CYP isoforms is unlikely to markedly increase GS-5734 levels as its metabolism is likely to be predominantly mediated by hydrolase activity. GS-5734 is a substrate for the organic anion transporter 1B1 (OATP1B1) and P-gp. However, the impact of these transporters on GS-5734 disposition is likely minimized by the parenteral route of administration. GS-5734 is an

inhibitor of CYP3A4, OATP1B1, and OATP1B3 in vitro but its potential to be the perpetrator of clinically significant drug-drug interactions is limited by its rapid clearance. No potential for induction of enzymes or transporters via the PXR or AhR was detected in reporter cell lines. If unanticipated drug-drug interactions are observed, the short-term course of GS-5734 treatment may allow for temporary discontinuation of other interacting drugs.

In summary, GS-5734 administered IV exhibits a favorable and consistent pharmacokinetic profile as well as efficient delivery of high levels of the pharmacologically active nucleoside triphosphate metabolite into cells relevant for Ebola virus replication, supporting clinical exploration of the drug as a novel agent for the treatment of EVD.

Further information is available in the IB.

1.2.2.2 Preclinical Efficacy

The in vivo efficacy of GS-5734 has been assessed in 2 studies in rhesus monkeys acutely infected with Ebola virus (variant Kikwit/1995), a high-mortality model that mimics the course of EVD in humans. GS-5734 administered IM at 3 to 10 mg/kg once daily following the onset of plasma viremia resulted in significantly prolonged survival time of Ebola virus-infected animals without a major impact on the symptoms of EVD. In an independent study, infected animals treated with 3 to 10 mg/kg of GS-5734 administered once daily by IV injection for 12 days beginning 3 days post Ebola virus inoculation exhibited 100% survival combined with a significant suppression of EVD clinical symptoms including amelioration of behavioral depression, changes in hematology and coagulation parameters, as well as serum chemistry markers of kidney and liver toxicity, and no signs of drug-related toxicities. In comparison, all vehicle-treated animals succumbed to Ebola virus infection by day 9.

The profound impact on survival and clinical symptoms of EVD in Ebola virus-infected drug-treated animals was due to the potent suppression of virus replication. Animals treated with 10 mg/kg GS-5734 beginning Day 3 post infection exhibited pronounced suppression of plasma viral RNA levels with a mean reduction of −3.9 log10 on Day 5 compared to the vehicle-treated control group. Plasma viral RNA in three out of six animals treated with 10 mg/kg GS-5734 decreased below the lower limit of quantification (LLOQ; 8×10^4 RNA copies/mL) by Day 5. By Day 12, all animals treated with 10 mg/kg GS-5734 had plasma viral RNA below the lower limit of detection for the RT-qPCR assay. Consistent with the profound suppression of Ebola virus replication, animals treated with 10 mg/kg GS-5734 showed minimal to no physical signs of EVD as indicated by low cumulative physical symptom scores. Fifty percent of animals treated with 10 mg/kg GS-5734 remained free of any physical signs of EVD throughout the 28-day duration of the study. No genotypic resistance was detected in vivo using deep sequencing of the entire EBOV RNA pol (L) gene from infected rhesus monkeys treated with various GS-5734 dosing regimens.

Similar efficacy of GS-5734 at 10 mg/kg associated with 100% survival rate and profound antiviral effect was also demonstrated in rhesus monkeys infected with Ebola virus isolated from the most recent West African outbreak (variant Makona/2014).

Further information is available in the IB.

1.2.2.3 Preclinical Toxicology

Parent nucleoside GS-441524 exhibits low in vitro cytotoxicity in several types of human cell lines and primary cells (CC50 = 74 to > 200 μM) and low potential for mitochondrial toxicity based on results from biochemical and cell-based assays.

The diastereomeric prodrug mixture, GS-466547 (GS-5734 and its diastereomer at phosphorous in a ~1:1 ratio), and the parent nucleoside, GS-441524, have a low potential for off-target activity. Neither compound significantly inhibited binding to a panel of 87 biological targets (receptors, ion channels, enzymes) at a concentration of 10 μM. No notable inhibition of the potassium (hERG) channel was observed with GS-466547 or GS-441524 at 30 μM. In Ames screening tests, GS-466547 and GS-441524 were not mutagenic. Based on these results, GS-5734 is considered non genotoxic.

Safety pharmacology studies were conducted to examine the potential effects of GS-5734 on the respiratory system, central nervous system, and cardiovascular system after IV administration. In a respiratory safety study in rats, GS-5734 had no effect on tidal volume or minute volume; however, respiration rates were increased from 0.75 to 6 hours post-dose in animals administered ≥ 20-mg/kg GS-5734. Respiration rates returned to control levels by 24 hours post-dose, resulting in a no observed effect level (NOEL) for respiratory function in male rats of 5 mg/kg, at exposures ~2.1-fold above the estimated GS-441524 C_{max} at the 150-mg clinical dose. Single IV (slow bolus) injection administration of GS-5734 had no effect on the central nervous system of rats at dose levels up to 50 mg/kg, the highest dose tested, and was estimated to be at least 17-fold above the GS-441524 C_{max} at the proposed 150-mg dose. In a cardiovascular safety study in monkeys, single IV (slow bolus) injection of GS-5734 had no effect on cardiovascular parameters up to 10 mg/kg, the highest dose tested. At the 10-mg/kg NOEL, GS-441524 exposures were ~2.5-fold above the C_{max} at the proposed 150-mg dose. The lack of in vivo cardiovascular effect is consistent with the weak in vitro inhibition of hERG channel by GS-5734. The concentration that results in 20% inhibition (IC20; 7.5 μM) and IC50 (28.9 μM) are at least 16-fold and 63-fold, respectively, above the estimated free drug concentration (0.458 μM) at C_{max} of the dose of 150 mg.

The nonclinical toxicology profile of GS-5734 has been characterized through the conduct of repeat-dose studies in rats and cynomolgus monkeys with once-daily dosing up to 2 weeks in duration, studies to evaluate the genotoxic potential of the compound, and a hemolysis/blood compatibility study. Following repeated dosing in rats and monkeys, the kidney was identified as the target organ. In both species, clinical chemistry, urinalysis, and/or urinary biomarkers were early predictors of the observed kidney changes.

In rats administered GS-5734 via daily IV (slow bolus) injection for 2 weeks, decreases in body weight gain and food consumption as well as clinical pathology and microscopic findings indicative of kidney injury and/or dysfunction in males administered ≥ 5 mg/kg/day and in females administered ≥ 20 mg/kg/day were observed. Effects on body weight gain and food consumption as well as clinical pathology and microscopic findings were generally reversible after a 4-week recovery period. Female rats were generally less sensitive to GS-5734, and the low dose of 5 mg/kg/day was considered the NOAEL in females.

In monkeys administered GS-5734 via daily IV (slow bolus) injection for 2 weeks, there were no changes suggestive of an effect in the kidney and the NOAEL was the high dose of 10 mg/kg/day. Cynomolgus monkeys administered the diastereomeric prodrug mixture GS-466547 once daily via IM injection for 7 days had clinical and anatomic pathology changes compatible with renal injury at 15 mg/kg/day; the NOAEL for systemic toxicity was 7.5 mg/kg/day.

The vehicle used in the IV repeat-dose toxicity studies and the hemolytic potential and plasma compatibility study contained 12% [w/v] betadex sulfobutyl ether sodium (SBECD) in water, pH 3.5 ± 0.1, similar to the vehicle for the proposed Phase 1 clinical study. The toxicity of SBECD has been well characterized in the GS-5734 toxicology studies, and in several peer-reviewed publications.[21-23] SBECD-related microscopic findings of diffuse tubule cell vacuolation and focal/multifocal tubule cell hypertrophy in the kidney of rats and monkeys were not considered adverse nor associated with any clinical pathology effects indicative of changes in kidney function, and have been previously described.[22,23] There was no notable exacerbation of the SBECD-related effects when administered with GS-5734.

Further information is available in the IB.

1.2.3 Summary of Relevant Clinical Studies

1.2.3.1 Phase 1 Single Ascending Dose Study GS-US-399-1812

In the placebo-controlled, double-blind, first-in-human study GS-US-399-1812, healthy volunteers were administered single doses of GS-5734 or vehicle in the following dose cohorts:

Table 1. Dosing cohorts in GS-US-399-1812.

Cohort	Subjects	Dose Level
1 (n = 10)	8 Active 2 PTM	3 mg GS-5734 or PTM
2 (n = 10)	8 Active 2 PTM	10 mg GS-5734 or PTM
3 (n = 10)	8 Active 2 PTM	30 mg GS-5734 or PTM
4 (n = 10)	8 Active 2 PTM	75 mg GS-5734 or PTM
5 (n = 10)	8 Active 2 PTM	150 mg GS-5734 or PTM
6 (n = 10)	8 Active 2 PTM	225 mg GS-5734 or PTM

Administration of single GS-5734 doses of 225 mg or less are complete. A summary of preliminary, unaudited, unblinded data from the study follows.

Sixty healthy adult males and nonpregnant, nonlactating females of nonchildbearing potential were enrolled in the study. Forty-eight subjects received GS-5734 and 12 subjects received placebo; all 60 subjects completed the study. Subjects had a median age of 49 years (range: 24-55 years). Fifty-five percent of subjects were male. Most subjects (90.0%) were white, and most were Hispanic or Latino (95%). The median (first, third quartile [Q1, Q3]) body mass index (BMI) was 27.4 (25.3, 29.0) kg/m^2, and the median (Q1, Q3) creatinine clearance was 112.98 (103.92, 126.33) mL/min.

A total of 13 of 48 subjects who received GS-5734 and 1 of 12 subjects who received placebo had ≥ 1 treatment-emergent adverse event (AE). No AE was reported for > 1 subject within an individual treatment group. Constipation was the only AE reported for > 1 subject in the study, occurring in 3 of 48 subjects who received GS-5734 (1 subject each in the 3, 30, and 225 mg groups) and 0 subjects who received placebo. Most AEs were Grade 1 (mild) in severity; 2 subjects, 1 who received GS-5734 75 mg and 1 who received placebo, had AEs (pre-syncope and pain in extremity, respectively) that were Grade 2 (moderate) in severity. Two subjects had AEs that were considered related to study drug by the investigator: One subject who received GS-5734 10 mg had dizziness and 1 subject who received GS-5734 225 mg had ear discomfort and pruritus generalized. All of these AEs were Grade 1 in severity, began on Day 1, and resolved on the same day.

No subject had a treatment-emergent laboratory abnormality of maximum severity Grade 4 during the study. One subject, who received GS-5734 225 mg, had a treatment-emergent Grade 3 laboratory abnormality, comprising a Grade 3 lipase value of 255 U/L on Day 2. This subject also had a Grade 1 amylase value (133 U/L) on Day 2. All other lipase and amylase values for this subject were within the normal range, and she had no other signs or symptoms of pancreatitis. The only treatment-emergent Grade 2 laboratory abnormalities observed in > 1 subject within a treatment group were elevated cholesterol (2 subjects GS-5734 3 mg, 1 subject GS-5734 30 mg, 1 subject placebo) and elevated low-density lipoprotein (LDL) cholesterol (3 GS-5734 3 mg, 1 GS-5734 75 mg, 1 GS-5734 225 mg, 1 placebo). All subjects with treatment-emergent Grade 2 elevated total and LDL cholesterol had Grade 1 elevations at ≥ 1 pre-dose time point. Elevated cholesterol also was the most common treatment-emergent Grade 1 laboratory abnormality observed during the study (2 subjects each in the GS-5734 3, 10, and 75 mg groups; 1 subject each in the GS-5734 30 and 150 mg groups; 0 subjects in the placebo group).

Three subjects who received GS-5734 had ALT elevations above the upper limit of normal (ULN) on Day 5, including 1 subject who received GS-5734 3 mg, 1 subject who received GS-5734 150 mg, and 1 subject who received GS-5734 225 mg. The subject who received GS-5734 150 mg also had an AST elevation above the upper limit of normal on Day 5. The elevated transaminase values were < 1.25 x ULN and, therefore, did not meet Grade 1 toxicity grading criteria. These transaminase elevations resolved to within the normal range by the time repeat testing was performed 2 days later (on Day 7). Follow-up visit (Day 14) results were consistent with pretreatment results for all 3 subjects. No notable changes from baseline were observed in total bilirubin, albumin, alkaline phosphatase, or international normalized ratio (INR) values for these subjects. No AEs were reported for the 3 subjects.

Overall, no consistent patterns of laboratory abnormalities or changes from baseline in laboratory parameters were noted during the study. Also, no patterns of clinically relevant changes in vital signs or shifts in 12-lead ECGs were observed during the study.

The plasma PK parameters of GS-5734 and the nucleoside analog metabolite GS-441524 after a single 3- to 225-mg dose of GS-5734 administered in the fasted state as a 2-hour IV infusion are presented in Table 2 and Table 3. These preliminary data indicate that GS-5734 and GS-441524 exposures were approximately dose-proportional following single-dose administration of GS-5734 3 to 225 mg. The peak plasma concentrations of GS-5734 and GS-441524 were achieved approximately 2 and 3.5 to 5 hours, respectively, after the start of infusion. GS-5734 had a short median $t_{1/2}$ of ≤ 1 hour, and GS-441524 had a longer median $t_{1/2}$ of approximately 13 to 31 hours across the studied dose range.

