Title

A Phase Ia Clinical Study of HIV Entry Inhibitor CPT31: Single Ascending Dose Study of Safety, Tolerability, Immunogenicity, and Pharmacokinetics in Healthy Adults

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Protocol

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SPONSOR APPROVAL

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INVESTIGATOR AGREEMENT

I have read the protocol and agree to conduct the study as described herein.

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STUDY IDENTIFICATION

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SYNOPSIS

Title of study: A Phase Ia clinical study of HIV entry inhibitor CPT31: single ascending dose study of safety, tolerability, immunogenicity, and pharmacokinetics in healthy adults
Objectives: The primary objective of the study is: • to assess the safety and tolerability of single subcutaneous (SC) doses of CPT31 in healthy subjects. The secondary objectives of the study are: • to characterize the single SC dose pharmacokinetics (PK) of CPT31 • to identify and characterize antibodies to CPT31
Study design: This will be a single SC dose study. Doses will be administered in an escalating manner following satisfactory review by a Protocol Safety Review Team (PSRT) of the safety, tolerability and PK data through Day 6 from the lower dose levels. This study will comprise a placebo-controlled, double-blind, single-dose, sequential-group design in healthy subjects. Each subject will participate in 1 treatment period and reside in the Clinical Research Unit (CRU) from Day -1 through Day 6. It is planned for 6 subjects per dose level group to receive SC CPT31 and 2 subjects to receive matching placebo. Each group will be divided into 2 cohorts, with each cohort being dosed 72 hours apart. Sentinel dosing will take place in the first cohort, which will comprise 2 subjects, with 1 subject receiving CPT31 and 1 subject receiving placebo. The second cohort will comprise 6 subjects, with 5 subjects receiving CPT31 and 1 subject receiving placebo. Blood samples for PK analysis and assessment of immunogenicity will be collected predose and up to 5 days postdose, with an additional immunogenicity sample taken at the Follow-up visit.
Number of subjects: 32 healthy subjects will be studied in 4 groups (Groups A1 to A4).
Diagnosis and main criteria for inclusion: Healthy male and female subjects aged 18 to 55 years (inclusive) with a body mass index of 18.0 to 32.0 kg/m^2 (inclusive).
Investigational products, dose, and mode of administration: Test products: 15 mg/mL CPT31 solution Proposed dose levels: 0.01, 0.04, 0.12, and 0.24 mg/kg Administration route: SC
Reference product and mode of administration: Reference product: placebo solution Administration route: SC
Duration of subject participation in the study: Planned Screening duration: approximately 4 weeks Planned study duration (Screening to Follow-up): approximately 8 weeks

Endpoints:

Pharmacokinetics:

Blood and urine samples will be collected for the analysis of plasma and urinary concentrations of CPT31. Pharmacokinetic parameters will be derived by noncompartmental analysis. The PK parameters will include area under the plasma concentration-time curve (AUC) from time zero to infinity ($AUC_{0-\infty}$), AUC from time zero to the time of the last quantifiable concentration ($AUC_{0-tlast}$), maximum observed plasma concentration (C_{max}), time of the maximum observed plasma concentration (T_{max}), apparent plasma terminal elimination half-life ($t_{1/2}$), apparent total plasma clearance (CL/F), apparent volume of distribution (V_z/F), amount of drug excreted in the urine (A_e), percentage of dose excreted unchanged (F_e) in urine, and renal clearance (CL_R).

Other PK parameters may also be added.

Safety:

Adverse events, clinical laboratory evaluations (hematology, clinical chemistry, urinalysis), 12-lead electrocardiograms, vital signs measurements, physical examinations, and anti-CPT31 antibodies, e.g., immunoglobulin (Ig)G, IgM.

Statistical methods:

Pharmacokinetics:

Individual plasma concentrations of CPT31 and PK parameters will be listed and summarized using descriptive statistics. Individual urine concentrations of CPT31 will be listed. Individual and mean CPT31 concentration-time profiles will be presented graphically.

Where data are available, CPT31 dose proportionality will be examined across the dose groups. The PK parameters will be analyzed for dose proportionality using a power model approach or analysis of variance (ANOVA) model, as appropriate.

Safety:

Safety parameters will be listed and summarized using descriptive statistics. The frequency of subjects with QTcB >470 ms or change from baseline >30 ms will be summarized for each treatment. No formal statistical analysis of safety data is planned.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
A_e	amount of drug excreted in the urine
AE	adverse event
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	analysis of variance
ART	antiretroviral therapy
AUC	area under the plasma concentration-time curve
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity
$AUC_{0-tlast}$	area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration
AUC_{0-24}	area under the plasma concentration-time curve from time zero to 24 hours postdose
$AUC_{0-\tau}$	area under the plasma concentration-time curve over a dosing interval
cART	combination antiretroviral therapy
CFR	Code of Federal Regulations
CL/F	apparent total plasma clearance
CL_R	renal clearance
C_{max}	maximum observed plasma concentration
C_{min}	minimum observed plasma concentration
CRO	Contract Research Organization
CRU	Clinical Research Unit
CSA	clinical study agreement
CNS	central nervous system
Da	Daltons
DAIDS	Division of AIDS
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture
FDA	Food and Drug Administration
F_e	percentage of drug excreted unchanged
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
hr	hour
HED	human equivalent dose
HIV	human immunodeficiency virus
IB	Investigator's Brochure

IC_{50}	Concentration producing 50% inhibition
IC_{90}	Concentration producing 90% inhibition
ICF	Informed Consent Form
ICH	International Council for/Conference on Harmonisation
Ig	immunoglobulin
IMP	investigational medicinal product
IRB	Institutional Review Board
ISR	injection site reaction
IUD	intrauterine device
IV	Intravenous(ly)
kg	kilogram
Mg	milligram
MATE	multidrug and toxin extrusion
MTD	maximum tolerated dose
NIH	National Institutes of Health
NOAEL	no observed adverse effect level
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
PBMC	Peripheral blood mononuclear cells
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PrEP	pre-exposure prophylaxis
PSRT	Protocol Safety Review Team
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using Fridericia's method
$RA_{AUC0-\tau}$	observed accumulation ratio based on $AUC_{0-\tau}$
RA_{Cmax}	observed accumulation ratio based on C_{max}
SAE	serious adverse event
SC	subcutaneous(ly)
SHIV	simian/human immunodeficiency virus
STI	sexually transmitted infection
$t_{1/2}$	apparent plasma terminal elimination half-life
$TCID_{50}$	concentration producing 50% infection in tissue culture
TK	toxicokinetic(s)
T_{max}	time of the maximum observed plasma concentration
TMF	Trial Master File
V_z/F	apparent volume of distribution

1. INTRODUCTION

1.1. Overview

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) remain serious global health issues. Since the epidemic began, 77 million people have become infected with HIV (1.8 million in the U.S.), and 35 million people have died from AIDS-related illnesses (~700,000 in the U.S.). In 2017, the most current year for which data are available, 1.8 million new cases of HIV infection were diagnosed (~40,000 in the U.S.). Globally, about 9.4 million or ~25% of HIV-positive individuals are not yet diagnosed (170,000 or ~15% in the U.S.)[1,2].

For many patients, combination antiretroviral therapy (cART) has transformed HIV into a chronic manageable illness, however, despite cART approximately 20% of patients do not achieve complete viral suppression[3]. Many cART patients experience serious side effects (including liver toxicity, pancreatitis, cardiotoxicity, central nervous system [CNS] toxicity, rash, and loss of bone density) that reduce quality of life. Additionally, patients often struggle with lifelong daily dosing. These factors negatively affect patient adherence to cART. In a national survey of HIV clinicians, 72 respondents indicated that ~21% of their patients are poorly adherent (Navigen unpublished clinician survey). Published reports estimate adherence as low as 53% in certain populations [4]. Poor adherence results in suboptimal viral suppression, which can lead to drug resistance, virologic failure, and increased HIV transmission rates.

CPT31 is a novel D-peptide HIV entry inhibitor that binds to the gp41 trimer pocket region and addresses many of the limitations of current cART. Natural L-peptides, composed of L-amino acids, are often poor drugs because they are susceptible to proteolytic degradation. The resulting high dose requirements, cost, and delivery challenges have limited the otherwise outstanding potential of peptides to block protein-protein interaction interfaces in diverse diseases. D-peptides, composed entirely of mirror-image D-amino acids, hold great promise as therapeutic agents, since they are resistant to proteolysis. D-peptides also have the advantage of being minimally immunogenic, likely due to their inability to be processed for major histocompatibility complex presentation[5].

D-peptides are ideal HIV entry inhibitors since the HIV fusion machinery (i.e., gp41) is an accessible extracellular target and has been clinically validated by Fuzeon® (enfuvirtide) studies. Fuzeon is an L-peptide derived from the gp41 C-peptide region that binds the gp41 N-trimer and prevents a critical conformation change that drives virus and target-cell membrane fusion. It represented a therapeutic breakthrough as the first Food and Drug Administration (FDA)-approved HIV entry inhibitor but has had limited lasting impact on HIV treatment due to the practical limitations of L-peptide drugs. Two other entry inhibitors are currently approved for use. Selzentry® (maraviroc) blocks HIV entry by binding to a cellular coreceptor, CCR5, and thus does not inhibit strains that use an alternative coreceptor, CXCR4, necessitating expensive and time-consuming tropism testing prior to initiation of therapy. Trogarzo® is a recently approved (2018) monoclonal antibody that inhibits entry by binding the primary HIV receptor CD4. It is currently approved for patients with multidrug resistant HIV and is administered every 14 days.

Navigen, Inc. is developing CPT31 to both treat patients with HIV type 1 infection and prevent HIV infection in healthy individuals. The study outlined in this protocol exclusively focuses on establishing the safety, tolerability, pharmacokinetic, pharmacodynamic, and immunologic profiles of a simple (non-depot) subcutaneous (SC) injectable formulation. However, nonclinical studies with a long-acting formulation of CPT31 (CPT31-LA) are underway in parallel, and we ultimately envision CPT31 to be administered as a long-acting injectable in an extended-release (depot) formulation, differentiating it from current marketed treatments that require one or more pills to be administered daily. Based on current nonclinical data for CPT31, expected advantages over current and pipeline anti-HIV compounds include the following:

- High potency against all major circulating HIV clades and no cross-resistance with existing entry inhibitors;
- Strong barrier to resistance resulting from an exceptionally high binding affinity to a highly conserved region of HIV;
- Low antigenicity;
- Minimal side effects due to high specificity to an extracellular HIV-specific target;
- Pharmacokinetic (PK) profile that potentially supports monthly dosing with extended-release formulation; and
- More convenient dosing that will lead to better compliance in some patient populations.