Table 2. GS-US-399-1812: GS-5734 Mean (%CV) Plasma PK Parameters Following a Single 2-hour IV Infusion of GS-5734 in Healthy Subjects (Cohorts 1-6; Analysis Set: PK)

PK Parameter	GS-5734 3 mg (n = 8)	GS-5734 10 mg (n = 8)	GS-5734 30 mg (n = 8)	GS-5734 75 mg (n = 8)	GS-5734 150 mg (n = 8)	GS-5734 225 mg (n = 8)
C_{max} (ng/mL)	57.5 (31.1)	220.8 (31.2)	693.9 (18.6)	1626.0 (38.6)	2280.0 (30.1)	4421.3 (16.0)
T_{max} (hr)[a]	2.03 (2.01, 2.04)	2.01 (2.00, 2.03)	2.02 (2.00, 2.03)	2.03 (2.03, 2.05)	2.00 (1.98, 2.04)	1.97 (1.95, 1.98)
$t_{1/2}$ (hr)[a]	-	0.66 (0.54, 0.79)	0.81 (0.61, 0.91)	0.90 (0.82, 1.07)	0.99 (0.92, 1.06)	1.05 (0.96, 1.21)
AUC_{inf} (hr•ng/mL)	-	230.0 (28.4)	773.9 (22.9)	1999.6 (27.1)	2976.1 (19.0)	5274.7 (11.6)

a Median (Q1, Q3)

Table 3. GS-US-399-1812: GS-441524 Mean (%CV) PK Plasma Parameters Following a Single 2-hour IV Infusion of GS-5734 in Healthy Subjects (Cohorts 1-6; Analysis Set: PK)

PK Parameter	GS-5734 3 mg (n = 8)	GS-5734 10 mg (n = 8)	GS-5734 30 mg (n = 8)	GS-5734 75 mg (n = 8)	GS-5734 150 mg (n = 8)	GS-5734 225 mg (n = 8)
C_{max} (ng/mL)	3.2 (10.9)	9.4 (28.4)	34.3 (30.9)	85.8 (23.9)	152.0 (23.6)	256.6 (30.2)
T_{max} (hr)[a]	5.00 (4.00, 5.00)	3.57 (3.00, 5.00)	4.00 (3.25, 4.00)	4.50 (3.50, 5.00)	4.00 (3.50, 4.00)	3.50 (3.00, 4.00)
$t_{1/2}$ (hr)[a]	12.89 (7.35, 14.23)[b]	22.01 (19.83, 25.30)	27.33 (22.68 29.62)	26.88 (25.44, 28.90)	27.38 (25.94, 28.77)	30.59 (29.46, 31.13)
AUC_{inf} (hr•ng/mL)	55.2 (27.6) [b]	264.0 (26.7)	1013.8 (31.1)	2471.8 (22.7)	4642.5 (16.2)	7348.6 (20.7)

a Median (Q1, Q3)
b n = 5

The urinary excretion of GS-5734 as unchanged drug and as the GS-441524 metabolite was also evaluated in this study for 48 hours from the start of study drug infusion. Following administration of single 3- to 225-mg doses of GS-5734, < 10% of the dose was recovered in the urine as unchanged drug and approximately 34% to 38% of the dose was recovered as GS-441524 over 48 hours.

Exposure in PBMCs following GS-5734 administration was assessed in this study by measuring total GS-441524 levels, which serve as a surrogate for total intracellular phosphorylated species of GS-441524. Detectable GS-441524 levels were present in PBMCs after GS-5734 administration at all doses tested (3 to 225 mg). The median T_{max} of GS-441524 in PBMCs was variable, ranging from 2 to 9 hours. GS-441524 exposure in PBMCs was persistent for ≥ 144 hours (6 days; median $t_{1/2}$ ~38 hours), and a concentration of ≥ 5 μM was maintained for the first 24 hours following a single 150-mg dose.

1.2.3.2 Phase 1 Multiple Dose Study GS-US-399-1954

Study GS-US-339-1954 is an ongoing (study conduct complete, data analysis ongoing) randomized, blinded, placebo-controlled, Phase 1 study designed to evaluate the safety and tolerability of multiple IV doses of GS-5734 compared with placebo and to evaluate the PK of GS-5734 and its metabolites following multiple IV doses of GS-5734 in healthy adult subjects. The study was conducted at a single center in the United States.

Two dose cohorts were evaluated in the study. Following screening and Day −1 procedures, eligible subjects were confined to the study center beginning on Day −1 and were randomized 2:1 within each cohort to receive GS-5734 150 mg or matching placebo, respectively. Each cohort comprised 2 groups (Groups 1a and 1b and Groups 2a and 2b) of 6 subjects each (4 GS-5734, 2 placebo). Each subject received GS-5734 or placebo administered IV over a 1-hour period once daily for 7 days in Cohort 1 and 14 days in Cohort 2. Subjects were discharged on Day 9 for Cohort 1 and Day 16 for Cohort 2 and then returned 7 days after discharge for an in-clinic follow-up visit.

Twenty-four healthy subjects were enrolled in the study. Sixteen subjects received > 1 dose of GS-5734 and 8 subjects received > 1 dose of placebo. Twenty-two subjects completed the study. One subject in Cohort 1 who received GS-5734 and 1 subject in Cohort 2 who received placebo discontinued study drug prematurely, both after the fourth dose. Subjects had a median age of 47 years (range: 19-55 years). Fifty-eight percent of subjects were male. Most subjects (83.3%) were white, and all were Hispanic or Latino. The median (Q1, Q3) BMI was 27.5 (25.8, 28.2) kg/m^2, and the median (Q1, Q3) estimated glomerular filtration rate (by the Cockcroft-Gault equation) was 107.96 (101.38, 129.75) mL/min.

No Grade 3 or 4 AEs, SAEs, AEs leading to study drug or study discontinuation, or deaths were reported during the study. However, 1 subject who received GS-5734 discontinued study drug after having multiple AEs considered to be related to study drug by the investigator, as described further below. The only AEs reported for > 1 subject in a treatment group were constipation (3 subjects [37.5%] GS-5734 7-day, 0 subjects GS-5734 14-day, 0 subjects placebo) and

dyspepsia (1 [12.5%] GS-5734 7-day, 2 [25.0%] GS-5734 14-day, 0 placebo). All of the AEs reported during the study were Grade 1 in severity.

Nine subjects, 6 of whom received GS-5734 and 3 of whom received placebo, had ≥ 1 AE that was considered related to study procedures by the investigator. The AEs included pain in extremity (1 subject GS-5734 7-day, 2 subjects GS-5734 14-day); contact dermatitis (1 GS-5734 7-day, 1 placebo); pruritus (1 GS-5734 7-day, 1 placebo); infusion site extravasation, infusion site pain, and infusion site hemorrhage (each 1 GS-5734 7-day); ecchymosis (1 GS-5734 14-day); and dermatitis and iron deficiency anemia (each 1 placebo). The start dates of the procedure-related AEs ranged from Day 1 to Day 15. Time to resolution ranged from < 1 day to 52 days.

Four subjects, 3 of whom received GS-5734 and 1 of whom received placebo, had AEs that were considered related to study drug by the investigator: One subject in the GS-5734 7-day group had constipation, nausea, vomiting, decreased appetite, headache, and tremor. This subject discontinued study drug after dosing on Day 4 (reported reason for discontinuation was subject decision). The nausea, vomiting, headache, and tremor (left leg and left hand) all started on Day 3; the tremor resolved on the same day, and the other 3 AEs resolved between Days 4 and 7. The decreased appetite started on Day 5 and resolved on Day 7, and the constipation started on Day 8 and resolved on Day 9. Two subjects in the GS-5734 14-day group had dyspepsia that was considered related to study drug by the investigator. The dyspepsia started on Day 4 for both subjects and resolved on Day 19 for 1 subject and Day 20 for the other subject. One subject in the placebo group had diarrhea that was considered related to study drug by the investigator. The diarrhea started on Day 7 and resolved on Day 12.

No subjects had a Grade 3 or 4 treatment-emergent laboratory abnormality during the study. Four subjects had treatment-emergent ALT elevations of maximum toxicity Grade 2, and an additional 5 subjects had ALT elevations of maximum toxicity Grade 1. These 9 subjects with graded ALT elevations included 2 subjects in the GS-5734 7-day group, 6 subjects in the GS-5734 14-day group, and 1 subject in the placebo group. Overall, 14 of 16 subjects who received GS-5734 (6 in the 7-day group and all 8 in the 14-day group) and 2 of 8 subjects who received placebo had a post-dose increase in ALT to a value ≥ 1.5 × their pre-dose ALT value. The median time to onset for the ALT elevations for these 16 subjects was 6 days, with a range of 5 to 23 days. The median time to resolution of the ALT elevation to < 1.5 the pre-dose value was 14 days, with a range of 3 to 64 days.

Among the 9 subjects with Grade 1 or 2 ALT elevations, 7 (1 in the GS-5734 7-day group and 6 in the GS-5734 14-day group) also had concomitant elevated AST levels. The start dates of the graded ALT and AST elevations varied, ranging from 4 to 24 days after the day of first dose administration. The ALT and AST values returned to within the normal range during the study for all 9 subjects. None of the 9 subjects with graded ALT or AST elevations had abnormalities in total bilirubin, alkaline phosphatase, or albumin during the study. Seven of the subjects also had Grade 1 increased prothombin time (PT), which resolved to within the normal range during the study for all of the subjects. No clinically significant change in INR occurred in the 7 subjects. None of the laboratory results for the 9 subjects with transaminase elevations indicated systemic sign of drug reaction.

Four of the subjects with treatment-emergent graded transaminase elevations had AEs on study: One subject was the subject in the GS-5734 7-day group described above with the treatment-related AEs (constipation, nausea, vomiting, decreased appetite, headache, and tremor) who discontinued study drug after the fourth dose. She also had non-treatment-related AEs of pain in extremity (a study procedure-related AE), tremor (bilateral hand and left leg), back pain, hematochezia, hemorrhage, viral infection, and oral herpes. This subject had a peak ALT value of 123 U/L and a peak AST value of 112 U/L, both occurring on Day 7. Another subject, also in the GS-5734 7-day group, had a non-treatment-related AE of constipation. The third subject, who was in the GS-5734 14-day group, had non-treatment-related AEs of ECG T wave inversion (considered not clinically significant by the investigator) and pain in extremity (left and right antecubital pain). The fourth subject, also in the GS-5734 14-day group, had a treatment-related AE of dyspepsia and a non-treatment-related AE of dizziness. These latter 3 subjects did not discontinue study drug or the study.

The only other treatment-emergent Grade 2 laboratory abnormality observed in > 1 subject in a treatment group was elevated total cholesterol, which was observed in 2 subjects in the placebo group, both of whom had pre-dose Grade 1 elevations. Of note, Grade 1 laboratory abnormalities of increased PT occurred in 10 of 16 subjects who received GS-5734 (4 in the 7-day group, 6 in the 14-day group); no subjects who received placebo had Grade 1 increased PT. For all but 2 of these subjects, the increases were observed at multiple post-dose time points. The increases resolved to within the normal range during the study for all of the subjects. As described above, 7 of the 10 subjects also had graded transaminase elevations during the study. No clinically significant change from baseline in INR was observed in any of the treatment groups; the INR ranged from 0.9 to 1.3 across all subjects and time points.

Overall, no other consistent patterns of laboratory abnormalities or changes from baseline in laboratory parameters were noted during the study. Also, no patterns of clinically relevant changes from baseline in vital signs or shifts in 12-lead ECGs were observed during the study.

The plasma PK parameters of GS-5734, the nucleoside analog metabolite GS-441524, and the intermediate metabolite GS-704277 after multiple 150-mg doses of GS-5734 administered in the fasted state as 1-hour IV infusions over 7 (Cohort 1) or 14 days (Cohort 2) are presented in Table 4, Table 5, and Table 6. Consistent with a short $t_{1/2}$ (~1 hour) relative to the once-daily dosing, accumulation of GS-5734 did not occur. The nucleoside analog metabolite GS-441524 had a longer $t_{1/2}$ of approximately 21 hours, with an accumulation ratio for AUC after multiple daily dosing of approximately 1.9, reaching steady-state by Day 7; no further accumulation was observed from Days 7 to 14. The intermediate metabolite GS-704277 had a $t_{1/2}$ of approximately 1.6 hours. Slight accumulation of this metabolite was observed after 7 days of dosing (accumulation ratio for AUC of 1.4), and further accumulation was observed after 14 days of dosing (accumulation ratio of ~1.9). The mechanism of this accumulation is currently unknown.

Table 4. GS-US-399-1954: GS-5734 Mean (%CV) Plasma PK Parameters Following Multiple 1-hour IV Infusions of GS-5734 150 mg in Healthy Subjects (Cohorts 1 and 2; Analysis Set: PK)

PK Parameter	GS-5734 Day 1 (n = 8)	GS-5734 Day 7 (n = 8)	GS-5734 Day 14 (n = 8)
Cohort 1 (7 days dosing)			
C_{max} (ng/mL)	2922.5 (21.4)	3048.6 (21.2)	NA
T_{max} (hr)[a]	1.01 (0.50, 1.05)	1.05 (1.02, 1.05)	NA
$t_{1/2}$ (hr)[a]	0.93 (0.79, 0.99)	0.97 (0.94, 1.10)	NA
AUC_{tau} (hr•ng/mL)	2347.1 (19.2)	2476.0 (19.9)	NA
Cohort 2 (14 days dosing)			
C_{max} (ng/mL)	3413.8 (26.2)	3197.5 (15.5)	3380.0 (23.5)
T_{max} (hr)[a]	1.04 (1.03, 1.05)	1.05 (1.03, 1.07)	1.05 (1.05, 1.05)
$t_{1/2}$ (hr)[a]	0.83 (0.78, 0.91)	1.00 (0.91, 1.03)	1.03 (0.93, 1.14)
AUC_{tau} (hr•ng/mL)	2803.6 (17.8)	2731.3 (16.9)	2858.7 (20.2)

NA = not applicable
a Median (Q1, Q3)

Table 5. GS-US-399-1954: GS-441524 Mean (%CV) PK Parameters Following Multiple 1-hour IV Infusions of GS-5734 150 mg in Healthy Subjects (Cohorts 1 and 2; Analysis Set: PK)

PK Parameter	GS-441524 Day 1 (n = 8)	GS-441524 Day 7 (n = 8)	GS-441524 Day 14 (n = 8)
Cohort 1 (7 days dosing)			
C_{max} (ng/mL)	146.4 (25.9)	231.7 (17.7)	NA
T_{max} (hr)[a]	3.00 (2.00, 4.00)	3.00 (2.00, 4.00)	NA
$t_{1/2}$ (hr)[a]	23.10 (17.14, 24.40)	23.69 (21.56, 23.84)	NA
AUC_{tau} (hr•ng/mL)	1996.3 (15.4)	3642.1 (16.3)	NA
Cohort 2 (14 days dosing)			
C_{max} (ng/mL)	130.8 (22.0)	230.0 (19.4)	230.5 (21.1)
T_{max} (hr)[a]	4.00 (2.50, 4.00)	3.50 (2.50, 4.00)	2.75 (2.00, 3.50)
$t_{1/2}$ (hr)[a]	16.39 (15.08, 21.80)	24.06 (20.25, 33.32)	21.44 (20.23, 22.00)
AUC_{tau} (hr•ng/mL)	1903.1 (16.7)	3599.5 (15.4)	3620.7 (15.9)

NA = not applicable
a Median (Q1, Q3)

Table 6. GS-US-399-1954: GS-704277 Mean (%CV) PK Parameters Following Multiple 1-hour IV Infusions of GS-5734 150 mg in Healthy Subjects (Cohorts 1 and 2; Analysis Set: PK)

PK Parameter	GS-441524 Day 1 (n = 8)	GS-441524 Day 7 (n = 8)	GS-441524 Day 14 (n = 8)
Cohort 1 (7 days dosing)			
C_{max} (ng/mL)	290.6 (21.7)	386.1 (31.6)	NA
T_{max} (hr)[a]	1.05 (1.03, 1.06)	1.07 (1.02, 1.17)	NA
$t_{1/2}$ (hr)[a]	1.41 (1.19, 1.73)	1.75 (1.37, 1.97)	NA
AUC_{tau} (hr•ng/mL)	563.1 (21.1)	753.7 (29.0)	NA
Cohort 2 (14 days dosing)			
C_{max} (ng/mL)	274.3 (20.7)	387.6 (26.3)	525.4 (23.0)
T_{max} (hr)[a]	1.05 (1.03, 1.11)	1.17 (1.07, 1.17)	1.17 (1.05, 1.17)
$t_{1/2}$ (hr)[a]	1.68 (1.53, 1.75)	1.68 (1.43, 1.83)	1.61 (1.47, 1.80)
AUC_{tau} (hr•ng/mL)	549.9 (22.8)	789.3 (29.4)	1077.8 (26.4)

NA = not applicable
a Median (Q1, Q3)

Following administration of multiple 150-mg doses of GS-5734 for 7 or 14 days, approximately 8% and 12%, respectively, of the dose was recovered in the urine as unchanged drug and approximately 41% and 48%, respectively, of the dose was recovered as GS-441524 over the 24-hour collection period. The levels of GS-441524 persisted throughout the 24 hour-collection period in all subjects after 7 and 14 days of dosing.