These advantages support CPT31 use (especially as a long-acting injectable) as a component in a first-line combination therapy in HIV+ individuals. Additionally, because CPT31 has a unique mechanism of action, it could be used as "salvage" therapy for multi-drug-resistant HIV patients. Finally, CPT31's advantages make is a candidate for use by HIV negative individuals as pre-exposure prophylaxis (PrEP) to reduce the likelihood of HIV transmission.

This study will be the first time that CPT31 is administered to humans.

1.2. Summary of Nonclinical Pharmacology

The *in vitro* antiviral activity of CPT31 against laboratory and clinical isolates of HIV-1 was assessed in peripheral blood lymphocytes, human osteosarcoma cells expressing CCR5 (HOS CCR5), and CXCR4-positive HeLa cells (TZM-bl). *In vitro*, CPT31 has a concentration producing 90% inhibition (IC_{90}) ≤10 nM in 96% of HIV strains in an international panel of 60 primary isolates representing clades A, B, C, and D, and circulating recombinant forms CRF01_AE and CRF02_AG, in TZM-B1 cells.

In human peripheral blood mononuclear cells (PBMCs), CPT31 inhibited HIV-1_{NL4-3} with an IC_{90} of ~0.06 nM (reverse transcriptase assay). There was a 3.5-12.8-fold potency loss against HIV-1_{NL4-3} in the presence of 10-40% dialyzed human serum. Extrapolation to 100% human serum predicts an IC_{90} of ~2 nM. The average potency of CPT31 in PBMCs infected with additional isolates from various clades (in the absence of human serum) was ~2-fold less than measured against HIV-1_{NL4-3} (comparing concentration producing 50% inhibition [IC_{50}] since it is less susceptible than IC_{90} due to the relatively large PBMC assay variability).

To achieve full virologic suppression, Navigen is targeting a trough CPT31 serum concentration of ≥4× the serum-adjusted mean IC_{90} of diverse strains in human PBMCs, which, based on the above data, gives an estimated target trough CPT31 serum concentration of ~16 nM (145 ng/mL).

The efficacy of CPT31 in a rhesus macaque model was assessed using both pre-exposure prophylactic and treatment protocols. A virulent simian/human immunodeficiency virus (SHIV) strain was developed for evaluating the effectiveness of HIV envelope-targeting inhibitors such as CPT31[6]. This SHIV-AD8 contains the HIV R5-tropic env AD8, derived from a primary clade B strain. SHIV-AD8 is more pathogenic than commonly used SHIV strains; therefore, it provides a more robust test for CPT31 efficacy. As expected, this strain is highly sensitive to CPT31 with an IC_{50} <20 pM in a standard pseudovirion entry assay.

Male rhesus macaques (n = 4) were given CPT31 via intramuscular injection daily for a total of 10 days at dose levels of 3, 0.5, and 0.125 mg/kg/day. The animals were challenged on Day 4 intrarectally with 1000 × concentration producing 50% infection in tissue culture ($TCID_{50}$) SHIV-AD8. Treatment continued after challenge through Day 10. Animals first were dosed at 3 mg/kg/day for 10 days, and plasma virus levels were monitored for 12 weeks post-challenge. After documenting lack of infection, the study was repeated in the same 4 animals at a dose level of 0.5 mg/kg/day. After 12 weeks and absence of seroconversion, the study was repeated at a dose level of 0.125 mg/kg/day. At this dose level, 3/4 animals seroconverted within 10 days. One hundred percent of historical vehicle control animals seroconvert within 1-week post-challenge in this model.

A therapeutic efficacy study was also completed. Three naïve rhesus macaques were infected with SHIV-AD8 (1000× $TCID_{50}$, rectal challenge) and monitored for 14 weeks until stable viremia was achieved (median 10^5 copies/mL). CPT31 was then dosed at 3 mg/kg/day for 30 days. Rapid ~2-log reductions in plasma viremia were observed. However, as expected, virus levels rebounded after ~2 weeks due to drug resistance (as confirmed by sequencing). A similar result is observed with modern first-line HIV drugs when used as monotherapy against uncontrolled infection[7].

To more closely mimic a traditional therapeutic setting where virus is suppressed by a combination of highly active drugs, a monotherapy maintenance study was performed in which CPT31 was introduced when virus was undetectable. Combination ART was utilized to suppress virus levels in infected rhesus macaques to undetectable levels, and then treatment was transitioned to CPT31 monotherapy to determine if it could prevent viral rebound. CPT31 (3 mg/kg/day) maintained viral suppression in all 4 animals for the duration of the 12 weeks of monotherapy. Significantly, there were no clinical signs of toxicity observed in these animals (including injection site reactions [ISRs]), and clinical chemistry and hematology parameters were all within normal ranges.

1.3. Summary of Safety Pharmacology

Following single doses of up to 20 mg/kg/day CPT31, there were no effects on cardiovascular, respiratory, or central nervous systems in cynomolgus monkeys. No effects on respiratory and central nervous systems were observed in Sprague-Dawley rats at doses up to 60/40 mg/kg/day.

Detailed descriptions of the studies can be found in the Investigator's Brochure (IB)[8].

1.4. Summary of Toxicology

1.4.1. Toxicology in Rats

A study was conducted to evaluate toxicity after a single dose of CPT31 when administered via SC injection to male Sprague-Dawley rats at 0, 10, 30, 60, and 100 mg/kg.

All animals survived to scheduled sacrifice at 24 hours postdose. In the 100 mg/kg dose group, 1/3 animals appeared slow and sensitive to touch at 8 hours postdose, and 3/3 animals were slightly lethargic with increased respiration rates at 24 hours postdose. All CPT31-treated animals had mild to moderate ISRs. No other adverse clinical or necropsy findings were observed. Based on these results, the maximum tolerated dose (MTD) of CPT31 in male Sprague-Dawley rats following single-dose SC administration is 100 mg/kg.

A Good Laboratory Practice (GLP)-compliant 14-day repeat-dose toxicity study evaluated the toxicity (including respiratory and CNS safety pharmacology endpoints) as well as the toxicokinetics (TK) of CPT31 in male and female Sprague-Dawley rats at dose levels of 0, 10, 20, or 60/40 mg/kg/day, administered daily via SC injection. The dose solution strength for all groups was 15 mg/mL. The 60 mg/kg/day dose was not tolerated, and several animals were found dead or were sacrificed moribund on Days 4-8. Dosing in this group was suspended on Day 4 and resumed on Day 7 at the reduced dose of 40 mg/kg/day in surviving animals (although these animals were inadvertently not dosed on Day 10 due to a protocol deviation). All animals that survived past Day 8 survived until their scheduled sacrifice on Day 15.

CPT31 was well tolerated in 63/64 animals in the 10 and 20 mg/kg/day dose groups. A single male animal (R0110) in the low (10 mg/kg/day) dose group was sacrificed moribund on Day 6. In all dose groups, the cause of either death or moribund condition in 16 of 18 animals that did not survive the dosing phase (including Animal R0110) was renal tubular degeneration with correlating clinical pathology changes. The cause of death in the other 2 of 18 animals was hepatic coagulative necrosis. No safety pharmacology (respiratory and CNS) findings were observed even in the high-dose group animals (60/40 mg/kg/day). The findings in Animal R0110 prevented assignment of a no observed adverse effect level (NOAEL) in this study. Notably, Animal R0110 was a statistical outlier in terms of its plasma drug level at terminal sacrifice (24-hours post last dose), which was ~3-fold higher than its dose-group peers (Grubb's test, GraphPad Prism® software).

A 28-day GLP-compliant repeat-dose study evaluated the toxicity and TK of CPT31 in Sprague-Dawley rats at dose levels of 0, 1, 3, and 6 mg/kg/day, administered once daily by SC injection. All CPT31 dose solutions were 5 mg/mL. All animals survived until their scheduled sacrifice. CPT31-related effects included an increased incidence of nodules at the dose sites in males administered 6 mg/kg/day and increased incidence of scabs in the cervical and thoracic skin area of females administered ≥ 3 mg/kg/day; decreased body weight gain in females administered ≥ 3 mg/kg/day and males administered 6 mg/kg/day; decreased food consumption for animals administered 6 mg/kg/day; clinical pathology effects of minimal to mild severity consistent with inflammatory response (higher neutrophil count and fibrinogen

and lower albumin and albumin:globulin ratio) in males administered ≥1 mg/kg/day and females administered ≥ 3 mg/kg/day; and increases in mean IL-1β levels on Day 7 in animals administered 6 mg/kg/day and on Day 28 in males administered ≥ 1 mg/kg/day and females administered ≥ 3 mg/kg/day. CPT31-related non-adverse microscopic findings were observed in the SC injection sites, kidney, and spleen. Microscopic findings included edema and/or macrophage infiltrates in the SC injection sites (correlating with macroscopic thickening) in animals administered ≥ 1 mg/kg/day, slight to moderate focal renal tubular degeneration in males administered ≥ 3 mg/kg/day, minimal to slight focal mononuclear cell infiltration in kidney of males administered ≥ 1 mg/kg/day, and slightly to moderately increased extramedullary hematopoiesis in spleen of males administered ≥ 1 mg/kg/day. All effects exhibited full or partial (SC injection site findings) recovery during the recovery phase. Due to the mild severity of findings and the lack of impact on the health and wellbeing of animals administered up to 6 mg/kg/day, effects for doses up to 6 mg/kg/day were considered non-adverse. Thus, the NOAEL in this study was 6 mg/kg/day. This dose level corresponded to maximum observed plasma concentration (C_{max}) and area under the concentration-time curve (AUC) from time zero to 24 hours postdose (AUC_{0-24}) values of 6,900 ng/mL (764 nM) and 92,300 hr*ng/mL (10,223 hr*nM), respectively, on Day 28 of the dosing phase.