The $t_{1/2}$ of total GS-441524 phosphorylated metabolites in PBMCs was approximately 40 hours (Table 7). Upon multiple once-daily dosing, the accumulation of GS-441524 in PBMCs was approximately 2.6- to 3.5-fold.

Table 7. GS-US-399-1954: Intracellular GS-441524 Phosphorylated Metabolites Mean (%CV) PK Parameters in PBMC Following Multiple 1-hour IV Infusions of GS-5734 150 mg in Healthy Subjects (Cohorts 1 and 2; Analysis Set: PK)

PK Parameter	GS-5734 Day 1 (n = 8)	GS-5734 Day 7 (n = 8)	GS-5734 Day 14 (n = 8)
Cohort 1			
C_{max} (ng/mL)	2.8 (20.5)	12.5 (20.7)	NA
T_{max} (hr)[a]	6.00 (1.03, 9.00)	6.00 (1.00, 12.00)	NA
$t_{1/2}$ (hr)[a]	35.26 (33.82, 36.70)	37.11 (32.22, 40.07)	NA
AUC_{tau} (hr•ng/mL)	90.9 (20.1)	245.1 (18.9)	NA

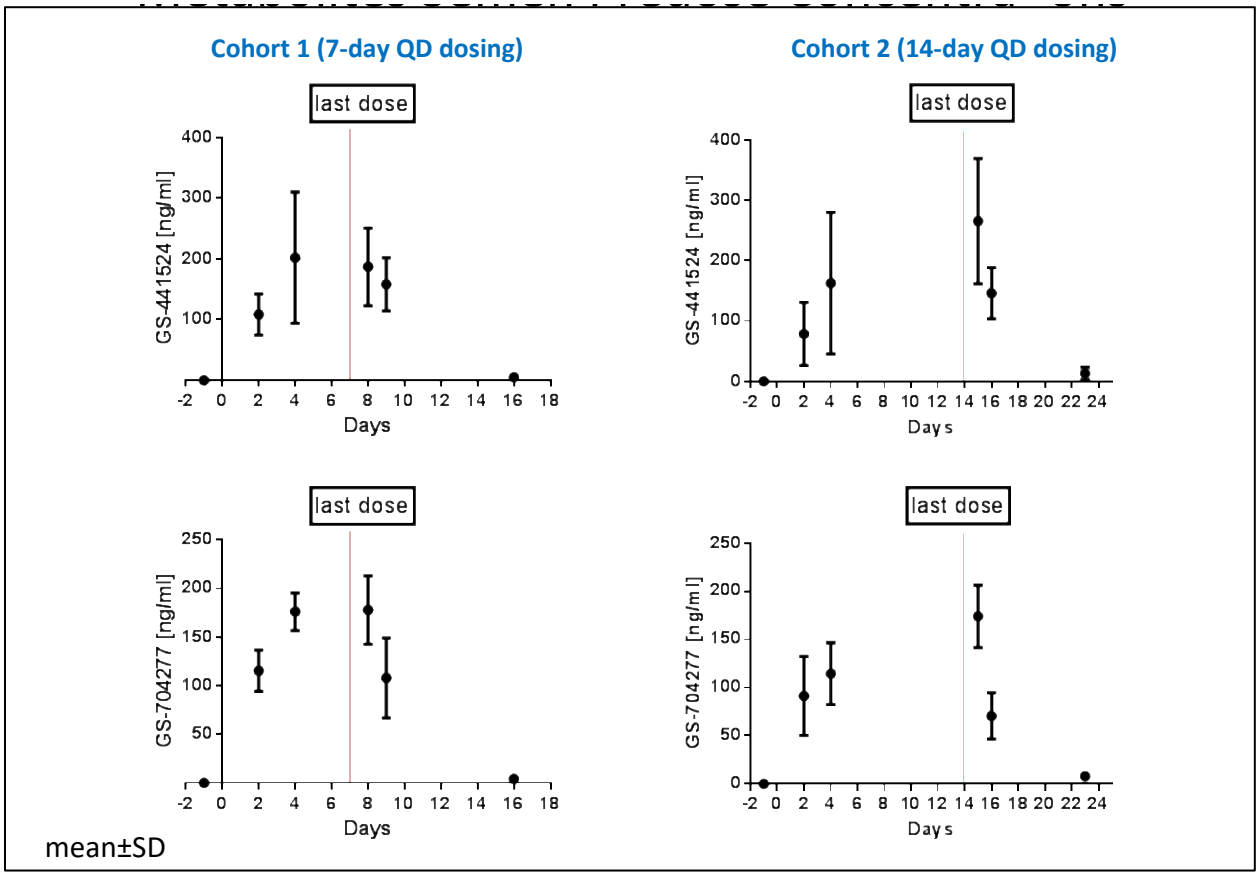
Cohort 2			
C_{max} (ng/mL)	4.2 (31.4)	22.7 (38.2)	21.6 (32.4)
T_{max} (hr)[a]	1.04 (1.03, 3.53)	1.06 (1.05, 1.07)	1.06 (1.05, 6.00)
$t_{1/2}$ (hr)[a]	36.36 (35.97, 41.81)	34.38 (34.38, 34.38)	40.16 (28.35, 56.14)
AUC_{tau} (hr•ng/mL)	107.4 (17.9)	392.2 (20.5)	388.0 (33.8)

NA = not applicable
a Median (Q1, Q3)

1.2.3.3 Pharmacokinetic Semen Data from the Phase 1 Multiple Dose Study GS-US-399-1954

Parent drug GS-5734 is undetectable in semen (BLQ) due to its short half-life. Levels of pre-dose (trough) GS-441524 in semen reaches 200–250 ng/mL after multiple dosing. The C_{trough} of GS-441524 in plasma ~110 ng/mL at steady-state. The semen C_{trough} levels were approximately 200 ng/mL at steady state which is about 2x higher than plasma C_{trough} (~111 ng/mL) at steady-state. Both GS-441524 and GS-704277 metabolites were present in semen for up to 7 days after the last dose.

Figure 3. Metabolites: Semen Predose (C_{trough}) Concentrations.



1.2.4 Additional GS-5734 Human Experience

To date, GS-5734 has been administered on an expanded access basis to 2 patients with Ebola infection, a 39-year-old female diagnosed with recrudescent Ebola meningitis and a neonatal patient with acute Ebola infection. These patients are described below.

Recrudescent Ebola Meningitis Case

This 39-year-old female was originally treated in the United Kingdom for acute EVD in December 2014 and January 2015. She subsequently recovered from her acute EVD and remained in the United Kingdom. In early October 2015, the patient presented with malaise, headache, photophobia, nausea, and vomiting. She underwent lumbar puncture, which revealed a pleocytosis, and PCR tests were positive for EBOV in her blood and cerebrospinal fluid (CSF) (cycle thresholds [Cts] of 29 and 23.7, respectively). She was diagnosed with Ebola meningitis and admitted for inpatient treatment on 08 October 2015. GS-5734 was requested for treatment of her case on 09 October 2015.

Therapy with GS-5734 150 mg IV once daily (every 24 hours) was initiated on 11 October 2015. EBOV PCR of blood became negative on 12 October 2015, after the first dose of GS-5734 (Ct > 40). On the basis of a lack of evident toxicity, both in the patient's case and in the ongoing single ascending dose Study GS-US-399-1812, the dose was increased to 225 mg IV once daily on 13 October 2015. The patient received daily GS-5734 225 mg IV for an additional 12 days without incident. She completed a 14-day course of GS-5734 on 24 October 2015 (2 days of 150 mg IV once daily plus 12 days of 225 mg IV once daily). Examination of CSF on 21 October 2015 (Day 11 of GS-5734 therapy) demonstrated a decrease in EBOV PCR from a pretreatment Ct of 23.7 to a Ct of 35.5 on 21 October 2015. EBOV PCR of CSF obtained on 28 October 2015, 4 days after GS 5734 was discontinued, was negative (Ct > 40). Per communication with the treating physician, the patient tolerated GS-5734 well. She experienced no AEs related to GS-5734 during her treatment, and there were no apparent laboratory, vital sign, or ECG abnormalities attributed to GS-5734. Her neurologic status and the symptoms and signs of Ebola meningitis were resolving as of 20 November 2015.

Acute Ebola Virus Case

This neonatal black female was born on 27 October 2015 to an Ebola-infected mother. The patient's mother arrived on 23 October 2015 for treatment at the Nongo Ebola treatment center (ETC) in Conakry Guinea, operated by Médecins Sans Frontières. The patient's 2 siblings also presented with symptoms of acute EVD on the same day and were transferred to a different facility for treatment. The family had recently been exposed to Ebola as a result of contact with a family member who died due to EVD as part of the Forecariah branch of the Ratoma Ebola transmission chain in Guinea [24]. The patient's mother self-reported a pregnancy of 7 months gestation at the time of admission to the Nongo ETC. The mother was diagnosed with EVD on 24 October 2015 and had a high viral load (Ct = 14.9, PCR method not specified). She delivered an infant girl in the ETC on 27 October 2015 and subsequently died a few hours later.

Upon delivery on 27 October 2015, the neonate appeared mature and weighed 3 kg, consistent with a term or near-term gestation. The patient's capillary and umbilical blood was positive for EBOV by PCR on her first day of life, and she was diagnosed with EBOV infection as a result of

transplacental transmission. Because of the poor prognosis for neonatal EVD (100% case fatality reported), ZMapp™ was obtained for the patient on a compassionate use basis. The patient was treated with 4 ZMapp 150-mg infusions between 28 October and 06 November 2015 (Day 2 to Day 11 of life). Because she had persistent Ebola viremia and there was a concern for Ebola CNS disease, the patient was then treated with a 12-day course of GS 5734 10 mg IV daily from 14 to 25 November 2015 (Day 19 to 30 of life). Blood, skin, urine, and salivary specimens were consistently negative by PCR for EBOV following initiation of GS-5734 therapy. She had no apparent AEs or evidence of laboratory abnormalities related to GS-5734 administration. The patient recovered and was discharged from the treatment center on 28 November 2015.

1.3 Rationale for Study

Ebola virus persists in at least a proportion of survivors of EVD. Viable Ebola virus has been recovered from the ocular fluid of an expatriate survivor months after clearance of viremia.[25] Moreover, Ebola virus was detected by PCR in the semen of a substantial proportion of 100 male survivors in Sierra Leone, 65% at 4-6 months and 26% at 7-9 months,[11] and 33-38% of PREVAIL III male participants with repeat semen sample test results after 6 months of participation in PREVAIL III.[26] In October 2015, a female expatriate survivor who had recovered from EVD over 9 months earlier with documented clearance of viremia, experienced an acute febrile illness and meningoencephalitis with high levels of Ebola virus detected in her cerebrospinal fluid and blood plasma. This case suggests that persistence of Ebola virus in non-circulatory compartments may be driving lingering symptoms associated with EVD convalescence, be a source for secondary transmission, and serve as a reservoir which can lead to recrudescence of severe EVD.

Ebola cluster outbreaks continue to occur in Liberia, Sierra Leone, and Guinea, with molecular evidence of transmission documented from the Ebola survivor community. Epidemiologic and molecular sequencing data support male survivor sexual transmission to susceptible females. Though sexual transmission appears to be uncommon, it has been responsible for at least a portion of the 12 Ebola cluster outbreaks that WHO attributes to transmission from survivors to others in their communities. During a period when few cases of primary EVD are being reported, a study of GS-5734 in EVD male survivors with evidence of Ebola virus persistence in semen provides an important opportunity to study the antiviral activity, longer-term genital clearance, safety, and pharmacokinetics of GS-5734. Furthermore, the effect of this agent on the dynamics of residual virus persisting in compartments such as the genital tract can be explored as can changes in clinical symptoms associated with the Post-Ebola Syndrome.

Thorough characterization of the safety and tolerability of GS-5734 will also inform future study and use of this agent in Sub-Saharan Africa. Demonstration of an antiviral effect of GS-5734 in EVD survivors with detectable Ebola virus in genital fluids would support the use of this agent in this population and may be an important tool in preventing recurrent Ebola virus outbreaks in West Africa.

While supportive care and treatment of specific symptoms improves survival, there currently are no effective therapeutic agents available for the treatment of EVD. The availability of an effective antiviral agent with a favorable risk/benefit profile would address a serious unmet medical need for the treatment of adults and children infected with Ebola virus.

1.4 Rationale for Dose Selection

The dose selection of 100 mg IV/day for 5 days, with a built-in dose de-escalation, is based upon safety data from the GS-5734 phase I studies in normal human subjects, the clinical pharmacokinetic profile in plasma and semen, and written guidance from the Unites States Food and Drug Administration (FDA). In late March 2016, the FDA reviewed the data from the Gilead Sciences Phase I studies GS-US-399-1812 and GS-US-399-1954. In GS-US-399-1812, the single ascending dosing study, three subjects (one subject each receiving 3 mg, 150 mg, and 225 mg doses) had transient elevations of transaminases not exceeding Grade 0. In GS-US-399-1954, Grade 1 and Grade 2 transaminase elevations occurred in 8 of 16 subjects receiving 7- or 14-day multi-dosing of GS-5734 150 mg/day (2 of 8 healthy subjects in the 7-day cohort and 6 of 8 subjects in the 14-day cohort). The ALT elevations were up to 10 times the subjects' baseline values and returned to normal limits. The onset of transaminase elevations out of the normal range occurred as early as day 5 and elevations in transaminases above baseline occurred between days 2 and 5. Based on the transaminase elevations, the FDA has placed a partial clinical hold on IND 125,566, prohibiting the performance of studies without a favorable risk-benefit ratio for subjects. The FDA specified that clinical studies may be performed in EVD survivors who have evidence of persistent viral shedding (e.g. documented in semen or vaginal fluid) and/or post-Ebola syndrome. The FDA specified that the duration of treatment with GS-5734 may not exceed 5 days and the dose may not exceed 150 mg/day IV. Multiple-dose clinical studies may not be performed in survivors of EVD who have no evidence of persistent viral shedding or post-Ebola syndrome as the risk-benefit balance is not favorable for these subjects. Data from 2,723 Liberian PREVAIL III participants (including men, women and children) from 1083 survivors and 1640 close household and sexual contacts, demonstrate no difference between the two groups with respect to serum transaminase levels (Table 8). Importantly, these levels of transaminases are within the same range as the baseline transaminase levels in healthy subjects enrolled in Phase 1 studies with GS-5734.