1.4.2. Toxicology in Monkeys

A study was conducted to evaluate the toxicity of escalating SC doses of 10, 20, 40, and 80 mg/kg CPT31 doses on Days 1, 4, 8, and 12, respectively to one male and one female cynomolgus monkey. The 3- to 4-day interval between the dose days served as a washout period. The dose level of 80 mg/kg was well tolerated in the male animal. The female animal administered 80 mg/kg CPT31 showed midline hemorrhage with swelling, and the animal was sacrificed as scheduled on Day 15. The cause of hemorrhage was not identified. Other clinical pathology findings in this female animal were consistent with inflammation, including increased white blood cell and neutrophil counts, moderately decreased total protein, albumin, albumin:globulin ratio, and mildly increased globulin. Based on the clinical observations and correlating clinical pathology changes, a dose level of 80 mg/kg/day was considered adverse; therefore, 40 mg/kg/day is the MTD when CPT31 is administered as a single dose.

Another study was conducted to evaluate the toxicity of CPT31 when given as repeat doses via SC injection to cynomolgus monkeys. Dose groups were 10, 40, or 80 mg/kg/day CPT31 for 14 days via SC injection in the scapular region. Administration of 40 or 80 mg/kg/day was terminated after Day 2 due to toxicity. CPT31-related unscheduled sacrifice or death occurred for males administered 40 or 80 mg/kg/day and females administered 80 mg/kg/day. CPT31-related clinical observations for these animals included hypoactivity, hunched posture, decreased reactivity to stimulus, pallor, high or subnormal temperature, lethargy, irregular respiration, reduced food consumption, and moribund condition. Clinical observations at the dose sites included moderate edema and bruising which were localized to the dose site or spread across the entire quadrant designated for injection, although only 2 of 4 dose sites were utilized. Clinical observations correlated with major clinical pathology findings in animals administered ≥40 mg/kg/day, which were generally consistent with inflammation and coagulopathy. Bone marrow hypocellularity in males administered 40 mg/kg/day correlated with decreased peripheral blood cell counts. CPT31-related, non-adverse, SC dose site reactions were observed in both sexes administered 10 mg/kg/day

and males administered 40 mg/kg/day. Effects for animals administered 10 mg/kg/day were considered non-adverse. Thus, the NOAEL is 10 mg/kg/day.

A GLP-compliant 14-day repeat-dose study evaluated the toxicity (including cardiovascular, respiratory, and CNS safety pharmacology endpoints) as well as TK of CPT31 in cynomolgus monkeys at dose levels of 0, 5, 10, or 20 mg/kg/day administered daily via SC injection. Two groups of animals were treated at 10 mg/kg/day: one at 5 mg/mL and one at 15 mg/mL CPT31 solution to compare the effects of dose concentration. All remaining groups were treated at 15 mg/mL CPT31 solution. The study also evaluated the reversibility, persistence, or delayed occurrence of any effects during a 14-day recovery phase. All animals survived until scheduled sacrifice. Major findings included CPT31-related renal tubular degeneration and necrosis with correlating clinical chemistry changes, and dose-dependent clinical laboratory changes suggestive of inflammation that correlated with SC ISRs. CPT31-related increases in mean IL-6 levels above the lower limit of quantitation and above control means were noted at 3 hours postdose on Day 7 of the dosing phase in females administered 10 mg/kg/day (15 mg/mL) and animals administered 20 mg/kg/day. The finding in animals administered 20 mg/kg/day was consistent with the increase in C-reactive protein on Days 7 and 15 of the dosing phase. No CPT31-related changes were noted in IL-1α, IL-1β, or TNF-α in animals administered ≥5 mg/kg/day. The NOAEL in this study was 10 mg/kg/day regardless of dose solution strength. This dose level corresponded to mean (across male and female groups) C_{max} and AUC_{0-24} values of 25,400 ng/mL (2,813 nM) and 380,000 hr∗ng/mL (42,086 hr∗nM), respectively on Day 14 of the dosing phase.

1.5. Summary of Nonclinical Pharmacokinetics

Following intravenous (IV) or SC administration (1 mg/kg) in Sprague-Dawley rats, the apparent plasma terminal elimination half-life ($t_{1/2}$) of CPT31 was 3.2 and 5.5 hours, respectively, and SC bioavailability was 53%. Similarly, following IV or SC administration (1 or 3 mg/kg, respectively) in cynomolgus macaques, the $t_{1/2}$ of CPT31 was 7 and 18.6 hours, respectively, and SC bioavailability was 78%. CPT31 given IV to Sprague-Dawley rats or monkeys has a low volume of distribution (134 mL/kg and 96 mL/kg, respectively), suggesting that it is largely confined to the plasma compartment. Plasma protein binding studies revealed that 5 µM CPT31 is ≥96% bound in rat, minipig, dog, monkey, and human plasma.

Both C_{max} and AUC of CPT31 upon SC administration to Sprague-Dawley rats are linear (but not fully dose proportional) at increasing dose levels up to 40 mg/kg. Terminal half-life is consistently between 4 and 8 hours at dose levels ranging from 3-75 mg/kg.

A distribution/excretion study using radioactive [^{125}I]-CPT31 suggested that CPT31 is eliminated via renal clearance, as expected for a mid-molecular weight compound (average 9,029 Daltons). Composed of D-peptides, CPT31 is not expected to enter peptide catabolic pathways.

Non-parametric superposition of available cynomolgus macaques PK data (Table 1) and allometric scaling to humans predicts that a 0.03 mg/kg SC injection every third day would give a trough level ~13-22 nM (117-199 ng/mL). Therefore, this dose is tentatively set as the expected human therapeutic dose in order to achieve a trough CPT31 level of ≥4 × the

serum-adjusted IC_{90} against diverse strains in human PBMCs (~16 nM), as described above. However, the PK profile in humans remains to be established, and the estimated effective dosing will change accordingly.

Table 1: Pharmacokinetic Parameters of CPT31 in Cynomolgus Macaques

Day	Dose (mg/kg/day)	Sex	AUC_{0-24} (hr*ng/mL)	C_{max} (ng/mL)
1	5	M	252 000	15 100
		F	248 000	16 100
	10	M	356 000	23 000
		F	393 000	25 700
	20	M	686 000	41 600
		F	627 000	37 800
14	5	M	264 000	16 700
		F	270 000	17 500
	10	M	369 000	25 500
		F	391 000	25200
	20	M	396 000	30 800
		F	676 000	54 500

Abbreviations: AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours postdose; C_{max} = maximum observed plasma concentration; hr = hour; T_{max} = time of the maximum observed plasma concentration.

There is little potential for significant CPT31 drug-drug interactions involving cytochrome P450 based on direct inhibition studies in liver microsomes. There is also low intrinsic clearance of CPT31 in microsomes as well as low to medium CPT31 intrinsic clearance in hepatocytes. In all species tested (rat, minipig, dog, monkey, and human), clearance rates are greater in hepatocytes than in liver microsomes, indicating the possibility of a non-cytochrome P450-mediated mechanism of biotransformation. Furthermore, CPT31 did not significantly inhibit key cell transporters (P-glycoprotein, Breast Cancer Resistance Protein, organic anion transporting polypeptide ([OATP]1B1, OATP1B3, organic cation transporter [OCT]2, OAT1, OAT3, multidrug and toxin extrusion [MATE]1, MATE2-K), indicating low therapeutic potential for drug-drug interactions as a result of transporter inhibition.

Putative metabolites formed during non-NADPH-dependent CPT31 clearance observed in rat, monkey, and human microsomes were sought and not detected in a study using liver microsomes. By contrast, another study sought putative CPT31 metabolites formed during clearance in rat, monkey, and human hepatocytes. While partial degradation in all species was observed, no specific species attributable to CPT31 metabolism were detected.

1.6. Study Rationale

This is the first time CPT31 will be administered to humans. The principal aim of this study is to obtain safety and tolerability data when CPT31 is administered SC as a single dose to healthy subjects. This information, together with the PK data, will help establish the doses and dosing regimen suitable for future studies in patients.

1.7. Benefit-risk Assessment

Subjects in this study will not gain any clinical benefits. Study subjects and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of safe and effective interventions to treat and/or prevent HIV transmission.

The risks of participation are primarily those associated with adverse reactions to the investigational medicinal product (IMP), although there may also be some discomfort from collection of blood samples and other study procedures.

Phlebotomy may lead to discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection. Good venipuncture practices reduce the pain and likelihood of bruising and secondary infection. Precautions will be taken with subjects who have a history of fainting or other vasovagal events during blood draws, including having the subject lie down during the procedure.

Subcutaneous injections can cause injection site swelling, warmth, and discomfort. These ISRs generally resolve within 3 days. Inadvertent administration of study product into a vein or into a nerve can occur. Injections are only administered by trained designated study personnel, and numerous precautions are taken to minimize this risk.

Electrocardiograms (ECGs) have no major risks. However, mild pain may be associated with removal of the electrodes and mild skin irritation may develop at the sites of electrode attachment.

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

The primary objective of the study is:

- to assess the safety and tolerability of a single SC dose of CPT31 in healthy subjects

The secondary objectives of the study are:

- to characterize the single SC dose PK of CPT31
- to identify and characterize antibodies to CPT31

2.2. Endpoints

2.2.1. Primary Endpoints

The primary safety endpoints for this study are as follows:

- incidence and severity of adverse events (AEs)
- incidence of laboratory abnormalities, based on hematology, clinical chemistry, and urinalysis test results

- 12-lead electrocardiogram (ECG) parameters
- vital signs measurements
- physical examinations
- anti-CPT31 antibodies, e.g., immunoglobulin (Ig)G, IgM

2.2.2. Secondary Endpoints

The single ascending dose PK outcome endpoints of CPT31 are as follows:

- AUC from time zero to infinity ($AUC_{0-\infty}$)
- AUC from time zero to the time of the last quantifiable concentration ($AUC_{0-tlast}$)
- C_{max}
- time of the maximum observed plasma concentration (T_{max})
- $t_{1/2}$
- apparent total plasma clearance (CL/F)
- apparent volume of distribution (V_z/F)
- renal clearance (CL_R)
- amount of drug excreted in the urine (A_e)
- percentage of dose excreted unchanged in the urine (F_e)

Other PK parameters may also be reported.

3. INVESTIGATIONAL PLAN

This will be a single SC dose study.

3.1. Overall Study Design and Plan

This study will comprise a placebo-controlled, double-blind, single-dose, sequential-group design. Overall, it is planned for 32 healthy subjects to be studied in 4 groups (Groups A1 to A4), with each group consisting of 8 subjects. In each group, 6 subjects will be randomized to receive SC CPT31 and 2 subjects will be randomized to receive matching placebo.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration.

Each subject will participate in 1 treatment period only. Subjects will reside at the Clinical Research Unit (CRU) from Day -1 (the day before dosing) to Day 6. All subjects will return for a Follow-up visit 28 to 30 days after dosing.

Based on the ongoing review of the safety, tolerability, and PK results, additional nonresidential visits may be required. The number of additional visits per subject will not exceed 3 and will not extend beyond 30 days after dose administration.

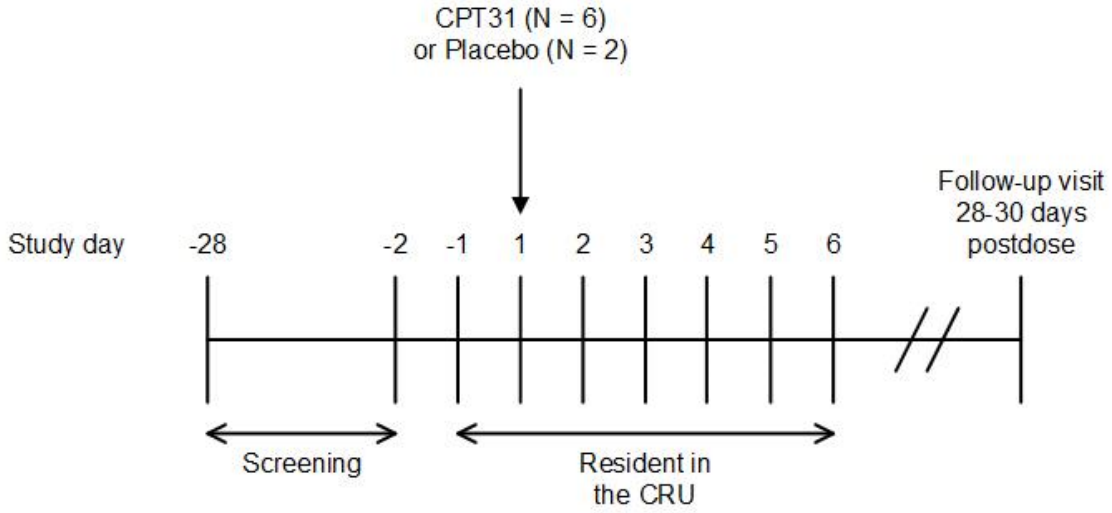
All groups will be divided into 2 cohorts, with each cohort being dosed 72 hours apart. The first (sentinel) cohort will comprise 2 subjects, with 1 subject receiving CPT31 and 1 subject receiving placebo. The second cohort will comprise 6 subjects, with 5 subjects receiving CPT31 and 1 subject receiving placebo.

The maximum total duration of study participation for each subject (from Screening through Follow-up visit) is anticipated to be approximately 8 weeks.

Dose escalation will occur a minimum of 7 days after all subjects complete all Day 6 assessments in the previous dose group.

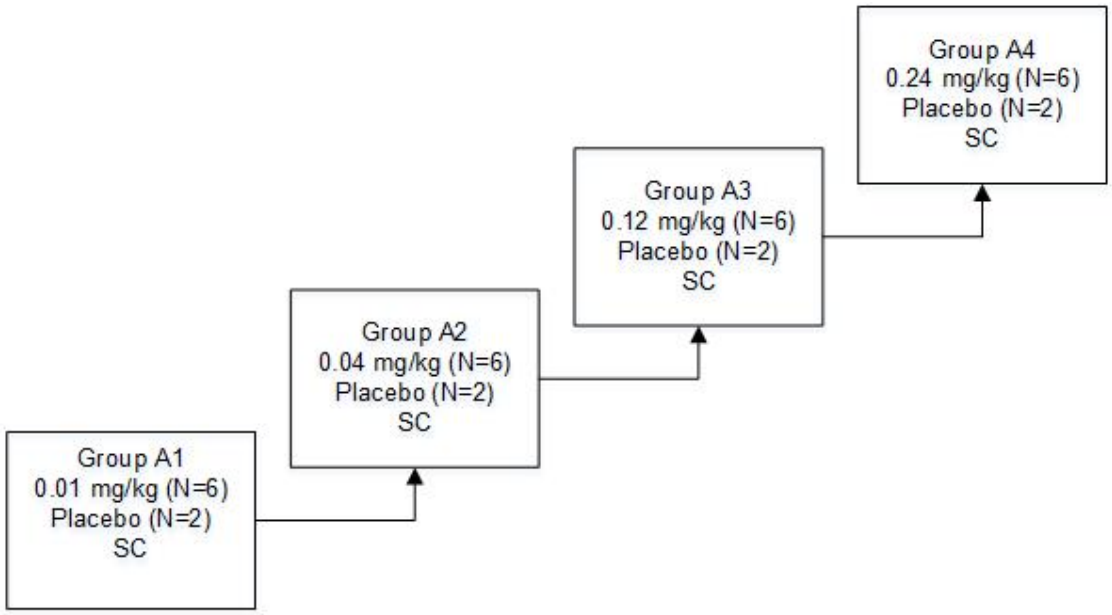
An overview of the study design is shown in Figure 1 and the planned dose levels in are shown in Figure 2. A Schedule of Assessments is presented in Appendix 6.

Figure 1: Study Schematic



Abbreviations: CRU = Clinical Research Unit, N = number of subjects.

Figure 2: Planned Dose Levels



Abbreviations: N = number of subjects; SC = subcutaneous.

3.2. Study Start and End of Study Definitions

The start of the study is defined as the date the first enrolled subject signs an Informed Consent Form (ICF). The point of enrollment occurs at the time of subject number allocation. The end of the study is defined as the date of the last subject's last assessment (scheduled or unscheduled).

3.3. Additional Groups

Following review of the safety, tolerability, and PK data, additional dose groups may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included.

The requirement for additional groups will be decided by the Protocol Safety Review Team (PSRT), comprised of the Investigator, Sponsor Representative, Medical Monitor, and NIH Division of AIDS (DAIDS) Medical Monitor). Changes will be documented in the Trial Master File (TMF), and the IRB will be notified.

3.4. Discussion of Study Design, Including the Choice of Control Groups

A sequential-group, ascending-dose design has been chosen for safety reasons as CPT31 is in the early stages of clinical development, with this study being the first time it will be administered to humans. Subcutaneous doses have been chosen for the study, as this is the intended clinical route of administration.

Continuous 12-lead ECG monitoring will be included for Groups A3 and A4 in order to allow assessment of the relationship between CPT31 concentrations and QT interval

corrected for heart rate (QTc), with a view to supporting a potential thorough QT study waiver.

Based upon the nonclinical data, the planned duration of the study is considered adequate to achieve the study objectives.

This study will be double-blind and placebo-controlled in order to avoid bias in the collection and evaluation of data during its conduct. Placebo has been chosen as the control treatment to assess whether any observed effects are treatment-related or simply reflect the study conditions.

Conducting the study in healthy subjects mitigates the potential confounding effects of the disease state and concomitant medications.

3.4.1. Dose Interval

Dosing at each dose level will be such that 2 subjects (1 CPT31 and 1 placebo) will be dosed 72 hours before the remaining 6 subjects. After dosing the first 2 subjects on a separate day, a minimum of a 5-minute dosing interval for the remaining 6 subjects in each dose-ascending cohort is considered acceptable. Continuation to dose the remaining 6 subjects will be made by the PSRT.

3.5. Selection of Doses in the Study

Based on activity in human PBMCs infected with diverse primary isolates, the estimated effective serum trough concentration of CPT31 is 16 nM (145 ng/mL) (4x the serum-adjusted IC_{90} in PBMCs).

Human PK modeling predicts that a dose of 0.03 mg/kg administered every third day will be optimal to maintain CPT31 serum trough levels in a narrow range (~13-22 nM, 117-199 ng/mL) that is close to the target trough concentration of 16 nM while minimizing total exposure (C_{max} ~50 nM, 452 ng/mL) over the course of an envisioned future short-duration (~2-week) multiple ascending dose human study. We propose a starting dose of 0.01 mg/kg in this first-in-human single ascending dose study, with subsequent dose escalations to 0.04 mg/kg (4-fold increase), 0.12 mg/kg (3-fold increase), and 0.24 mg/kg (2-fold increase). The safety margins for the 0.01 mg/kg human dose relative to rat and monkey NOAELs are 97 and 320, respectively (Table 2).

Table 2: Human Safety Margin Estimates for CPT31

Study Type	Species	Duration	NOAEL		HED (mg/kg)*	Human Safety Margins**
			mg/kg	mg/m^2		
Safety Pharmacology						
CNS	Rat	Single dose	60	360	9.7	970
CNS	Monkey	14 days	20	240	6.5	650
Respiratory	Rat	Single dose	60	360	9.7	970
Respiratory	Monkey	14 days	20	240	6.5	650
Cardiovascular	Monkey	14 days	20	240	6.5	650

General Toxicology						
Repeat-dose	Rat	14 days	ND	ND	ND	ND
	Rat	28 days	6	36	0.97	97
	Monkey	14 days	10	120	3.2	320

CNS = central nervous system; HED = human equivalent dose; ND = not determined; NOAEL = no observed adverse effect level.

* Based on allometric conversion between species based on mg/kg dose levels. Allometric conversion factors used for rat and monkey were 6.2 and 3.1, respectively. Assumes body weights for rat, monkey, and human of 0.15, 3, and 60 kg, respectively[9,10].

** Calculated by dividing the HED by the proposed CPT31 clinical start dose of 0.01 mg/kg.

The planned IMP dose levels for the study are shown in Table 3.