Table 8. Median (with the first and third quartile in parentheses) ALT and AST levels in 2,723 Liberian participants in PREVAIL III: 1083 Ebola survivors and 1640 household and sexual contacts.

	Contacts	Survivors
ALT (U/L)	7 (4-11)	7 (3-13)
AST (U/L)	13 (11-20)	15 (10-19)

Note: Self-identification was used to determine survivor status.

Based on the FDA guidance in the partial clinical hold letter, and on the Liberian PREVAIL III Ebola survivor participant transaminase data, the dose selected for this study is 100 mg/day for 5 days with dose de-escalation criteria for individuals and early planned DSMB assessment of data. The 100-mg dose represents a 33% reduction from the 150-mg dose allowed by FDA as the highest dose that can be investigated under the partial clinical hold. Based on the comparison of systemic drug exposure levels, a 100-mg human dose is estimated to be equivalent to approximately a 5-mg/kg dose in NHPs. While the efficacy of a 5-mg/kg dose of GS-5734 has not been tested in NHPs acutely infected with Ebola, this dose is expected to be efficacious given that 10 and 3 mg/kg are associated with 100% and 33%-67% survival rates, respectively, and both dose levels demonstrated statistically significant reduction in systemic Ebola viremia

compared to control placebo-treated animals. The pharmacokinetic data from the Phase I multi-dose cohort study demonstrated that the concentration of GS-5734 metabolites in seminal fluid are approximately two times that of plasma levels (~220 ng/mL vs. ~100 ng/mL). Thus, a 100-mg dose of GS-5734 for 5 days may have less hepatotoxicity than the 150-mg dose for 7 days, while maintaining antiviral activity given the levels of the GS-5734 metabolites in seminal fluid are two times those of plasma.

In summary, the dose of 100-mg GS-5734 to be administered by IV infusion for 5 days was selected due to the anticipated more favorable toxicity profile compared to the 150-mg dose of GS-5734 for 7 days combined with a good potential for antiviral activity manifested in genital tract.

1.4.1 Individual Dose De-escalation Plan

In both Phase 1 clinical studies, when hepatic enzyme (ALT/AST) elevations occurred, levels generally began to rise by Day 4 or 5, (though transaminase data between day 2 and 5 are not available in the multi-dose cohort study) and generally did not exceed the normal range until day 4 and beyond. A dose of GS-5734 of 100 mg daily over 5 days has been selected for this study, with an individual built-in dose de-escalation strategy for infusion days 2-5 for Grade 1 elevation in transaminases (1.25 to < 2.5 x ULN DAIDS Table for Grading the Severity of Adverse Events November 2014). If the criteria for dose reduction are triggered, the Safety Medical Officer will notify the unblinded pharmacist. For Grade 1 elevations in AST and ALT subjects on the GS-5734 arm will have their dose of study medication reduced to 75 mg IV once daily for the remainder of infusion days and subjects on the placebo are will have the vehicle reduced by 25% (see Section 5.5.3 for details). If a participant experiences a Grade 2 increase in AST or ALT during the infusion days they will be switched to normal saline.

1.4.2 Cohort Dose De-escalation Evaluation

The Data and Safety Monitoring Board (DSMB) will reviewed early safety data to make recommendations on the need to reduce the starting dose for all future subjects, and to reduce the current dose for those currently being infused (see Section 11.7). The early DSMB review in August 2016 concluded there was no need for a cohort dose reduction. There had been 34 subjects enrolled at the time of DSMB review and no serious adverse events (SAEs) nor recommended changes to the protocol by the DSMB.

1.5 Rationale for Length of Treatment-Phase

The objective of the treatment phase is to assess the anti-viral activity of a five day course of GS-5734 assessed by comparing the ANR between the two study arms. The mechanism and source of seminal viral persistence is unknown. If viral Ebola virus RNA is present it is assumed that viral replication is taking place somewhere in the genital track, perhaps the prostate. It is also not known if the viral replication is continuous or intermittent, but the intermittent shedding suggests that replication may be intermittent as well. If the mechanism of anti-viral activity is through polymerase inhibition and GS-5734 is effective in stopping viral replication, clearance of seminal Ebola virus RNA may take time. It is not known how long clearance of seminal Ebola RNA might take after viral replication ceases. The concentration of GS-5734 in seminal fluid metabolites and longer time to clearance from the seminal fluid suggest anti-viral activity

may occur over a longer period of time. The long intracellular half-life of active GS-5734 moiety of approximately 42 hours suggest that anti-viral activity may be exerted up to 5 times this half-life after the last dose and thus anti-viral activity may be present in the seminal compartment for up to 15 days. Measuring anti-viral activity out to 28 days will enable an additional 13 days for clearance of Ebola virus RNA. Considering all these issues assessing a 28 day period has been selected for assessing anti-viral activity.

1.6 Rationale for Use of the GeneXpert for Ebola Virus RNA Detection

The GeneXpert was selected for detection of Ebola virus RNA following extensive testing using clinically contrived samples and live Ebola virus in combination with field testing using clinical samples. To test the assay performance, semen samples were spiked with live virus and assay procedures were optimized and a head to head comparison was performed using identical contrived semen samples on the GeneXpert as well as the EZ1 and MGB assays currently in use in Liberia. Subsequently, a limit of detection (LOD) was determined for the NP gene target (10 copies/mL and GP gene target (100 copies/mL) in the GeneXpert assay. In addition, unspiked semen from over 30 unique healthy donors was tested to look for the potential of false positives (publication pending). Among all these control samples not a single false positive signal was detected for either NP or GP target specific PCR. GeneXpert devices were deployed to the Liberian Institute of Biomedical Research (LIBR). Incoming semen samples provided by male survivors were tested on both the GeneXpert as well as using the EZ-1 and MGB assays. A total of 417 samples were tested. Of these, 92 samples had bola virus RNA detected; 88 of the 92 had NP detected. Of the 92 samples positive 75 were detected on the GeneXpert only.

The GeneXpert Ebola assay has also been extensively tested for cross reactivity with a number of viral, bacterial and parasitic agents. In addition, the sample buffer has been demonstrated to inactivate filoviruses by multiple laboratories, minimizing the amount of manipulations required with potentially infectious substances and the use of the closed cartridge system reduces the potential for cross-contamination. In summary the GeneXpert is being selected as the preferred assay platform because: (1) the increased sensitivity compared to existing assays in use, (2) reduction in risk to staff deployed in the field handling potentially infectious substances; (3) reduced potential for introduction of contaminants and (4) the ease of use.

2 Study Objectives

2.1 Primary Objectives

2.1.1 Treatment Phase

To compare the antiviral activity over 28 days following the administration of 5 days of IV GS-5734 versus placebo in male Ebola Virus Disease survivors with evidence of Ebola virus RNA in their semen.

2.1.2 Follow-up Phase

To compare the effect of 5 days of IV GS-5734 versus placebo on the detection of Ebola virus RNA in semen of male survivors of Ebola Virus Disease over 24 weeks..

2.2 Secondary Objectives

2.2.1 Treatment Phase

- To evaluate the safety and tolerability of GS-5734 in male Ebola survivors with evidence of Ebola virus RNA in their semen.
- To evaluate the pharmacokinetics of GS-5734 (and metabolites as appropriate) in blood and PBMCs.
- To compare the antiviral activity over 28 days following the administration of 5 days of IV GS-5734 versus placebo in subjects with: a CT value of either the GP or NP targets of ≤ 39 in at least one semen sample during the 42-day pre-screening period and subjects with one versus two positive semen samples during the 42-day screening period.

2.2.2 Follow-up Phase

- To evaluate 24-week safety outcomes following IV GS-5734 treatment in male Ebola survivors.
- To compare individual changes from baseline to 24-week signs and symptoms related to "Post Ebola Syndrome" as identified in PREVAIL III.
- To compare the effect of GS-5734 and placebo on change from baseline to 24-week signs and symptoms related to "Post Ebola Syndrome" identified in PREVAIL III.
- To compare the effect of IV GS-5734 versus placebo on the sustained negativity rate for the detection of Ebola virus RNA in semen of male survivors of Ebola Virus Disease over 24 weeks.

2.3 Exploratory Objectives

- To compare the sensitivity of Ebola virus RNA detection using the GeneXpert standard versus "pellet" semen preparation methods.
- To compare changes from baseline in ophthalmologic findings between the GS-5734 arm and the placebo arm.
- To compare Ebola virus RNA sequence changes from screening semen samples to Day 28 and Week 24 semen samples when sample volume allows.
- To assess participant satisfaction during screening and study participation.
- To assess participant satisfaction during screening and study participation.

3 Study Design

3.1 Description of the Study Design

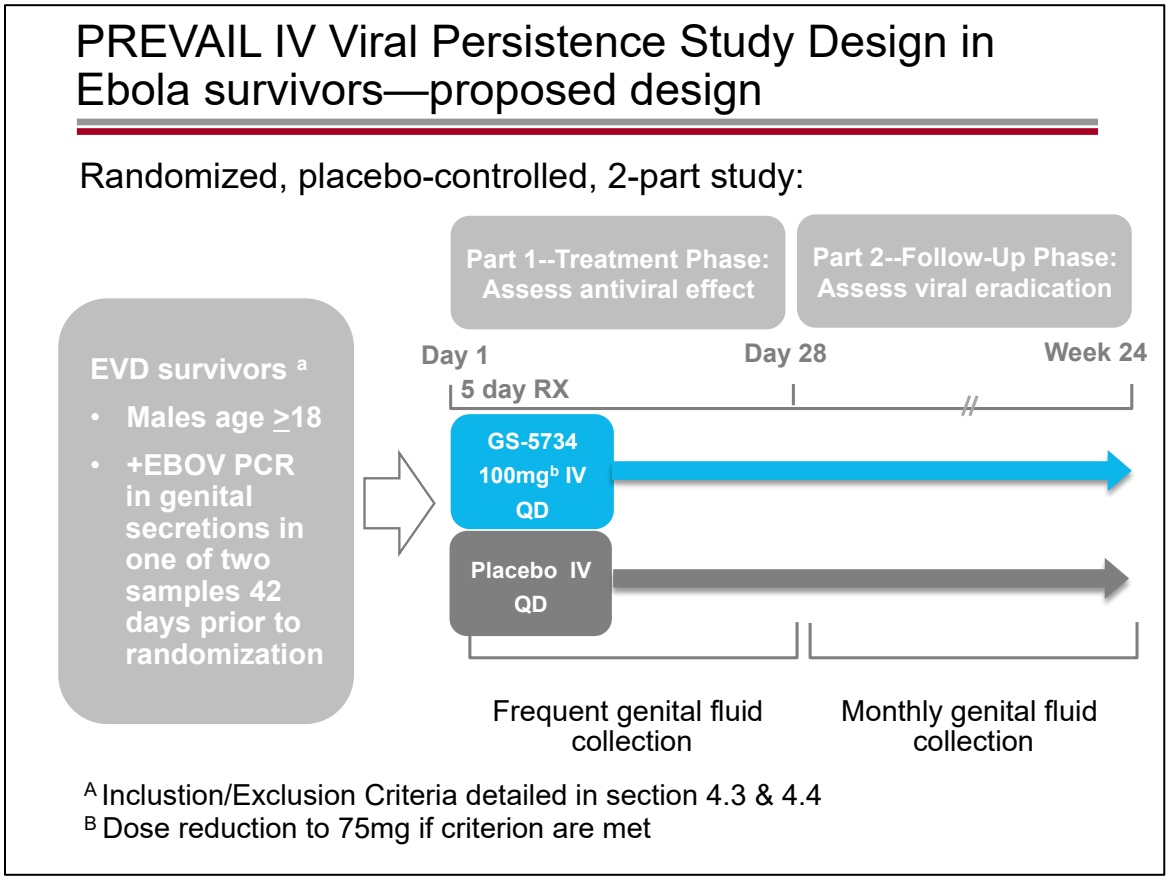
This study will be a double-blind, randomized, two-phase (treatment and longer-term follow-up), two-arm trial of GS-5734 versus placebo among Ebola survivors in Liberia and Guinea who show persistent Ebola virus RNA in their semen at screening (see Figure 4). Participants will be

randomized 1:1 to receive either 100 mg GS-5734 or placebo once daily by IV for 5 days. Participants will be stratified by country and on the basis of one versus two positive genital samples for Ebola PCR taken within 42 days prior to study enrollment.

Antiviral activity as well as safety and tolerability will be assessed during the treatment phase. Longer-term clearance of Ebola virus will be assessed during the follow-up phase. Primary analyses for the assessment of antiviral activity in the treatment phase will focus on the ANR over the first 28 days of the study, as well as clinical and laboratory AEs. ANR is defined to be the percentage of genital samples for which Ebola virus is not detected by PCR during a given interval (days 3-28 or weeks 8-24). A sample is considered negative by PCR if the NP and GP are undetectable by the GeneXpert assay. Primary analysis for the follow-up phase will focus on the ANR collected monthly from weeks 8 to 24.

Because data from the Phase 1 studies demonstrate an elevation in liver transaminases, individual subject dose de-escalation criteria have been incorporated into the study (see Section 5.5.3). For cohort dose reductions the DSMB will review early safety data and make a recommendation for dose adjustment as described below (see Section 11.7.1.1.1). If the dose-reduction criteria, Grade 1 elevations during infusion days 1-5, are reached, the dose of GS-5734 will be reduced to 75 mg/day IV for the rest of the infusion period. For Grade 2 elevations in AST or ALT during the study period, participants will be switched to normal saline.

Figure 4. Study design.



3.2 Study Endpoints

3.2.1 Primary Endpoints

3.2.1.1 Treatment Phase

ANR over days 4, 8, 11, 16, 24, and 28.

3.2.1.2 Follow-up Phase

ANR over weeks 8, 12, 16, 20, and 24.

3.2.2 Secondary Endpoints

3.2.2.1 Treatment Phase

- Proportion of Grade 1, Grade 2, Grade 3, or Grade 4 aspartate transaminase (AST) and alanine transaminase (ALT) levels at days 1, 2, 3, 4, 5, 8, 11, 16, 24, and 28.
- New Grade 3 and Grade 4 adverse events (AEs).
- Change from baseline in safety labs, including N(%) with an increase in grade.
- Change in targeted signs and symptoms (based on GS-5734 Phase I study AEs reported as associated with GS-5734 administration) including: constipation, nausea, vomiting, decreased appetite, headache, tremor, pain at infusion site, dyspepsia, and others new signs and symptoms reported by subjects.
- Incidence of SAEs.
- Frequency of discontinuation of study medication due to AEs or intolerance.
- Post-dose concentrations of GS-5734 and metabolites in serum on days 1 and 5.
- Pre-dose concentrations of GS-5734 and metabolites in serum on day 5.
- Steady-state PBMC-associated concentrations of GS-441524 and its phosphorylated metabolites on Day 5.

3.2.2.2 Follow-up Phase

- Sustained Negativity Rate at weeks 8, 12, 16, 20, and 24.
- Proportion of ALT or AST above baseline, at Grade 1 and above at weeks 8, 12, 16, 20, and 24.
- Incidence of SAEs.
- Change from baseline and 24-week signs and symptoms identified as part of the “Post Ebola Syndrome” from PREVAIL III data on completed review of symptoms and physical exam, depression, and post-traumatic stress disorder screening.

3.2.2.3 Exploratory Endpoints

- Proportion of Ebola viral RNA detection, either the GP or the NP gene targets, at all CT levels in semen.
- Average minimum CT levels at which Ebola virus RNA is detected for GP and NP gene targets in semen.
- Proportion of Ebola viral RNA detection of either GP or NP gene targets of CT ≥ 40.
- Proportion of abnormal ophthalmologic exams.
- Proportion of abnormal ophthalmologic exams with an inflammatory etiology at baseline which have improved or become worse between baseline and week 24.
- Proportion of Ebola virus RNA sequence changes in the polymerase and elsewhere between screening sample day 28 and 24 week semen samples.

4 Study Population

4.1 Rationale for Subject Selection

Adult male EVD survivors aged ≥18 years with persistent Ebola virus RNA in their semen based on PCR detection of either the NP or the GP Ebola virus gene targets using GeneXpert assay are eligible for this study (N=60). Based on preclinical and Phase I safety data, subjects with abnormal eGFR, liver transaminases, or prothrombin time will not be eligible for enrollment in PREVAIL IV. Women are excluded as the primary endpoint in this study involved detection of Ebola virus RNA in semen.

4.2 Recruitment Plan & Retention Plans

Participants may be recruited from the ongoing PREVAIL III (Ebola survivors) study using the PREVAIL Social Mobilization and Communications team and Participant Trackers who are assigned to follow individual subjects. Ebola survivors may also be recruited from other survivor care clinics, research programs, public health programs, or by self-referral.

4.3 Subject Inclusion Criteria

Individuals must meet all of the following criteria to be eligible for study participation:

- Men ≥18 years of age.
- One of two semen samples with Ebola virus RNA detection (defined as a positive PCR for NP or GP using the GeneXpert assay within 42 days prior to randomization).
- Willingness to be available for study evaluations for 6 months.
- Willingness to allow storage of biological samples.
- Willingness to be followed by a Participant Tracker.
- Willingness to refrain from alcohol consumption for study days -7 to 14.
- Willingness to comply with MOH & CDC guidance on using a condom for sexual activity and at least through week 24 of the study.

4.4 Subject Exclusion Criteria

Individuals meeting any of the following criteria will be excluded from study participation:

- Estimated glomerular filtration rate (see Appendix C) less than 60 mL/min/1.73m^2.
- History of significant renal disease.
- History of significant liver disease.
- Evidence of liver disease on physical exam such as ascites.
- AST or ALT, greater than the upper limit of normal, a prothrombin time 1.1 times greater than the upper limits of normal, or a total bilirubin > 1.5 times the upper limits of normal (per DAIDS toxicity tables version 2.0 Nov. 2014).
- Presence of Grade 2 or higher abnormalities for: low hemoglobin, low white blood count (WBC), low platelets, or low or high potassium (per DAIDS toxicity tables version 2.0 Nov. 2014).
- Presence of greater than Grade 2 abnormalities for low or high sodium (per DAIDS toxicity tables version 2.0 Nov. 2014).
- Any condition that, in the opinion of the investigator, would compromise the safety of the study subject or staff, or would prevent proper conduct of the study.

4.5 Justification for Exclusion of Special Populations

Exclusion of children: Because there are insufficient data regarding GS-5734 dosing or AEs available in adults to judge the potential risk in children, children are excluded from this study.

5 Study Agent/Interventions

5.1 Disposition and Dispensation

Sufficient quantities of GS-5734 and placebo to match vials will be shipped to the study site from Gilead Sciences Clinical Supplies Management (or its designee). Any remaining GS-5734 injection concentrated solution and/or diluted GS-5734 solution for infusion will be disposed per local applicable hazardous waste disposal policies and procedures, or will be maintained in the PREVAIL pharmacy.

5.1.1 Formulation, Packaging, and Labeling

GS-5734 will be supplied as a sterile, single-use, preservative-free, clear, colorless to slightly yellow, aqueous-based concentrated solution containing 5 mg/mL GS-5734 that is to be diluted into IV infusion fluids (250-mL normal saline) prior to IV infusion. In addition to the active ingredient, GS-5734 injection, concentration 5 mg/mL, contains the following inactive ingredients: water for injection, betadex sulfobutyl ether sodium (SBECD), and hydrochloric acid and/or sodium hydroxide. Hydrochloric acid and/or sodium hydroxide are used to adjust the formulation to a pH of 3.0 to 4.0.

The supplied matching placebo concentrate for solution for infusion is identical in physical appearance to the active formulation and contains the same inactive ingredients.

GS-5734 injection and placebo to match is filled in 50-mL glass vials enclosed with a rubber stopper and aluminum seal with a propylene flip-off cap. Each single-use vial contains sufficient volume to allow withdrawal of 30 mL of 5 mg/mL of GS-5734 concentrate or placebo.

All labels for GS-5734 and placebo to match vials shall be labeled to meet all applicable requirements of the US Food and Drug Administration (FDA) and EU Guidelines to Good Manufacturing Practice, Annex 13 Investigational Medicinal Products and/or other local regulations as applicable.

5.2 Study Agent Storage and Stability

GS-5734 and placebo to match vials should be stored frozen prior to use. Frozen storage is defined as storage in a place in which the temperature is maintained between −25 °C and −10 °C. Vials should be thawed prior to use at room temperature. See the pharmacy manual for thawing and storage information.

5.3 Randomization Procedures

Once eligibility has been confirmed, subjects will be eligible for randomization. Prior to or during the day 1 visit, the investigator or designee will randomize the subject. Subjects will be assigned a unique subject number and randomized in a 1:1 ratio to GS-5734 or placebo. For subjects participating in PREVAIL III, the PREVAIL III subject number will also be used for this study.

5.4 Blinding and Unblinding Procedures

Study team members and subjects will be blinded to treatment arm assignment throughout the duration of the study. The Sponsor Medical Monitor can request unblinding in the event of a suspected unexpected serious adverse reaction (SUSAR).

5.5 Preparation, Administration, and Dosage of Study Agents

5.5.1 Preparation

GS-5734 injection and corresponding placebo to match are hypertonic and must be diluted prior to administration. Refer to the study pharmacy manual for preparation instructions.

5.5.2 Dosing and Administration

The study treatment groups are as follows:

- Treatment Arm A: GS-5734 100 mg IV over 1 hour, once daily for 5 days.
- Treatment Arm B: Placebo IV over 1 hour, once daily for 5 days.

5.5.3 Dose Adjustments

Individual dose de-escalation will take place as follows with the volume of intravenous study agents remaining the same throughout the 5 day dosing period to retain the blind:

- Due to the potential for hepatotoxicity related to GS-5734 treatment, if a subject experiences a Grade 1 elevation of ALT or AST during the 5-day dosing period, the GS-5734 dose will be reduced from 100 mg/day to 75 mg/day for the remainder of the 5-day dosing period.

- If a subject in either treatment group experiences a Grade 2 elevation of ALT or AST, he will be switched to normal saline.
- Placebo recipients who experience Grade 1 elevations in AST or AST will have the amount of drug vehicle reduced by 25%.

Note: PREVAIL IV will use the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.0, November 2014, which can be found at: http://rsc.tech-res.com/document/safetyandpharmacovigilance/daids_ae_grading_table_v2_nov2014.pdf .

Consideration for cohort dose reduction from 100 mg/day to 75 mg/day via IV:

- The DSMB reviewed early safety data and did not make a recommendation for a cohort dose adjustment as described (see 11.7.1.1.1).

5.6 Study Product Accountability Procedures

The study pharmacist will be responsible for maintaining an accurate record of the unused study agent and an accountability record of study agent supplies for this study. Electronic documentation as well as paper copies will be used. Accountability instructions will be included in the pharmacy manual.

5.7 Concomitant Medications

Prescription medications taken by subjects as maintenance medications for chronic medical conditions, such as hypertension, can be continued during the study and will be recorded on the concomitant medication form throughout the study. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

5.8 Discouraged Medications

Unless advised by a study physician otherwise, study participants will be counseled to refrain from taking: non-maintenance, non-prescription medications, over the counter medications, herbal supplements. Paracetamol, also known as Tylenol or acetaminophen, is particularly discouraged.

Subjects will be advised to refrain from drinking alcohol for 7 days prior to initiation of IV study agents through day 14 of the study.

6 Study Schedule

All study visits will take place at the study sites in Liberia and Guinea per study manual of operations. A tabular presentation of the schedule of evaluations is presented in Appendix B.

6.1 Baseline Ophthalmologic Exam (4 months prior to enrollment)

The baseline ophthalmologic exam will be done within four months of study enrollment. The ophthalmologic exam will include the same set of tests and evaluations included in the PREVAIL III Ebola Natural History and data may be taken from PREVAIL III if the ophthalmologic exam was conducted within four months prior to enrollment in PREVAIL IV. If the ophthalmologic exam was done longer than four months prior to PREVAIL IV study

enrollment or participants were not previously enrolled in PREVAIL III the ophthalmologic exam will be repeated.

6.2 Optional Pre-Screening (prior to screening)

The following can be performed during the pre-screening period:
Note: It is anticipated that Pre-Screening will be used in Guinea.

- Sign pre-screening informed consent for PREVAIL IV.
- Semen collection for Ebola viral RNA detection using the GeneXpert Ebolavirus Assay.

  Note: Semen specimens collected in the context of PREVAIL III are acceptable for meeting eligibility criteria for PREVAIL IV if they occur within the -42-day screening window prior to randomization. An individual with one semen sample with Ebola virus RNA detected on GeneXpert testing within the -42-day window from anticipated randomization during Pre-Screening or on PREVAIL III can undergo screening and have the second semen sample collected during Week 0 (within -21 days prior to day 1). Men coming from other studies or programs with or without a history of detectable seminal Ebola viral RNA will have their semen retested in a PREVAIL research lab using the GeneXpert. Due to intermittent detection of Ebola viral RNA in PREVAIL III, repeat testing is allowable at two-week intervals for determination of eligibility in PREVAIL IV.
- Men within PREVAIL III not currently enrolled in the semen study are eligible for enrollment in PREVAIL IV. In Liberia, men can participate in the semen screening at PREVAIL sites or if preferable via at-home semen donation. In Guinea, men can participate in semen screening either at fixed sites or through mobile screening units.

6.3 Screening/Week 0 (within 21 days prior to day 1)

At screening, the study will be described to the subjects. Subjects will sign the full study PREVAIL IV informed consent document before any study procedures (beyond semen screening conducted under the Pre-screening informed consent) are performed. The following will be performed at the screening visit:

- Physical exam/signs, medical history (including EVD history), demographics and contact information, and symptoms (unless completed within the -42 day screening window under PREVAIL III)
- Vital signs
- Concomitant medications
- Depression questionnaire
- Post-Traumatic Stress Disorder Questionnaire (for those not co-enrolled in PIII)
- Stigma and Discrimination Questionnaire (for those not co-enrolled in PIII)
- Blood draw for:
  - ALT/AST
  - CBC with differential
  - Chemistries (Na, K, Cl, CO_2, creatinine, glucose, eGFR), total and direct bilirubin

  - Lipids: triglycerides, cholesterol (HDL, LDL)
  - Prothrombin time (PT), activated Partial thromboplastin time (aPTT), INR, D-dimer
  - Ebola antibody levels
  - HIV and syphilis testing (for Liberian participants not co-enrolled in PREVAIL III and all participants in Guinea)
- Participants with only one semen sample positive for detection of Ebola viral RNA will provide another sample within 21 days prior to randomization. Participants who have had two semen samples with at least one positive for detection of Ebola virus RNA within the -42 day window prior to randomization, but the last sample was taken >21 days prior to enrollment, will provide an additional semen sample if they are able. Note that the results of this sample will not affect eligibility for the study.
- Randomization will be performed after eligibility is confirmed

6.4 Treatment Phase (day 1 to day 28)

6.4.1 Day 1

Pre-dose: The following will be performed prior to study agent infusion:

- Targeted physical exam for signs and symptoms since screening
- Review of concomitant medications
- Review of baseline labs
- Labs per Schedule of Events (SOE) which includes storage of serum sample for future serologic hepatitis B and hepatitis C testing
- Vital signs (within 30 minutes prior to infusion)
- Insertion of IV catheter

Infusion: After completion of the pre-dose procedures and review of laboratory test results study agent will be infused over 1 hour.

Post-dose: The following will be performed after completion of the study agent infusion:

- Subjects will be observed during and for 60 minutes following the infusion.
- Vital signs (approximately 30 and 60 minutes after completion of the infusion)
- AE assessment
- Physical exam (as needed to assess new signs and symptoms)
- When site facility conditions allow processing of PK samples, one blood draw for PK analysis of GS-5734 and metabolites concentration in plasma (within 5 min after the end of infusion). For PK analysis sample schedule see Table 9.

6.4.2 Days 2, 3, 4, and 5

Pre-infusion:

- Targeted physical exam to assess new signs and symptoms since previous day's infusion
- Review of concomitant medications

- Review of prior day AST and ALT (by designee)
- Vital signs (within 30 minutes prior to infusion)
- AE assessment
- Insertion of IV catheter if outpatient
- Insure patency of IV catheter if an inpatient
- Blood draw for:
  - Per SOE
  - When site facility conditions allow processing of PK samples, one blood sample for GS-5734 metabolites concentrations in plasma pre-dose (Day 4 only). For PK analysis sample schedule see Table 9.

Infusion: Study agent infusion over 1 hour, with close observation of subject during infusion and for 60 minutes after completion of infusion.