Table 3: Planned Investigational Medicinal Product Dose Levels

Group	Subject Numbers	Dose
A1	0101 – 0108	0.01 mg/kg or placebo
A2	0109 – 0116	0.04 mg/kg or placebo
A3	0117 –0124	0.12 mg/kg or placebo
A4	0125 – 0132	0.24mg/kg or placebo

Dose levels should not increase by more than 5-fold for predicted nonpharmacologically active dose levels and by more than 3-fold for predicted pharmacologically active dose levels (≥0.03 mg/kg by SC injection every third day).

The highest dose level may exceed the top dose shown in the above table, provided the dose escalation stopping rules are not exceeded (Section 3.7).

Details of all doses administered in the study will be documented in the TMF.

3.6. Dose Escalation

Doses will be administered in an escalating manner following satisfactory review by the PSRT of the safety, tolerability, and PK data (up to 5 days postdose) from the lower dose levels. Any clinically significant results will be discussed before dose escalation continues. Interim PK data will also be reviewed in terms of dose escalation and to confirm that the study design remains appropriate. Doses may be reduced and may be lower than the starting dose. There will be a minimum of 7 days between completion of Day 6 assessments in the previous dose group and dosing in the next dose group to allow sufficient time for an adequate safety review.

Dose escalation will only occur if data from all participants in the dosing group have been reviewed by the PSRT.

An interim safety report, summarizing results from all available safety assessments, will be sent to the Sponsor prior to the start of each successive group.

3.7. Dose Escalation Stopping Rules

Following discussion among the PSRT, dose escalation will stop if:

- Clinically relevant signs or symptoms of similar nature occur in 2 or more subjects in a group that, in the opinion of the Investigator, warrant stopping of dose escalation.
- One or more subjects experience a serious AE (SAE) that is considered to be related to IMP.
- Two or more subjects experience a Grade 3 or 4 AE or laboratory abnormality that is considered to be at least possibly related to IMP.
- There is evidence of clinically significant increases in liver function tests (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, and/or gamma-glutamyl transferase), defined as 3 times the upper limit of normal in 3 or more subjects in a group (confirmed with repeat testing).
- Clinically significant increases in serum creatinine or blood urea nitrogen, defined as 1.5 times and 2 times the upper limit of normal, respectively (confirmed with repeat testing), in 3 or more subjects in a group.

3.8. Overall Study Stopping Rules

The study may be discontinued at the discretion of the PSRT if any of the following criteria are met:

- AEs unknown to date (i.e., not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration)
- increased frequency, severity, and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined at Day -1 as baseline signs and symptoms)
- medical or ethical reasons affecting the continued performance of the study
- difficulties in the recruitment of subjects
- cancelation of drug development.

The study will be discontinued if 2 or more subjects experience a Grade 3 or 4 AE or laboratory abnormality that is considered to be at least possibly related to IMP.

The study may be stopped at any time prior to completion by the sponsor, FDA, IRB, and/or doctors participating in the study.

4. SELECTION OF STUDY POPULATION

4.1. Healthy Subjects

4.1.1. Inclusion Criteria

Subjects must satisfy all the following criteria at the Screening Visit unless otherwise stated:

1. Males or females, of any race, between 18 and 55 years of age, inclusive.
2. Body mass index between 18.0 and 32.0 kg/m^2, inclusive.
3. In good health, determined by no clinically significant findings from medical and surgical history, physical examination, 12-lead ECG, vital signs measurements, and clinical laboratory evaluations (congenital nonhemolytic hyperbilirubinemia [e.g., suspicion of Gilbert's syndrome based on total and direct bilirubin] is not acceptable) at Screening and/or Day -1 as assessed by the Investigator (or designee).
4. Females will not be pregnant or have been within the previous 3 months, or lactating, and females of childbearing potential and males will agree to use contraception, as detailed in Appendix 4.
5. Able to comprehend and willing to sign an ICF and to abide by the study restrictions.

4.1.2. Exclusion Criteria

Subjects will be excluded from the study if they satisfy any of the following criteria at the Screening Visit unless otherwise stated:

1. Significant history or clinical manifestation of any metabolic, allergic, dermatological, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neurological, respiratory, endocrine, or psychiatric disorder, as determined by the Investigator (or designee).
2. A ≥Grade 2 laboratory abnormality at Screening or Day -1 as defined by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 dated July 2017.
3. Estimated glomerular filtration rate (eGFR per CKD-Epi equation) of <90 ml/min/1.73 m^2.
4. Known sensitivity to CPT31 or any of its components.
5. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator (or designee).
6. History of alcoholism or drug/chemical abuse within 2 years prior to Day -1.
7. Alcohol consumption of > 21 units per week for males and > 14 units for females. One unit of alcohol equals 12 oz (360 mL) beer, 1½ oz (45 mL) liquor, or 5 oz (150 mL) wine.
8. Positive urine drug screen at Screening or positive alcohol breath test result or positive urine drug screen on Day -1.
9. Positive HIV test as documented by Combo Ag/Ab HIV-1/HIV-2 immunoassay.
10. Positive hepatitis B surface antigen, positive hepatitis B core antibody with negative hepatitis B surface antibody test result, or positive hepatitis C antibody at Screening or within 3 months before first dose of study treatment.
11. Participation in a clinical study involving administration of an investigational drug in the past 30 days prior to dosing.

12. Use or intend to use any prescription or over the counter medications/products (including HIV medications being used for pre-exposure prophylaxis) other than hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives or acetaminophen up to 2 grams per day for no more than 3 consecutive days within 14 days prior to dosing, unless deemed acceptable by the Investigator (or designee).
13. Use of tobacco- or nicotine-containing products within 3 months prior to Day -1, or positive cotinine at Screening or Day -1.
14. Receipt of blood products within 2 months prior to Day -1.
15. Donation of blood from 3 months prior to Screening, plasma from 2 weeks prior to Screening, or platelets from 6 weeks prior to Screening.
16. Poor peripheral venous access.
17. Have previously completed or withdrawn from this study or any other study investigating CPT31 and have previously received the investigational product.
18. Subjects who, in the opinion of the Investigator (or designee), should not participate in this study.

4.1.2.1. Additional Exclusion Criteria

19. Have any clinically significant abnormal ECG results constituting a risk while taking the investigational product, as determined by the Investigator, such as any of the following, as determined by single 12-lead ECG:
    a. QT interval corrected for heart rate using Fridericia's method (QTcF) >450 ms for males and >470 ms for females, confirmed by calculating the mean of the original value and 2 repeats.
    b. QRS duration >120 ms confirmed by calculating the mean of the original value and 2 repeats.
    c. PR interval >220 ms confirmed by calculating the mean of the original value and 2 repeats.
    d. findings which would make QTc measurements difficult or QTc data uninterpretable.
    e. history of additional risk factors for torsades de pointes (e.g., heart failure, hypokalemia, family history of long QT syndrome).
    f. Risks related to bradycardia, for example, second or third degree atrioventricular block or sick sinus syndrome

4.2. Subject Number and Identification

Subjects will have a unique identification number used at Screening. Subjects will be assigned a subject number at the time of their randomization. Assignment of subject numbers will be in ascending order and no numbers will be omitted (e.g., Subjects 0101, 0102, etc.). Replacement subjects (Section 4.3) will be assigned a subject number corresponding to the

number of the subject he/she is replacing plus 1000 (e.g., Subject 1101 replaces Subject 0101).

Subjects will be identified by identification or subject number only on all study documentation. A list identifying the subjects by subject number will be kept in the Site Master File. Photos of subjects may be taken for subject identification.

4.3. Subject Withdrawal and Replacement

A subject is free to withdraw from the study at any time. In addition, a subject will be withdrawn if any of the following criteria are met:

- change in compliance with any inclusion/exclusion criterion that is clinically relevant and affects subject safety as determined by the Investigator (or designee)
- noncompliance with the study restrictions that might affect subject safety or study assessments/objectives, as considered applicable by the Investigator (or designee)
- any clinically relevant sign or symptom that, in the opinion of the Investigator (or designee), warrants subject withdrawal.

If a subject is withdrawn, the Sponsor will be notified and the date and reason(s) for the withdrawal will be documented in the subject's electronic Case Report Form (eCRF). If a subject is withdrawn, efforts will be made to perform all follow-up assessments, if possible (Appendix 6). Other procedures may be performed at the Investigator's (or designee's) and/or Sponsor's discretion. If the subject is in-house, these procedures should be performed before the subject is discharged from the clinic. The Investigator (or designee) may also request that the subject return for an additional Follow-up visit. All withdrawn subjects will be followed until resolution of all their AEs or until the unresolved AEs are judged by the Investigator (or designee) to have stabilized.

Subjects who are withdrawn for reasons not related to study drug may be replaced following discussion of the PSRT Subjects withdrawn as a result of AEs thought to be related to the study drug will generally not be replaced.

5. STUDY TREATMENTS

5.1. Description, Storage, Packaging, and Labeling

The IMPs (15 mg/mL CPT31 histidine formulation [50 mM histidine, 200 mM mannitol, pH 7.2 solution for SC injection] and placebo) will be supplied by the Sponsor (or designee), along with the batch/lot numbers and Certificates of Analysis. All IMPs will be provided in single-use vials.

All IMPs will be stored at 2ºC to 8ºC at the study site in a location that is locked with restricted access.

The IMPs will be labeled in accordance with national laws and regulations.

5.2. Study Treatment Administration

All IMP vials will be removed from storage at 2ºC to 8ºC for between 5 to 60 minutes prior to dose preparation. The IMP vials will be swirled for approximately 3-5 seconds, IMP will be drawn up into 1 or more BD tuberculin syringes (0.3 or 1.0 mL), and syringes containing IMP will be used within 6 hours after dose preparation. Each dose of CPT31 and placebo will be administered by SC injection into the abdomen while the subject is in a semi-supine or sitting position. Subjects will be fasted for at least 8 hours prior to dosing and for at least 2 hours after dosing; a meal will be provided after the 2-hour postdose assessments are completed.

Subjects will be administered the IMP while semi-supine or seated and will remain semi-supine or seated for at least 30 minutes after dosing, except as necessitated by the occurrence of an AE(s) and/or study procedures.

The doses and estimated injection volumes are detailed in Table 4. Rounding may be used based on measurable volumes in the appropriate syringe.