Post-infusion:

- Vital signs (approximately 30 and 60 minutes after completion of infusion)
- Physical exam (as needed to assess new signs and symptoms)
- When site facility conditions allow processing of PK samples, blood draw (Day 5 only) for:
  - One blood sample for GS-5734 and metabolites concentrations in plasma (within 5 minutes after the end of infusion). For plasma PK analysis sample schedule see Table 9.
  - Blood sample for PBMC GS-441524 and phosphorylated metabolites concentration within 1 hour after the infusion. (Note this may be drawn with the blood sample to be taken 5 minutes after the infusion.) For PBMC PK analysis sample schedule see Table 9.
- Semen collection (Day 4 only) for:
  - Ebola virus PCR

Table 9. Summary of blood samples to be collected for PK analysis.[a]

Sample	Day 1		Day 4		Day 5	
	Pre-infusion	Post-infusion	Pre-infusion	Post-infusion	Pre-infusion	Post-infusion
Blood for plasma PK		X[b]	X[c]			X[b]
Blood for PBMC PK						X[d]

a When site facility conditions do not allow processing of PK samples, these samples will not be drawn and PK analysis will be omitted.

b Collect immediately after the end of infusion (within 5 min if possible)

c Collect anytime within 1 hour before initiation of infusion

d Collect within 1 hour after the end of infusion

6.4.3 Days 8, 11, 16, 24, and 28 (see Appendix B for windows)

- Targeted Physical exam (as needed to assess new signs and symptoms)
- Review of concomitant medications
- Vital signs
- AE assessment
- Blood draw for other labs per SOE
- Semen collection for: Ebola virus PCR
- Participant satisfaction survey (day 28 only)

6.5 Follow-up Phase

6.5.1 Weeks 8, 12, 16, 20, and 24 (see Appendix B for windows)

- Targeted Physical exam (as needed to assess new signs and symptoms) except for week 24 which will be a complete review of systems, physical exam to include a depression screen, and a post-traumatic stress screen.
- Review of concomitant medications
- Vital signs
- AE assessment
- Blood draw for labs per SOE
- Semen collection for Ebola virus PCR
- Participant satisfaction survey (week 24 only)

6.5.2 Week 24 (within ± 6 week window)

- Ophthalmologic exam

Subjects will be completed with the study after the week 24 visit and ophthalmologic visit, unless offered closeout treatment as described below.

6.6 Closeout Treatment for Subjects on Placebo Arm

If the treatment is shown to be efficacious and to have acceptable safety and tolerability, the subjects who received placebo who continue to have detectable EBOV virus RNA in their semen at or after 6 months on study will be offered treatment with GS-5734.

7 Study Procedures/Evaluations

7.1 Clinical Procedures/Evaluations

Blood draw: Blood will be drawn according to standard procedures for laboratory evaluations and storage. The amount of blood drawn for research purposes will not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period.

Semen collection: Collection of semen will occur at PREVAIL clinical study sites, as described in the study manual of procedures.

Ophthalmologic Evaluations: Ophthalmologic evaluations will take place at the JFK PREVAIL site in Liberia and the Ambroise Paré Clinic site in Guinea, and will only involve non-invasive assessments (e.g., visual acuity, intraocular pressure).

7.2 Laboratory Evaluations

Per the SOE, laboratory evaluations include routine chemistry, total and direct bilirubin, liver transaminases (ALT/AST), lipids, prothrombin (PT), activated partial thromboplastin time (aPTT), and D-dimer, CBC with differential and platelet counts, semen for assessment of EBOV RNA, blood and PBMCs for GS-5734 and metabolites, and Ebola antibody levels.

7.2.1 Specimen Preparation, Handling, and Shipping

Semen samples collected as part of this study are likely to contain Ebola virus RNA. It is not known if the presence of EBOV RNA represents viable Ebola virus. Thus, appropriate protective precautions will be taken by all personnel involved in obtaining, handling, and shipping of these semen specimens, as currently recommended by the Centers for Disease Control and Prevention, the Liberian Ministry of Health, and the Guinean Ministry of Health.

Blood samples collected for GS-5734 and metabolite concentrations in plasma and PBMCs will be processed and stored according to Gilead Sciences Laboratory Manual when site facility conditions allow proper processing; when conditions do not allow processing for PK analysis, samples will not be collected. Processed samples will be shipped to a contract laboratory recommended by Gilead Sciences that is qualified to perform quantitative analysis of GS-5734 and metabolites concentrations in human clinical samples.

8 Potential Risks and Benefits

8.1 Potential Risks

8.1.1 GS-5734

Early signs of kidney injury in humans can readily be detected and monitored using routine laboratory tests. In this study, the risk of clinically significant kidney injury in humans will be minimized by close monitoring of kidney function. Study subjects will be required to have liver function tests at or below the upper limit of normal at the time of screening. Subjects with a history of liver disease, renal disease or any other serious or active medical illness will be excluded from participation. Serum chemistry assessments, including liver function testing, will be closely monitored during the study period.

Based on a review of these Phase I data, a dose selection of 100 mg IV for 5 days has been selected with individual dose de-escalation built in for Grade 1 transaminase elevations during the 5-day dosing period. A DSMB review is planned after the first 8 randomized subjects (4:4 GS-5743:Placebo) complete study day 16, for safety and assessment of cohort dose reduction. The summarized risk mitigation strategy appears in Section 8.3.1.

To date in human studies, no serious AEs have occurred in healthy individuals who have received at least one dose of GS-5734. GS-5734 has been tested in humans as a single ascending dose over a dose range of 3 to 225 mg and in a multi-dose study of 150 mg/day for 7 or 14 days. In nonclinical animal studies, toxicity findings were consistent with dose dependent and reversible kidney injury and dysfunction.

While the clinical significance of the nephrotoxicity in animal species is unknown, no evidence of nephrotoxicity has been observed through single GS-5734 dose levels up to 225 mg in humans or in multi-dose studies of 150 mg/day for 7 and 14 days. The etiology of reversible kidney injury observed in rats is consistent with the ability of rat renal organic anion transporters (OATs), but not human OATs, to efficiently interact with blood metabolites of GS-5734, particularly GS-704277. This effect may lead to proportionally higher intracellular accumulation of drug metabolites in renal rat tubules, leading to kidney injury.

An Ebola meningitis patient who received 2 days of GS-5734 150 mg every 24 hours followed by 12 days of GS-5734 225 mg every 24 hours without apparent AEs or laboratory changes attributed to open-label GS-5734 therapy. Abnormal liver function tests have been observed in subjects receiving GS-5734 (as detailed in Section 1.2.3). In summary, in the single ascending dose group 3 subjects, 1 subject each in the 3-mg, 150-mg and 225-mg dose groups, had elevation of high ALT < 1.25x ULN on day 5 and returned to normal limits by day 7. Overall, 14 of 16 subjects who received multiple doses GS-5734 150 mg IV in the study had at least 1.5x increases in ALT from pretreatment levels, 6 subjects who received 7 days and all 8 subjects who received 14 days of study medication. Two of 8 placebo recipients had at least 1.5x increases in ALT from pretreatment levels. Grade 1 increased PT occurred in 10 subjects who received GS-5743 and no subjects who received placebo; 7 of the subjects also had graded transaminase elevations. No clinically significant changes from baseline were observed in total bilirubin, alkaline phosphatase, albumin, or INR in subjects who received GS-5734. There were no Grade 3 or Grade 4 transaminase elevations and ALT returned to normal range within 15 days for the 7-day dosing and by end of the study in the 14-day cohort.

Does levels were well-tolerated in the healthy volunteers enrolled in a single-ascending-dose study (GS-US-399-1812) and in healthy volunteers in the GS-5734 multi-dose study of 150 mg IV/day with cohorts of 7 or 14 days. One subject in the multi-dose 150 mg/day developed anorexia, constipation, emesis, headache, nausea, and shaking of the left leg and left hand considered related to study medication and discontinued study medication after the 4^{th} dose. This subject had accompanying Grade 2 AST and ALT elevations with normal bilirubin, alkaline phosphatase and PT/PTT/INR which returned to normal range. Additional symptoms related to GS-5734 treatment occurred in subjects who received GS-5734 included: abnormal sensation in the ear, dizziness, constipation, headache, loose stool, and indigestion. These symptoms resolved after GS-5734 was stopped

The 100 mg GS-5734 dose in this study will be administered with 6 g of SBECD, which is within the range of daily SBECD administration considered safe in humans. The 100-mg dose prepared in 0.9% saline will be hypertonic relative to human serum osmolality but approaches the normal physiological osmolar range for humans. GS-5734 100 mg administered as a 250-mL infusion once daily for 5 days is not anticipated to pose a safety risk to subjects enrolled in this study.

During animal studies, injection site reactions and associated inflammatory responses were seen with repeated daily intramuscular injections of GS-5734. These findings were not observed in animals receiving IV injection with a different formulation of the study medication. For this clinical study, GS-5734 will only be administered by the IV route. Historically, most injection site reactions from parenteral treatments in humans are self-limited in nature and can be treated with supportive care alone. No dose-limiting injection site reactions or immuno-allergic reactions were observed in the Phase I studies or in the Ebola meningitis patient during her 14-day course of GS-5734.

8.1.2 Placebo

There are no risks associated with placebo administration.

8.1.3 Blood Draw/Phlebotomy

The risks of drawing blood and IV catheter placement include pain, bruising, bleeding, fainting, and, rarely, infection. In addition, IV catheter placement may cause phlebitis.

8.1.4 Semen Collection

There are no risks associated with the collection of semen.

8.2 Potential Benefits

Participants may receive no benefit from participating in this study.

GS-5734 has shown antiviral activity against Ebola virus in vitro and in NHP models as described above. GS-5734 has been administered to a critically ill patient with recrudescent Ebola meningitis inclusive of Ebola virus viremia and to a neonate with perinatally acquired EVD who had persistent Ebola viremia following 4 doses of experimental ZMapp therapy. The adult patient received GS-5734 150 mg IV every 24 hours for 2 days and 225 mg every 24 hours for 12 days, and the neonate received GS-5734 10 mg IV every 24 hours for 12 days. Both patients recovered with no apparent adverse effects or evidence of laboratory abnormalities related to GS-5734 administration. Neither liver nor kidney toxicity was observed in either patient with active EVD previously treated with GS-5734 on a compassionate use basis.

Male Ebola survivors have demonstrated shedding of Ebola virus RNA in their semen beyond 18 months of the onset of their Ebola virus disease. It is not known if symptoms and signs associated with what is being called, “Post Ebola Syndrome” are associated with Ebola virus persistence. Individuals in the Ebola virus community are aware of the Ebola cluster outbreaks thought to be attributable to transmission from the survivor community. In focal group discussions, men with Ebola virus RNA found in their semen express worry that they may transmit the virus to their loved ones, concern that they want to father children but fears around intercourse without a condom. One survivor expressed, that he “wants his freedom back”. If GS-5734 is effective in reducing or eliminating Ebola virus from the semen of participants, the participants may experience the benefit of potentially less transmission risk to their families and communities. It is possible that those with post Ebola signs and symptoms may experience an improvement in these symptoms. However, there may be no benefit at all.

8.3 Risk-Benefit Assessment

The selected dose is thought to be a good balance between safety and potential for antiviral efficacy. A 10-mg/kg dose of GS-5734 administered intravenously is efficacious in Ebola virus-infected rhesus monkeys, providing 100% protection against the lethal effects of EVD. The pharmacokinetics of a single 10-mg/kg IV dose to rhesus monkeys and a single dose of 150 mg to adult human volunteers demonstrated similar systemic concentration-time exposure profiles with a short, biphasic half-life of the prodrug GS-5734 and a long terminal elimination half-life of the nucleoside metabolite GS-441524. PBMC exposures of total nucleoside were persistent in both monkey and humans, with suggestion of greater persistence in humans. In Phase I multi-dose studies overall, GS-5734 has a favorable pharmacokinetic profile, with concentrations of GS-5734 intermediate metabolites in semen approximately two-times that of plasma, thus evaluation of a 100-mg dose has potential to have antiviral activity in semen equivalent or slightly higher than the 150-mg dose in plasma, which correlates to 100% survival in the NHP EVD animal model.

Elevations in transaminases generally did not occur prior to day four or five with 150 mg IV of GS-5734. The 33% reduction in dosing to 100 mg IV/day for a reduced duration of 5 days is likely to mitigate risk of hepatotoxicity. In addition, the built-in dose de-escalation with concomitant monitoring of participants transaminase, PT/aPTT, and creatinine will enable close monitoring of patients by the study physicians and aggregate safety data by the DSMB. A risk mitigation strategy had been developed for the study (Section 8.3.1).

8.3.1 Risk Mitigation

Per Section 8.1.1, despite the absence of serious adverse effects in the GS 7-day and 14-day cohorts in the multi-dose Phase I studies of intravenous GS-5734 at a dose of 150 mg/day, the prevalence in both cohorts of transient Grade 1 and 2 elevations in hepatic transaminases, and temporally related though shorter duration of elevations in prothrombin times in subjects with transaminase elevations despite evidence of other hepatic effects is cause for a thoughtful risk-mitigation strategy. Liver transaminases and prothrombin time returned to normal limits during the study. The mechanism for the elevation in transaminases is not known. A risk-mitigation strategy for the target population and the target indication, e.g., persistence of Ebola virus in the semen of male survivors, warrant a risk mitigation strategy for PREVAIL IV. The risk-mitigation strategy for PREVAIL IV includes the following:

i) Restriction of study population to those without a history of significant renal or significant hepatic disease, or evidence of hepatic disease on physical exam.
ii) Restriction of the study population to those with normal results of the following within 21 days of initial dosing of study agent: ALT, AST,
iii) Exclusion of participants with: a total bilirubin > 1.5 times the upper limits of normal, or an estimated, GFR < 60 mL/min/1.73m^2, or a PT > 1.1 times the upper limits of normal.
iv) Reduction of the dose of intravenous GS-5724 from 150 mg per day used in the GS-5734 multi-dose Phase I Study, to 100 mg intravenous GS-5734 per day.
v) Built-in adjustments in dosing contingent on ALT or AST elevation:

(a) For a Grade I AST or ALT elevations: a 25% dose reduction of GS-5734 from 100 mg/day to 75 mg/day or 25% by volume reduction of placebo vehicle for the remainder of the dosing period with continued blinding of the study team.
(b) For a Grade 2 elevation in ALT or AST: cessation of administration of study agent and switch to normal saline with continued blinding of the study team.

vi) Reduction in dose duration from the Phase I GS-5734 multi-dose study of 7 days or 14 days of IV GS-5734 to 5 days of IV GS-5734.
vii) Enrollment is limited to 4 subjects per week during the first 4 weeks with active DSMB involvement looking at a weekly listing of AEs, protocol-determined frequency of dose de-escalation, and a formal assessment of the first 8 subjects on study inclusive of day 16 laboratory data for all subjects to assess the need for a cohort dose reduction (see Section 11.7.1.1.1 DSMB Early Safety Review Plan).

9 Research Use of Stored Human Samples, Specimens, or Data

Intended Use: Samples and data collected under this protocol may be used to better understand Ebola infection and Ebola viral persistence in apparent Ebola carriers. Scientists both inside and outside of Liberia and Guinea may be involved in studying samples and data from this study.