Table 4: Injection Volumes

Group	Dose Level (mg/kg)	Frequency	Estimated Injection Volume[a] (mL)
A1	0.01	Once	0.047
A2	0.04	Once	0.187
A3	0.12	Once	0.56
A4	0.24	Once	1.12

[a] Assuming a 70 kg subject.

5.3. Randomization

The randomization code will be produced by the Statistics Department at Covance using a computer-generated pseudo-random permutation procedure. Two subjects per group will be randomly assigned to receive placebo. For all groups, sentinel dosing will occur whereby 2 subjects (1 active and 1 placebo) will be dosed on 1 day and, providing no safety concerns arise, the remaining 6 subjects (5 active and 1 placebo) will be dosed after 72 hours.

Prior to the start of the study, the live randomization will be distributed according to the randomization specification form and will include the Covance CRU pharmacy staff.

5.4. Blinding

The following controls will be employed to maintain the double-blind status of the study:

- The placebo solution may not be identical in appearance to the CPT31 solution. The unblinded pharmacy staff member will prepare syringes for injection (placebo and CPT31 solution) and an unblinded study nurse will perform the injections. If color differences are apparent between active and placebo in dosing syringes, then subjects will be blindfolded prior to and during study drug/placebo injection. Other Covance staff, e.g., CRCs, will be asked to avert their eyes during study drug/placebo injection.

- The Investigator and other members of staff involved with the study will remain blinded to the treatment randomization code during the assembly procedure.
- Interim bioanalytical data will be provided to the PSRT in a blinded manner.

To maintain the blind, the Investigator will be provided with a sealed randomization code for each subject, containing details of their treatment. These individual sealed envelopes will be kept in a limited access area that is accessible 24 hours a day. In order to manage subject safety or to support dose escalation decisions (in the event of possibly treatment-related SAEs or Grade ≥3 AEs), the decision to unblind resides solely with the Investigator. Whenever possible and providing it does not interfere with or delay any decision in the best interest of the subject, the Investigator will discuss the intended code-break with the Sponsor. If it becomes necessary to break the code during the study, the date, time, and reason will be recorded in the subject's source data and on the individual envelope and will be witnessed by a second person.

5.5. Treatment Compliance

The following measures will be employed to ensure treatment compliance:

- All doses will be administered under the supervision of suitably qualified study site staff.
- At each dose preparation occasion, a predose and postdose inventory of IMP will be performed.

5.6. Drug Accountability

The Investigator (or designee) will maintain an accurate record of the receipt of the study supplies received. In addition, an accurate drug disposition record will be kept, specifying the amount dispensed to each subject and the date of dispensing. This drug accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by the Sponsor upon request.

Empty or partially used IMP vials will be discarded upon satisfactory completion of the compliance and accountability procedures. Any unused IMP vials will be retained until completion of the study.

At the completion of the study, all unused supplies will be returned to the Sponsor or disposed of by the study site, per the Sponsor's written instructions.

6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Concomitant Therapies

Subjects will refrain from use of any prescription or nonprescription medications/products (including HIV medications being used for pre-exposure prophylaxis) within 14 days of dosing, unless the Investigator (or designee) and/or Sponsor have given their prior consent.

Paracetamol/acetaminophen (2 g/day for up to 3 consecutive days), hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives are acceptable concomitant medications. The administration of any other concomitant medications during the study is prohibited without prior approval of the Investigator (or designee), unless its use is deemed necessary for treatment of an AE. Any medication taken by a subject during the course of the study and the reason for its use will be documented in the source data.

All concomitant medications, over-the-counter preparations, vitamins and nutritional supplements, recreational drugs, and herbal preparations reported throughout the course of the study will be recorded in the source documents and on eCRFs designated for that purpose.

6.2. Diet

While confined at the study site, subjects will receive a standardized diet at scheduled times that do not conflict with other study-related activities. Subjects will be fasted overnight (at least 8 hours) before collection of blood samples for clinical laboratory evaluations that include the lipid panel (see Appendix 6).

On the days with intensive PK assessments (Day 1), meals will be identical for each group.

On Day 1, subjects will be fasted for at least 8 hours prior to dosing, and at least 2 hours after dosing; a light breakfast will be provided after the 2-hour postdose assessments are completed. Breakfast time will be staggered between subjects to ensure that it is taken at the same time with respect to dosing.

Meals will be provided as appropriate at other times. Water will be freely available at all times.

Caffeine-containing foods and beverages will not be allowed from 48 hours before Day -1 until Discharge.

Consumption of alcohol will not be permitted from 72 hours prior to Day -1 until the Follow-up visit.

6.3. Smoking

Subjects will not be permitted to use tobacco- or nicotine-containing products within 3 months prior to Day -1 until the Follow-up visit.

6.4. Exercise

Subjects are required to refrain from strenuous exercise from 7 days before Day -1 until the Follow-up visit and will otherwise maintain their normal level of physical activity during this time (i.e., will not begin a new exercise program nor participate in any unusually strenuous physical exertion).

6.5. Blood Donation

Subjects are required to refrain from donation of blood from 3 months prior to Screening, plasma from 2 weeks prior to Screening, and platelets from 6 weeks prior to Screening until 3 months after the Follow-up visit.

7. STUDY ASSESSMENTS AND PROCEDURES

Every effort will be made to schedule and perform the procedures as closely as possible to the nominal time, giving considerations to appropriate posture conditions, practical restrictions, and the other procedures to be performed at the same timepoint.

The highest priority procedures will be performed closest to the nominal time. The order of priority for scheduling procedures around a timepoint is (in descending order of priority):

- dosing
- blood samples
- urine samples (for CPT31 assay, males only)
- any other procedures (ECGs will be scheduled before vital signs measurements).

Where activities at a given timepoint coincide, consideration must be given to ensure that the following order of activities is maintained: ECGs, vital signs, blood draws.

7.1. Pharmacokinetic Assessments

7.1.1. Sample Collection and Processing

Blood samples (approximately 2 × 1 mL) will be collected by venipuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 6. Furthermore, up to 3 additional blood samples may be taken from each subject, with the maximum volume of blood withdrawn per subject not exceeding the limit detailed in Appendix 3. Any changes to the scheduled times of PK assessments will be agreed with the Sponsor and documented in the TMF. Samples taken from subjects who received placebo will not be analyzed.

Procedures for collection, processing, and shipping of PK blood samples will be detailed in a separate document.

Urine samples, for male subjects only, will be collected into pre-weighed polyethylene containers over the time intervals indicated in the Schedule of Assessments in Appendix 6. Procedures for collection, processing, and shipping of urine samples will be detailed in a separate document.

7.1.2. Analytical Methodology

Plasma and urine concentrations of CPT31 will be determined using validated analytical procedures. Specifics of the analytical methods will be provided in separate documents.

7.2. Immunogenicity Assessments

Blood samples (approximately 2 × 1 mL) will be collected by venipuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 6. The timings may be subject to change based on the ongoing review of the data.

7.3. Safety and Tolerability Assessments

7.3.1. Adverse Events

Adverse event definitions, assignment of severity and causality, and procedures for reporting SAEs are detailed in Appendix 1.

The condition of each subject will be monitored from the time of signing the ICF to final discharge from the study. Subjects will be observed for any signs or symptoms and asked about their condition by open questioning, such as "How have you been feeling since you were last asked?", at least once each day while resident at the study site and at each study visit. Subjects will also be encouraged to spontaneously report AEs occurring at any other time during the study.

All nonserious AEs, whether reported by the subject voluntarily or upon questioning, or noted on physical examination, will be recorded from initiation of study drug until study completion. Serious AEs will be recorded from the time the subject signs the ICF until study completion. The nature, time of onset, duration, and severity will be documented, together with an Investigator's (or designee's) opinion of the relationship to study drug. Photos may be taken of visible side effects, such as a skin rash, for the study records.

Adverse events recorded during the course of the study will be followed up, where possible, until resolution or until the unresolved AEs are judged by the Investigator (or designee) to have stabilized. This will be completed at the Investigator's (or designee's) discretion.

7.3.2. Clinical Laboratory Evaluations

Blood and urine samples will be collected for clinical laboratory evaluations (including clinical chemistry, hematology, urinalysis, coagulation, and serology) at the times indicated in the Schedule of Assessments in Appendix 6. Clinical laboratory evaluations are listed in Appendix 2. Additional clinical laboratory evaluations will be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of clinical laboratory safety evaluations is required.

Subjects will be asked to provide urine samples for drugs of abuse screen and cotinine test, and will undergo an alcohol breath test at the times indicated in the Schedule of Assessments in Appendix 6. For all female subjects, a pregnancy test will be performed at the times indicated in the Schedule of Assessments in Appendix 6.

An Investigator (or designee) will perform a clinical assessment of all clinical laboratory data.

7.3.3. Vital Signs

Supine blood pressure, supine pulse rate, respiratory rate, and oral body temperature will be assessed at the times indicated in the Schedule of Assessments in Appendix 6. Vital signs may also be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of vital signs is required.

Day 1 predose blood pressure, pulse rate, and respiratory rate will be measured in triplicate at approximately 2-minute intervals. The median value will be used as the baseline value in the data analysis. All subsequent measurements will be performed singly and repeated once if outside the relevant clinical reference ranges. Oral body temperature will be measured singly.

Subjects must be supine for at least 5 minutes before blood pressure and pulse rate measurements.

7.3.4. Electrocardiogram

7.3.4.1. 12-Lead Electrocardiogram

Resting 12-lead ECGs will be recorded after the subject has been supine and at rest for at least 5 minutes at the times indicated in the Schedule of Assessments in Appendix 6. Single 12-lead ECGs will be repeated 2 more times, and an average taken of the 3 readings, if either of the following criteria apply:

- QTcF value > 500 msec
- QTcF change from the baseline (predose) is > 60 msec.

Additional 12-lead ECGs may be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of ECGs is required. The Investigator (or designee) will perform a clinical assessment of each 12-lead ECG.

Day 1 predose 12-lead ECGs will be measured in triplicate at approximately 2-minute intervals. The mean value will be used as the baseline value in the data analysis. All subsequent measurements will be performed singly and repeated 2 more times if outside the relevant clinical reference ranges, as described above.