Storage: Access to stored samples will be limited using either a locked room or a locked freezer. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.

Tracking: Samples and data acquired under this protocol will be tracked per the PREVAIL data management system.

Disposition at the Completion of the Protocol:

- In the future, other investigators (both at NIH, in Liberia, Guinea and outside) may wish to study these samples and/or data. In that case, Institutional Review Board (IRB) approval from the NIAID IRB, the National Ethics Research Board in Liberia, and the National Ethics Committee for Health Research in Guinea must be sought prior to any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior IRB approvals.
- Samples will be maintained in the PREVAIL Repository in Liberia and at the Maferinyah and Conakry study sites in Guinea.

Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:

- Any unanticipated loss of samples or data will be reported to the IRBs.
- Subjects may decide at any point not to have their samples stored. In this case, the principal investigator will advise study personnel to destroy all known remaining samples and report what was done to both the subject and to the IRB.

10 Compensation for Inconvenience: Plan for Subjects

Participants in the study will be compensated for their time and inconvenience. Compensation will be provided at each regularly scheduled study visit/contact. The governments of each country will recommend the amount of compensation to be provided.

11 Assessment of Safety

A manual of operations will describe procedures for safety reporting. Below a brief summary is provided.

11.1 Definitions

Adverse Event: An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR): An AE that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR): An AE for which there is a reasonable possibility that the investigational agent caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. An SAR implies a lesser degree of certainty about causality than AR, which implies a high degree of certainty.

Causality (relatedness): Likelihood that the event is caused by the study agents. Causality will be assessed considering the factors listed under the following categories:

Reasonable possibility of relatedness

- reasonable temporal relationship

AND

- little evidence for a more likely alternative etiology

No reasonable possibility of relatedness

- does not have a reasonable temporal relationship

OR

- reasonable evidence for a more likely alternative etiology

Note: Other factors should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

Serious Adverse Event (SAE): An SAE is an AE that results in one or more of the following outcomes:

a. Death
b. A life-threatening event (i.e., an immediate threat to life)

c. Hospitalization
d. Prolongation of existing hospitalization
e. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
f. Congenital anomaly or birth defects in the offspring of a study participant
g. Other important medical events, that, based upon appropriate medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.)

Note: For this protocol laboratory-confirmed malaria events that do not require hospitalization will not be considered as SAEs.

Unexpected Adverse Event: An AE is unexpected if it is not listed in the IB or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is an AE that is serious, related to the study agent, and unexpected.

Severity: The Investigator will grade the severity of each AE according to the "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.0, November 2014, which can be found at:
http://rsc.tech-res.com/document/safetyandpharmacovigilance/daids_ae_grading_table_v2_nov2014.pdf

Unanticipated Problem (UP): A UP is any event, incident, experience, or outcome that

1. is unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document, IB, or other study documents; and
    b. the characteristics of the subject population being studied; and
2. has a reasonable possibility of relatedness to participation in the research (as defined above); and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND sponsor, an AE with a serious outcome will be considered increased risk.)

Unanticipated Problem that is not an Adverse Event (UPnonAE): A UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered non-serious UPs. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

11.2 Investigators' Documentation and Assessment of Adverse Events

All AEs occurring from the first dose of the study drug through the end of the treatment phase on day 28 will be documented, assessed, and recorded in the CRF. During the follow-up phase all Grade 3 and Grade 4 AEs, and all grade transaminase elevations, will be documented, assessed, and recorded on CRFs.

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

- documented in the subject's medical record/source document, and
- assessed with respect to seriousness, severity (intensity or grade), and causality (see criteria listed above).

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be documented as the AE.

All abnormal laboratory findings will be reviewed on a routine basis by the DSMB to identify potential safety signals.

11.3 Reporting Responsibilities and Timelines

Except for events limited to a laboratory abnormality, all UPs, SAEs, and deaths are recorded by the investigator on CRFs and submitted to the data center.

SAEs must be submitted within 24 hours after the investigator becomes aware the event has occurred. All deaths are considered SAEs and must be reported as such, as well as being reported on a Death CRF.

A pregnancy in a sexual partner during the treatment phase of the study will be reported to site investigators by participants and the outcome of the pregnancy will be recorded on a CRF.

Safety data will be submitted to the DSMB for safety monitoring as described below (Section 11.7) and to the IND sponsor when requested for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the FDA by the IND Sponsor within the legal timeframe:

- 7 calendar days in case of death or life-threatening events after the sponsor's initial receipt of the information; relevant complementary information should be collected and reported within 8 extra-days.
- 15 calendar days for all other events after the sponsor's initial receipt of the information; relevant complementary information should also be collected and reported within 8 extra-days.

11.4 Investigator Reporting Procedures to the IRBs

Investigators are responsible for reporting to the NIAID IRB, National Research Ethics Board (NREB) of Liberia, and National Ethics Committee for Health Research (CNERS) in Guinea as outlined below.

11.4.1.1 Definitions

Protocol Deviation: Any change, divergence, or departure from the IRB-approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur.

Serious Protocol Deviation: A deviation that meets the definition of an SAE or compromises the safety, welfare, or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as:

1. Serious: non-compliance that:
    a. increases risks, or causes harm, to participants;
    b. decreases potential benefits to participants;
    c. compromises the integrity of the NIH HRPP; or
    d. invalidates the study data.
2. Continuing: non-compliance that is recurring.
3. Minor: non-compliance that is neither serious nor continuing.

11.4.1.2 Expedited Reporting to the NIAID IRB, NREB, and CNERS

Serious and non-serious UPs, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. SAEs that are related to the research (i.e., meet causality definition of reasonable possibility of relatedness above) will be reported to the NIAID IRB, NREB , and CNERS within 7 calendar days of investigator's awareness, regardless of expectedness.

11.4.1.3 Waiver of Reporting Anticipated Protocol Deviations, Expected UPnonAEs, and Deaths to the NIAID IRB, NREB, and CNERS

Anticipated deviations in the conduct of the protocol will not be reported to the NIAID IRB, NREB, or CNERS unless they occur at a rate greater than anticipated by the study team. Expected AEs will not be reported to the NIAID IRB, NREB, or CNERS unless they occur at a rate greater than that known to occur in Ebola survivors. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are UPs.

11.4.1.4 Annual Reporting to the NIAID IRB, NREB, and CNERS

The following items will be reported to the NIAID IRB, NREB, and CNERS in summary at the time of continuing review:

- Serious and non-serious UPs.
- Expected SAEs that are related to the research.
- SAEs that are not related to the research.
- All AEs, except expected AEs granted a waiver of reporting.
- Serious and non-serious protocol deviations.
- Serious, continuing, and minor non-compliance.
- Any trends or events which in the opinion of the investigator should be reported.

11.5 Sponsor's Reporting Responsibilities

SUSARs, as defined in 21 CFR 312.32 and determined by the IND sponsor, will be reported to FDA and all participating investigators as IND Safety Reports.

The IND sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

11.5.1 Resumption of the Study in the Event that the DSMB Recommends a Pause

If the study is paused (see Section 11.7.1.1), the IND sponsor, in collaboration with the principal investigator and the DSMB, will determine if it is safe to resume the study. The principal investigator will notify the IRBs of the decision on resumption of the study.

11.5.1.1 Discontinuation of Study Agent

Subjects who meet the pre-determined criteria for discontinuation of the study agent will continued to be followed for safety.

11.6 Withdrawal Criteria for an Individual Subject

An individual subject will be withdrawn for any of the following reasons:

- An individual subject's decision. (The investigator should attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- The investigator determines that continued participation in the study would not be in the best interest of the subject.

11.6.1 Replacement of Withdrawn Subjects or Subjects Who Discontinue Study Agent

The following subjects may be replaced:

- Subject who withdraw or are withdrawn from the study prior to administration of study agent on day 1 may be replaced.

11.7 Safety Oversight

11.7.1.1 Data and Safety Monitoring Board

The NIAID Intramural independent Data and Safety Monitoring Board (DSMB) with ad hoc representation from Liberia and Guinea will monitor the study. The DSMB will be composed of experts in clinical trials, ethics, and a representative from West Africa. None of the members of the study team will be part of the DSMB or have access to the closed data unless the DSMB made a recommendation for early stoppage based upon safety concerns. Prior to initiation and throughout the study the board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study

The trial will be conducted under the direction of an the protocol team. Members of the protocol team will be blinded to interim results by treatment group. The protocol team will monitor the enrollment and follow-up of participants, including the collection of event documentation and the completeness of study data. The study statistician will prepare open reports for the DSMB that contain this information along with pooled ANR summaries, pooled SAE information, pooled transaminase, prothrombin time, other lab data, and other pooled data (such as demographics). The study statistician will also prepare closed reports that break these data out by treatment group. The protocol team will meet with the DSMB during an open session prior to the DSMB's closed review of treatment differences. The study statistician will attend these closed sessions to help clarify the closed report. The protocol team may request that the DSMB convene if SAEs occur that are considered to be related to the study drug.

The DSMB will review the study prior to initiation and will review the safety data (laboratory changes, injection site reactions, AEs, dose de-escalations, dosing cessations, and SAEs) from the study every week via emailed weekly safety reports. In addition, the DSMB will meet to review safety data after a total of 8 participants have enrolled and completed 16 days on the study to enable assessment of safety data during anticipated drug exposure. After the initial meeting, the DSMB will continue to meet every 2-4 weeks depending on patient accrual. The DSMB will monitor safety closely throughout the trial and may pause enrollment in the event of deaths or SAEs that are considered drug-related. Based on their reviews, they will be asked to make a recommendation on whether enrollment should continue, pause, discontinue, or if there should be a cohort wide dose reduction.

The DSMB may also pause enrollment or request that participants be notified of an increased frequency of unanticipated or anticipated adverse effects (e.g., elevated transaminases). The DSMB will also review the completeness of follow-up and other aspects of study conduct. After each meeting they will recommend continuing the study as planned, modifying the study, or terminating the study.

The DSMB will be provided with the protocol and the pre-specified dose de-escalation and dose cessation criteria. All SAEs, all UPs, and all IND Safety Reports will be reported by the principal investigator to the DSMB at the same time they are submitted to the IRBs or IND sponsor. The principal investigator will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The principal investigator will notify the board at the time dose-reduction or cessation criteria are met. If the study is paused the DSMB, IND sponsor, and

Principal investigator will collaborate to determine if it is safe to resume the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

While the DSMB will be provided these guidelines for monitoring the study, they will be asked to use their expert and independent judgment concerning modification or early termination of the study. It is understood that this complex decision depends on multiple factors.

11.7.1.1.1 Early Safety Review Plan

The DSMB will review safety data after the first 8 participants (n=4/group) have completed the five day dosing period and lab safety data through day 16. It is expected that 8 will be enrolled on each arm at this time point, and 6 will have received 5 days of study product. Upon completion of the review, the board will make a recommendation to either continue the study at the 100-mg GS-5734 dose or reduce the dose to 75 mg for the remainder of the study. If the DSMB finds that a large proportion of participants on the active arm are meeting the criteria for de-escalation of dose, the DSMB should consider recommending the dose be reduced for all participants. As a guideline, if 4 or more of this first set of participants (4 through day 16; 6 through dosing; 8 enrolled) on the active arm have met the criteria for dose de-escalation or experienced grade 1 or higher elevations in AST or ALT, a de-escalation for the remainder of the study would be appropriate. Table 10 shows recommended thresholds for the DSMB decision to recommend study dose de-escalation, based on an assumed enrollment of 2/group (94 total) per week for the first 4 weeks, followed by 4 per group (8 total) on subsequent weeks. The table gives guidelines for DSMB meetings starting at approximately day 23, when 16-day follow-up data on the first 4 people per group should be available, and meeting every two weeks up to the point where there is at least 16-day follow-up available on approximately 50% of the study participants. At the last study point listed below, approximately study week 8, we estimate 48 of 60 participants will be enrolled. Under the assumptions used here, we would expect all participants to be enrolled within two weeks of this time-point, so it is unlikely that study-wide dose de-escalation at time points after this would be suggested.

Table 10. Suggested guidelines for dose de-escalation by the DSMB.

Approx study day (week)	Number of enrolled, per arm	Number of with 5 days dosing, per arm	Number of with 16 days follow-up, per arm	Number of Subjects that meet dose de-escalation criteria OR grade 1 or higher AST or ALT		Number of Subjects with Grade 2 or higher AST or ALT	
				Treatment Arm	Placebo Arm	Treatment Arm	Placebo Arm
23 (4)	8	6	4	≥4	0	≥1	0
37 (6)	16	12	8	≥5	0	≥1	0
51 (8)	24	20	16	≥6	0	≥2	0
65 (10)	30	28	24	N/A	N/A	N/A	N/A

Assumes enrollment of 2 subjects on active drug/week enrolled weeks 1-4 in each group, then 4/week

12 Site Monitoring Plan

According to the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines, Section 5.18, and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the consent process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections [OHRP]), FDA, and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g, consent forms) and pertinent hospital or clinical records readily available for inspection by the IRBs, the FDA, the site monitors, and the NIAID staff, or other designees, for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the principal investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

13 Statistical Considerations

13.1 Study Hypotheses

13.1.1 Treatment Phase

Male survivors of EVD who receive 5 days of IV GS-5734 will have Ebola virus RNA detected in a lower proportion of samples over the first 28 days, as compared to those who receive placebo.

13.1.2 Follow-up Phase

Male survivors of EVD who receive 5 days of IV GS-5734 will have Ebola virus RNA detected in a lower proportion of samples over the first 24 weeks, as compared to those who receive placebo.

13.2 Study Design

This study will use a parallel groups design.

13.2.1 Stratification and Randomization

All participants must have at least two semen samples before enrollment, at least one of which has detectable Ebola virus RNA, in order to be eligible for this study. Subjects will be stratified based on whether screening samples both had detectable Ebola virus RNA, or only one of the two had detectable Ebola virus RNA. Recruitment will focus on PREVAIL III participants with evidence of repeated samples with detectable Ebola virus RNA or CT values <39 on a single sample to try to enroll people likely to have detectable RNA on two screening samples, but participants may be enrolled from outside of PREVAIL III as well. Subjects will also be stratified based on the country in which they reside. There are substantial differences between countries in how the epidemic unfolded and how those with EVD were treated.

Subjects will be randomized 1:1 within the strata defined above.

13.3 Sample Size Justification

Preliminary data from PREVAIL III was used to estimate the rates of detectable virus in semen of survivors. Among 134 men who contributed at least two semen samples, we analyzed the last two semen samples per person. One hundred men had undetectable CT at both time points, 13 (10%) were detectable at both time points, and 21 (16%) had mixed detectability (detectable/undetectable or undetectable/detectable). Based on these estimates, 26% of male survivors may be eligible for the study.