7.3.4.2. Continuous 12-lead Electrocardiogram Monitoring

Continuous 12-lead ECG monitoring (Groups A3 and A4 only) using a digital recorder will take place at the times indicated in the Schedule of Assessments in Appendix 6.

All Continuous ECG data collected on study will be archived without extraction or analysis and will not be reported in the scope of this study.

Subjects will be supine for at least 10 minutes before the extraction timepoint and for 5 minutes from the start of each extraction timepoint (each extraction will last for 5 minutes). Environmental distractions (e.g., television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording.

When coinciding, vital signs assessments and PK sampling should always be performed after the ECG extraction time window. If a separate ECG machine is being used for safety assessments described in Section 7.3.4.1, that machine should be in place prior to the extraction window to permit safety ECGs to be recorded irrespective of the extraction window. If the machine is not in place prior to the extraction window, safety ECGs must be recorded after the extraction window. If an integral system is used, safety ECGs may be recorded irrespective of the extraction window.

7.3.5. Physical Examination

A full physical examination or symptom-directed physical examination will be performed at the timepoints specified in the Schedule of Assessments in Appendix 6.

7.3.6. Body Weight

Body weight (in light clothing) will be recorded at the times indicated in the Schedule of Assessments in Appendix 6.

8. SAMPLE SIZE AND DATA ANALYSIS

8.1. Determination of Sample Size

No formal statistical assessment, in terms of sample size, has been conducted as this is the first time CPT31 is being administered to humans. However, the number of subjects in the present study is common in early clinical pharmacology studies and is considered sufficient to achieve the objectives of the study. A common distribution between active and placebo subjects is 6+2. Buoen et al. (Evaluation of the Cohort Size in Phase I Dose Escalation Trials Based on Laboratory Data, J Clin Pharmacol (2003) 43:470-476) investigated the impact of cohort size on Type I error and power in Phase I dose escalation trials based on laboratory data and concluded that “the active cohort size in Phase I dose escalation trials should be between 6 and 10 active subjects.”

8.2. Analysis Populations

8.2.1. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of CPT31 and have evaluable PK data.

8.2.2. Safety Population

The Safety population will include all subjects who received at least 1 dose of study treatment (CPT31 or placebo).

8.3. Pharmacokinetic Analyses

Noncompartmental PK analysis will be performed on individual plasma and urine concentration data, using commercial software such as Phoenix® WinNonlin®. Plasma concentrations of CPT31 and PK parameters will be listed and summarized using descriptive

statistics. Urine concentrations of CPT31 will be listed. Individual and mean CPT31 concentration-time profiles will also be presented graphically.

Where data are available, CPT31 dose proportionality will be examined across dose groups. The PK parameters will be analyzed for dose proportionality using a power model approach or analysis of variance (ANOVA) model, as appropriate.

8.4. Safety Analysis

Safety parameters will be listed and summarized using descriptive statistics. The frequency of subjects with QTcB >470 ms or change from baseline >30 ms will be summarized for each treatment. No formal statistical analysis of safety data is planned. Each AE will be coded using the Medical Dictionary for Regulatory Activities.

8.5. Interim Analysis

No formal interim statistical analyses are planned for this study.

9. REFERENCES

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10. APPENDICES

Appendix 1: Adverse Event Reporting

Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and/or unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug.

Assessment of Severity

The Investigator will be asked to provide an assessment of the severity of the AE using the following criteria defined by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 dated July 2017:

- **Grade 1, Mild:** Usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Grade 2, Moderate**: Usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- **Grade 3, Severe**: Interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.
- **Grade 4, Potentially Life-threatening:** The subject was at immediate risk of death from the event as it occurred (i.e., does not include a reaction that might have caused death if it had occurred in a more serious form). For instance, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.
- **Grade 5, Death.**

Relationship to Study Treatment

The Investigator (or designee) will make a determination of the relationship of the AE to the study drug using a 4-category system according to the following guidelines:

- **Not Related**: The AE is definitely caused by the subject's clinical state or the study procedure/conditions.
- **Unlikely Related**: The temporal association between the AE and the drug is such that the drug is not likely to have any reasonable association with the AE.
- **Possibly Related**: The AE follows a reasonable temporal sequence from the time of drug administration but could have been produced by the subject's clinical state or the study procedures/conditions.

- **Related**: The AE follows a reasonable temporal sequence from administration of the drug, abates upon discontinuation of the drug, follows a known or hypothesized cause-effect relationship, and (if appropriate) reappears when the drug is reintroduced.

Follow-up of Adverse Events

Every reasonable effort will be made to follow up with subjects who have AEs. Any subject who has an ongoing AE that is possibly related or related to the IMP or study procedures at the Follow-up visit will be followed up, where possible, until resolution or until the unresolved AE is judged by the Investigator (or designee) to have stabilized. This will be completed at the Investigator's (or designee's) discretion. Any subject who has an ongoing AE that is not related or unlikely related to the IMP or study procedures at the Follow-up visit can be closed out as ongoing at the Investigator's discretion.

Adverse Drug Reactions

All noxious and unintended responses to an IMP (i.e., where a causal relationship between an IMP and an AE is at least a reasonable possibility) related to any dose should be considered as adverse drug reactions.

For marketed medicinal products, a response to a drug which is noxious and unintended, and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved IMP).

Serious Adverse Events

A serious AE (SAE) is defined as any untoward medical occurrence that at any dose either:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions)
- results in a congenital anomaly/birth defect
- results in an important medical event (see below).

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Instances of death or congenital abnormality, if brought to the attention of the Investigator at any time after cessation of the study treatment and considered by the Investigator to be possibly related to the study treatment, will be reported to the Protocol Safety Review Team (PSRT).

Definition of Life-threatening

An AE is life-threatening if the subject was at immediate risk of death from the event as it occurred (i.e., does not include a reaction that might have caused death if it had occurred in a more serious form). For instance, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.

Definition of Hospitalization

Adverse events requiring hospitalization should be considered serious. In general, hospitalization signifies that the subject has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate at the Clinical Research Unit (CRU). When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered as serious.

Hospitalization for elective surgery or routine clinical procedures, which are not the result of an AE, need not be considered AEs and should be recorded on a Clinical Assessment Form and added to the electronic Case Report Form. If anything untoward is reported during the procedure, this must be reported as an AE and either ‘serious’ or ‘nonserious’ attributed according to the usual criteria.

Serious Adverse Event Reporting

Food and Drug Administration reportable AEs are AEs that are associated with the use of the drug and are serious and unexpected. Food and Drug Administration-reportable AEs will be reported by the study site to the PSRT and the responsible Institutional Review Board (IRB).

The PSRT will be notified in writing (e.g., facsimile) within 24 hours of when an AE that is potentially FDA-reportable is first recognized or reported.

Subsequently, a written confirmation or summary of the AE (using FDA Form 3500A or equivalent) will be sent to the Sponsor within 3 working days of the original notification.

The IRB will be notified of any FDA-reportable AEs within the timeframe required by the IRB. The IRB Serious and Unexpected Adverse Experience Submission Form will be completed and submitted with the copy of the written confirmation or summary of the AE.

The responsibility for reporting SAEs will be transferred to the Sponsor 30 days after the end of the study.

Pregnancy

Pregnancy (maternal or paternal exposure to study drug) does not meet the definition of an AE. However, to fulfill regulatory requirements any pregnancy should be reported following the serious AE process to collect data on the outcome for both mother and fetus.

Appendix 2: Clinical Laboratory Evaluations

Clinical chemistry:	Hematology:	Urinalysis:
Alanine aminotransferase Albumin[a] Alkaline phosphatase Aspartate aminotransferase Blood urea nitrogen Calcium Chloride Creatinine Gamma-glutamyl transferase Glucose (random) Potassium Sodium Total bilirubin[b] Total protein[a] Uric acid Fibrinogen C-reactive protein **Cytokine panel** IL-1β IL-6 TNF-α **Lipid panel (fasted)[c]** Cholesterol Low density lipoprotein High density lipoprotein Triglycerides	Hematocrit Hemoglobin Mean cell hemoglobin Mean cell hemoglobin concentration Mean cell volume Platelet count Red blood cell (RBC) count RBC distribution width White blood cell (WBC) count WBC differential: Basophils Eosinophils Lymphocytes Monocytes Neutrophils **Coagulation profile:** Activated partial thromboplastin time International normalized ratio Prothrombin time	Bilirubin Blood Color and appearance Glucose Ketones Leukocyte esterase Nitrite pH Protein Specific gravity Urobilinogen Microscopic examination (if protein, leukocyte esterase, nitrite, or blood is positive)
Serology[d]:	**Drug screen[e]:**	**Hormone panel - females only:**
Anti-hepatitis B surface antibody Anti-hepatitis B core antibody Hepatitis B surface antigen Hepatitis C antibody Human immunodeficiency (HIV-1 and HIV-2) Combo Ag/Ab HIV-1/HIV-2 immunoassay	Including but not limited to: Amphetamines/methamphetamines Barbiturates Benzodiazepines Cocaine (metabolite) Methadone Phencyclidine Opiates Tetrahydrocannabinol/ Cannabinoids cotinine Alcohol[f] Alcohol breath test[f]	Follicle-stimulating hormone[d] (postmenopausal females only) Serum pregnancy test (human chorionic gonadotropin)[g]

[a] Calculated globulin and albumin:globulin ratio will be derived.
[b] Direct and indirect bilirubin will be analyzed if total bilirubin is elevated.
[c] Fasted lipid panel only included as indicated in the Schedule of Assessments (Appendix 6).
[d] Only analyzed at Screening.

[e] Urine test to include cotinine.
[f] Alcohol breath test (not included at Screening).
[g] Performed at Screening, Day -1, and Follow-up for all females.

Appendix 3: Total Blood Volume

The following blood volumes will be withdrawn for each subject.

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations	12.5	8	100
Serology	3.5	1	3.5
Coagulation	2.7	8	21.6
CPT31 pharmacokinetics	2	18	36
Immunogenicity	2	4	8
Total:			169.1

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 400 mL.

Appendix 4: Contraception Guidance

Definitions

Women of Childbearing Potential: premenopausal females who are anatomically and physiologically capable of becoming pregnant following menarche.