We use this data to estimate the proportion of men who might be eligible for the study as well as the “success” rate among placebos. The enrollment is limited by the number of available subjects with detectable Ebola virus in semen at screening. Based on semen testing in PREVAIL III, approximately 26% of male survivors will have detectable seminal Ebola viral RNA when tested longitudinally during the first 1-2 year window. This percentage may decrease with time.

Based on the power calculations below, complete data on 50 men (25/arm) should be adequate to find a difference under many assumptions. However, as the power calculations do not take into account missing data, nor is it clear what type of heterogeneity or treatment effect might be more realistic, the study will attempt to enroll at least 60 people (30/group), with a target of at least 20 participants with two screening samples with a detectable CT or at least 30 participants have an Ebola NP or GP CT value using the Cepheid of <39. If the total sample size of 60 is enrolled without 20 people with two detectable samples, or at least 30 participants with an Ebola NP or GP CT value using the Cepheid of <39 we will continue to enroll until one of these criteria is met up to a total of 120 people if possible.

13.3.1 Power Calculations

The table below lists the simulated power to find a significant difference in treatment-phase ANR and follow-up ANR for various true rates of ANR in the placebo and GS-5734 arms. With 50 people (25/arm), we will have reasonable power (>80%) to find a difference in both the treatment-phase ANR and the follow-up ANR if the effect of treatment is to cut the probability of a detectable sample by 50% for each person at each time, starting at day 3. If the effect of treatment is instead that 40% of people experience sterilization starting on day 3, such that their probability of a detectable sample drops to 0 (except for false positives), and the rest of the people are unaffected, then the power varies depending on the amount of heterogeneity in the probabilities.

ANR p-value based on t-test on proportion negative at 6 (day 3 to day 28) or 5 (week 8 to week 24) visits. All power estimates based on 1,000 simulations, 1% false positive rate, and 5% false negative rate, alpha=.05, no adjustments for multiple comparisons.

Table 11. Power for endpoints under varying assumptions.

N	P_i=P(detectable|person i) **Control group**	**Treatment Effect**	**Power d3-d28 ANR**	**Power w8-w24 ANR**
50	p_i=.5	.5 **(multiplicative on probability)** starting d5	.96	.98
	p_i ~Beta(10,10)		.93	.97
	p_i ~U(0,1)		.95	.98
	$p_i = \begin{cases} .1 \; with \; 50\% \; chance \\ .9 \; with \; 50\% \; chance \end{cases}$		.97	.99
50	p_i=.5	.4 **(Probability sterilize)** starting d5	.77	.84
	p_i ~Beta(10,10)		.85	.89
	p_i ~U(0,1)		.16	.23

	$p_i = \begin{cases} .1 \; with \; 50\% \; chance \\ .9 \; with \; 50\% \; chance \end{cases}$		.52	.67

13.4 Statistical Analyses

The primary analysis of treatment-period ANR will be conducted once subjects have completed their 28-day follow up and reviewed by the DSMB. The follow-up ANR can be conducted once all subjects have completed the week 24 visit. ANR will be computed as the proportion of assays in a specified window that are negative. For the primary analysis, any returned CT value will be considered a positive PCR.

The primary analysis will be based on an Intention-to-Treat framework, in which participants are analyzed in the group to which they are randomized, including participants who reduce or discontinue their individual dosing. In the event that the DSMB recommends a study-wide dose reduction, the primary analysis will remain all people randomized to treatment, regardless of dose, compared to all people randomized to control, but the DSMB can recommend increasing the sample size at that point so there are sufficient people at the lower dose for secondary analyses.

The primary analyses of ANR during the treatment phase and during the follow-up phase will be based on a two-sided permutation test comparing the groups (i.e. using a 2 sample t-test to define the test but use its permutation distribution to compute significance). This will be stratified by number of detectable samples during screening and country, as was done for randomization. No multiplicity adjustment will be imposed on the primary analyses of the ANR from the two different time periods.

As the expectation is that the study will be fully enrolled before we have sufficient data collected on the follow-up phase, there are no formal stopping rules for efficacy or futility.

This study could generate considerable missing data as a consequence of the dose de-escalation plan and unsuccessful donations at individual visits. If there is substantial missingness (more than just a few) then the previous analysis will need to adjust for differences in the number of observations among participants as the proportions from different participants will have different variances under the null hypothesis. This will be accomplished by replacing the 2 sample t-test with a t-test of the regression coefficient associated with an indicator variable for group membership (i.e. active or placebo) from a weighted linear regression analysis with weights given by the number of study visits for which a participant successfully made a donation (these weights are a consequence of inverse relationship between the variance of a binomial proportion and the number of trials).

13.4.1 Secondary Endpoints

Secondary analyses or safety and tolerability endpoints will include tabulation of grade 2 and higher laboratory values, including but not limited to ALT and AST, and adverse events by treatment arm.

Secondary analyses will examine the effect of defining a positive CT value to be any reading of 40 or lower, as well as the main analysis based on a per-protocol definition of treatment arm. Other secondary analyses will include per protocol analyses, Generalized Estimating Equations models for the serial binary endpoints of detectability, exact tests of proportions for binary endpoints such as Sustained Negativity Rate (no detectable Ebola RNA at any included time point), and censored regression models to compare CT trajectories will also be performed.

In the event that the study-wide dose is reduced to 75 mg, a secondary analysis will be restricted to people on both arms enrolled after the dose reduction.

13.5 Operational Futility

Since not all male survivors are interested in enrolling in PREVAIL III, any confirmed male survivor interested in participating in this study will be considered for screening/enrollment.

14 Ethics/Protection of Human Subjects

14.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks, and benefits. Subjects will be given the opportunity to ask questions and have them answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject’s record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

14.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRBs, the FDA, NIAID, OHRP, the pharmaceutical supporter, or the sponsor’s designee.

15 Data Handling and Record Keeping

15.1 Data Capture and Management

Study data will be maintained on CRFs and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' CRFs from PREVAIL III when available. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry onto CRFs will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

15.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP guidelines. Study records will be retained for a minimum of 5 to 7 years and in compliance with institutional, IRB, state, and USG federal, Liberian and Guinean medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by USG, Liberian, and Guinean federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from OCRPRO/NIAID.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

Evaluations/ Procedures	Pre-Screen[a]	Screening/ Week 0 (within 21 days prior to Day 1)	Day 1[f] (Infusion)		Day 2	Day 3	Day 4	Day 5	Day 8 (+1)	Day 11 (+1)	Day 16 (±2)	Day 24 (±2)	Day 28[f] (+3)	Wk. 8[g] (±14 days)	Wk. 12 (±14 days)	Wk. 16 (±14 days)	Wk. 20 (±14 days)	Wk. 24[g] (+5 days) End of Study
			Pre-dose	Post-dose														
Pre-Screening Informed Consent	X																	
Informed consent		X																
Demographics (participant and contacts)		X																
Targeted Physical exam/signs & symptoms			X	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	
Vital signs		X	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[d]	X[d]	X	X[d]	X[d]	X	X	X	X	X
Completed Medical Exam & ROS/ medication history		X																X
Medical and EVD history [a]		X																
Depression Questionnaire		X																X
PTSD Questionnaire [a]		X																X
Stigma and Discrimination Questionnaire [a]		X																
Randomize subject		X																
Infusion				X	X	X	X	X										

Assessment for Adverse events				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review of concomitant meds		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Participant satisfaction survey													X					X
Blood Samples																		
Chemistries, total and direct bilirubin, AST/ALT, and lipids		X	X		X[j]	X[j]	X[j]	X[j]	X	X	X	X	X	X	X	X	X	X
CBC with diff		X	X		X[j]	X[j]	X[j]	X[j]	X	X	X	X	X	X	X	X	X	X
Prothrombin time (PT), aPTT, INR, and D-dimer		X	X		X[j]	X[j]	X[j]	X[j]	X	X	X	X	X	X	X	X	X	X
Leftover serum and plasma storage		X	X[n]		X[j]	X[j]	X[j]	X[j]	X	X	X	X	X	X	X	X	X	X
GS-5734 PK and metabolites - plasma[o]				X[h]			X[j]	X[h]										
PBMCs for intracellular concentrations - PK[o]								X[i]										
eGFR		X																
Ebola antibody levels		X																X
Semen Samples Ebola PCR with CT for NP & GP	X[b]	X[m]					X[k]		X	X	X	X	X	X	X	X	X	X
Ophthalmologic Exam	X[e]																	X[l]
HIV and Syphilis Test		X[p]																

[a] Participants in Guinea can sign a pre-screening informed consent form for semen collection and testing. Procedures conducted under PREVAIL III will be accepted for PREVAIL IV pre-screening.
[b] Positive PCR for NP or GP using the GeneXpert assay at pre-screening in at least one of two or more semen samples within 42 days of randomization.
[c] On infusion days, vital signs will be obtained within 30 minutes prior to start of infusion and 30 minutes and 60 minutes after infusion completes.
[d] Performed only if needed to evaluate new symptoms/complaints.
[e] Baseline ophthalmologic exam to be conducted within 4 months of enrollment in PREVAIL IV. The baseline ophthalmologic exam can be extracted from PREVAIL III data if done within 4 months prior to enrollment in PREVAIL IV. Ophthalmologic exams must be repeated if conducted > 4 months prior to enrollment in PREVAIL IV or if they were not previously done.
[f] Beginning & end of Treatment phase with DSMB evaluation planned for end of treatment phase.
[g] Beginning & end of Follow up phase.
[h] Draw immediately after infusion if possible and not later than 5 minutes after infusion.

[i] Draw within 1 hour after infusion, but may be drawn with the PK plasma sample drawn within 5 minutes after infusion.
[j] Pre-infusion sample.
[k] Day 4 semen samples can +1 e.g. on days 4 or 5 but only one semen sample is needed during infusion.
[l] End of study ophthalmologic exam to be done at 24 weeks +/- 6 weeks
[m] An additional semen sample will be collected during the screening period if the participant has only had one sample with Ebola viral RNA detected or already had two previous semen tests with at least one positive detection of Ebola virus RNA and the last semen sample was collected greater than 21 days prior to enrollment. Note that the results of this sample **do not** affect subject eligibility. A participant with one previous semen sample with positive Ebola virus RNA detected within the -42-day window can provide the second required sample for randomization during the -21 day screening period, if they are able. The results of this sample will be used to stratify the participant and should be done on days -21 to -3.
[n] A portion of excess serum will be used for future serologic testing for hepatitis B and hepatitis C.
[o] When site facility conditions do not allow processing of PK samples, sample should not be collected.
[p] In Guinea and in Liberia for participants not co-enrolled in PREVAIL III.

Appendix C. eGFR Calculation

A normal serum creatinine does not necessarily reflect a normal Glomerular Filtration Rate (GFR). Serum creatinine alone is not a sensitive indicator for mild to moderate kidney injury. Estimate glomerular filtration rate (GFR) can be estimated e(GFR) from measured serum creatinine. Normal estimated GFR (eGFR) has been calculated from population level studies (NHANES III) in the US and have been extensively validated for Caucasians and African Americans and will be used in this study because similar data is not available for Liberia. The eGFR will be estimated using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI) for PREVAIL IV. The West African male population is muscular and lean with low BMI, in a tropical climate without climate control, and a cultural tendency not to drink water. The CKD-EPI has not been validated in the African non-American population. eGFR varies by age; the table below will be used to determine normal age eGFR to determine eligibility.

eGFR Method: The CKD-EPI equation for African American males is:

Black	Male	≤ 0.9	$GFR = 163 \times (S_{cr}/0.9)^{-0.411} \times (0.993)^{Age}$
Black	Male	> 0.9	$GFR = 163 \times (S_{cr}/0.9)^{-1.209} \times (0.993)^{Age}$

Ref: Levey AS, Stevens LA, et al. A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med. 2009; 150:604-612.

The equation does not require weight or height variables because the results are reported normalized to 1.73 m^2 body surface area, which is an accepted average adult surface area.

The table below shows population estimates for mean (average) estimated glomerular filtration rate (eGFR) by age. These means, derived from the NHANES III survey of over 10,000 individuals in the US, demonstrate that eGFR varies across age groups and that kidney function tends to decline with age.

Reference Table for Population Mean eGFRs From NHANES III

Age (Years)	Mean eGFR*
18[a]-29	116 mL/min/1.73 m^2
30-39	107 mL/min/1.73 m^2
40-49	99 mL/min/1.73 m^2
50-59	93 mL/min/1.73 m^2
60-69	85 mL/min/1.73 m^2
70+	75 mL/min/1.73 m^2

[a] Lower age range adjusted from 20years to 18 years of age

Appendix D: Delineation of Principal Investigator roles for the PREVAIL IV protocol

Overview: All investigators interact with subjects, participate in screening, oversee infusions, oversee study staff, review safety data, participate in design, and recruitment efforts. All PIs work with the study staff, the operations staff, the data management center, the pharmacists, and other members of the PREVAIL IV study team. The investigator team shares responsibility for subject safety and communication with the safety office.

Dr. Dennis resides in Liberia and is present at the site on a daily basis. She serves as the primary oversight to the medical monitors and the interface with the John F. Kennedy Hospital (JFK). Dr. Dennis provides daily leadership to the PREVAIL IV study team at JFK and Duport Road.

Dr. Bea resides in Guinea and provides oversight of the study on a daily basis. He serves as the oversight to the medical monitors and the interface with the sites and the Guinea MOH Trial Steering Committee.

Dr. Higgs has primary responsibility for the writing of the protocol and interface with the DSMB, Gilead, the FDA, and the IRBs. Dr. Higgs has overall responsibility for ensuring that the implementation of the protocol complies with applicable regulations and policies. Dr. Higgs travels back and forth to Liberia and Guinea.

In addition to the responsibilities outlined in the "overview" above, Dr. Fischer travels back and forth to Liberia and Guinea as needed and takes a lead role in recruiting subjects not affiliated with the PREVAIL studies previously. Dr. Fischer speaks French and English and is helpful in bridging the team into one functional unit. Dr. Fischer has primary responsibility over the emergency and safety SOPs.

Appendix E: Compensation for Inconvenience

Subjects will be compensated for their time and inconvenience as follows per current PREVAIL standards:

- Pre-screening information session and consent: $20
- Semen collection ($20/visit)
- Follow up visit for results without any additional tests/evaluations ($10)
- Screening (up to 4 separate visits): $20/each = up to $80
- Study Infusion Days 1-5: $30 (x5) = $150
- Follow-up visits during the treatment phase: $20 (x5) = $100
- Follow-up visits for the longer term follow-up phase: $20 per month x 5 = $100
- Study completion bonus after 15 visits over 6 months: $50

If the subjects presents for information only, they will be reimbursed $20. In addition, subjects may receive per diem compensation according to site-specific guidelines

Compensation for participants in Guinea will be in the equivalent GNF conversion.