Women of Nonchildbearing Potential:

1. **Surgically sterile:** females who are permanently sterile via hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy by reported medical history and/or medical records. Surgical sterilization to have occurred a minimum of 6 weeks, or at the Investigator's discretion, prior to Screening.
2. **Postmenopausal:** Females at least 45 years of age with amenorrhea for 12 months without an alternative medical reason with confirmatory follicle-stimulating hormone (FSH) levels of ≥ 40 mIU/mL. The amenorrhea should not be induced by a medical condition such as anorexia nervosa, hypothyroid disease or polycystic ovarian disease, or by extreme exercise. It should not be due to concomitant medications that may have induced the amenorrhea such as oral contraceptives, hormones, gonadotropin-releasing hormones, anti-estrogens, or selective estrogen receptor modulators. Women aged > 60 years old whose FSH values are not ≥ 40 mIU/L may be included at the discretion of the Investigator and in consultation with the Sponsor.

Fertile male: a male that is considered fertile after puberty.

Infertile male: permanently sterile male via bilateral orchiectomy.

Contraception Guidance

Female Subjects

Female subjects who are of nonchildbearing potential will not be required to use contraception. Female subjects of childbearing potential must be willing to use 2 methods (1 primary and 1 secondary method) of birth control from the time of signing the Informed Consent Form (ICF) until 90 days after the Follow-up visit. Primary (non-barrier) methods of contraception include:

- hormonal injection (as prescribed)
- combined oral contraceptive pill or progestin/progestogen-only pill (as prescribed)
- combined hormonal patch (as prescribed)
- combined hormonal vaginal ring (as prescribed)
- hormonal IUD
- surgical method performed at least 3 months prior to the Screening Visit:
    - bilateral tubal ligation

  - Essure® (hysteroscopic bilateral tubal occlusion) with confirmation of occlusion of the fallopian tubes
- hormonal implant
- vasectomized male partner (sterilization performed at least 90 days prior to the Screening Visit, with verbal confirmation of surgical success, and the sole partner for the female subject)

Secondary (barrier) methods of contraception include:

- male condom with spermicide
- female condom with spermicide
- over-the-counter sponge with spermicide
- cervical cap with spermicide (as prescribed)
- hormonal or non-hormonal intrauterine device (IUD)
- diaphragm with spermicide (as prescribed).

Female subjects of childbearing potential should refrain from donation of ova from Check-in (Day -1) until 90 days after the Follow-up visit.

Male Subjects

Male subjects will be surgically sterile for at least 90 days, with documented azoospermia, or when sexually active with female partners of childbearing potential will be required to use a male condom with spermicide (even if subject has a history of vasectomy) from Check-in until 90 days after the Follow-up visit. Sexual intercourse with female partners who are pregnant or breastfeeding should be avoided unless condoms are used from the time of the first dose until 90 days after the Follow-up visit. Male subjects are required to refrain from donation of sperm from Check-in until 90 days after the Follow-up visit.

Sexual Abstinence and Same-sex Relationships

For subjects who practice true abstinence, subjects must be abstinent for at least 6 months prior to Screening and must agree to remain abstinent from the time of signing the ICF until 90 days after the Follow-up visit.

A subject in a same-sex relationship at the time of signing the ICF must agree to refrain from engaging in a heterosexual relationship from the time of signing the ICF until 90 days after the Follow-up visit.

Appendix 5: Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines.
- Applicable International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
- Applicable laws and regulations.

The protocol, protocol amendments, Informed Consent Form (ICF), Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an Institutional Review Board (IRB) by the Investigator and reviewed and approved by the IRB before the study is initiated.

Any amendments to the protocol will require IRB and regulatory authority (as locally required) approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects. All protocol amendments must be approved by the NIAID Division of AIDS at the National Institutes of Health.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB.
- Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB, European Regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

Informed Consent

Prior to starting participation in the study, each subject will be provided with a study-specific ICF giving details of the study drugs, procedures, and potential risks of the study. Subjects will be instructed that they are free to obtain further information from the Investigator (or designee) and that their participation is voluntary and they are free to withdraw from the

study at any time. Subjects will be given an opportunity to ask questions about the study prior to providing consent for participation.

Following discussion of the study with Clinical Research Unit (CRU) personnel, subjects will sign 2 copies of the ICF in the presence of a suitably trained member of staff to indicate that they are freely giving their informed consent. One copy will be given to the subject, and the other will be maintained in the subject's records.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.

Subject Data Protection

Subjects will be assigned a unique identifier and will not be identified by name in electronic Case Report Forms (eCRFs), study-related forms, study reports, or any related publications. Subject and Investigator personal data will be treated in compliance with all applicable laws and regulations. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual subjects or Investigators will be redacted according to applicable laws and regulations.

The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject. The subject must also be informed that his/her study-related data may be examined by Sponsor or Contract Research Organization (CRO) auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB members, and by inspectors from regulatory authorities such as the Food and Drug Administration (FDA).

Disclosure

All information provided regarding the study, as well as all information collected and/or documented during the course of the study, will be regarded as confidential. The Investigator (or designee) agrees not to disclose such information in any way without prior written permission from the Sponsor.

Data Quality Assurance

The following data quality steps will be implemented:

- All relevant subject data relating to the study will be recorded on eCRFs unless directly transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.

- The Sponsor or designee is responsible for the data management of this study including quality checking of the data. Predefined agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a risk management register. Additional details of quality checking to be performed on the data may be included in a Data Management Plan.
- A Study Monitor will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator in accordance with 21 CFR 312.62(c) unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Investigator Documentation Responsibilities

All individual, subject-specific study data will also be entered into a 21 CFR Part 11-compliant electronic data capture (EDC) system on an eCRF in a timely fashion.

All data generated from external sources (e.g., laboratory and bioanalytical data), and transmitted to the Sponsor or designee electronically, will be integrated with the subject's eCRF data in accordance with the Data Management Plan.

An eCRF must be completed for each enrolled subject who undergoes any Screening procedures, according to the eCRF completion instructions. The Sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify the accuracy of the electronic data. The Investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

The Investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the Investigator reviewed and approved the data on the eCRF, data queries, and site notifications.

Publications

If on completion of the study the data warrant publication, the Investigator may publish the results in recognized (refereed) scientific journals subject to the provisions of the clinical study agreement (CSA). Unless otherwise specified in the CSA, the following process will occur:

If the Investigator expects to participate in the publication of data generated from this site, the institution and Investigator will submit reports, abstracts, manuscripts, and/or other presentation materials to the Sponsor for review before submission for publication or

presentation. The Sponsor will have 60 days to respond with any requested revisions including, without limitation, the deletion of confidential information. The Investigator will act in good faith upon requested revisions, except the Investigator will delete any confidential information from such proposed publications. The Investigator will delay submission of such publication or presentation materials for up to an additional 90 days in order to have a patent application(s) filed.

Appendix 6: Schedule of Assessments

Schedule of Assessments

Study Procedures	Screening		Treatment Period	Follow-up (28-30 days postdose)
	Day -28 to Day -2	Day -1	Days 1 to 6	
Informed consent	X			
Inclusion/exclusion criteria	X	X		
Demographic data	X			
Medical and surgical history	X	X[a]		
Urinary drug screen	X	X		
Alcohol breath test		X		
HIV, hepatitis B, and hepatitis C tests	X			
Serum pregnancy test[b]	X	X		X
Height and body weight	X[c]	X		
Study residency:				
Check-in		X		
Check-out			Day 6	
Nonresidential visit	X			X
Study drug administration:				
CPT31or placebo			Day 1 (0 h)	
Pharmacokinetics:				
Blood sampling[d]			Day 1 predose, 0.5 h (±3 min), 1 h (±6 min), 2 h (±12 min), 4 h (±30 min), 6 h (±30 min), 8 h (±30 min), 10 h (±30 min), 12 h (±30 min), 16 h (±30 min), Day 2 (24 h, ±30 min), Day 3 (48 h, ±4 h), Day 4 (72 h, ±4 h), Day 5 (96 h, ±4 h), and Day 6 (120 h, ±4 h)	
Urine sampling[d, i]			Day 1 predose (spot collection) and 0 to 4 h (±15 min), 4 to 8 h (±15 min), Day 1 to Day 2 (8 to 24 h, ±15 min), Day 2 to Day 3 (24 to 48 h, ±15 min), and Day 3 to Day 4 (48 h to 72 h ±15 min)	

Schedule of Assessments

Study Procedures	Screening Day -28 to Day -2	Day -1	Treatment Period Days 1 to 6	Follow-up (28-30 days postdose)
Immunogenicity:				
Blood sampling[d]			Day 1 predose, Day 2 (24 h), Day 5 (96 h)	X
Safety and tolerability:				
Adverse event recording	X	X	Ongoing	X
Prior/concomitant medication monitoring	X	X	Ongoing	X
Clinical laboratory evaluations (including coagulation)[d]	X[e]	X[e]	Day 2 (24 h), Day 3 (48 h), Day 4 (72 h), Day 5 (96 h), and Day 6 (120 h[e])	X
Supine blood pressure, supine pulse rate, respiratory rate and oral body temperature[d]	X	X	Day 1 predose, 2 h, 4 h, 12 h, Day 2 (24 h), Day 3 (48 h), Day 4 (72 h), Day 5 (96 h), and Day 6 (120 h)	X
12-lead ECG[d]	X		Day 1 predose, 2 h, 4 h, 12 h, Day 2 (24 h), Day 3 (48 h), Day 4 (72 h), Day 5 (96 h), and Day 6 (120 h)	X
Continuous 12-lead ECG			X[f]	
Physical examination[d]	X[g]		Day 1 predose, Day 2 (24 h), Day 3 (48 h), Day 4 (72 h), Day 5 (96 h), and Day 6 (120 h)[h]	X[h]

Abbreviations: ECG = electrocardiogram, h = hour; HIV = human immunodeficiency virus; min = minute.
[a] Interim medical history.
[b] In all females.
[c] Height measured at Screening only.
[d] Times (h) relative to dosing.
[e] Clinical laboratory tests to include lipid panel (fasted).
[f] Monitor for 12-lead ECG recording will be worn from 2 hours predose to 25 hours postdose on Day 1. Extraction timepoints will be 60, 45, and 30 minutes predose and at and 0.25 hours, 0.5 hours, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 24 hours postdose. Continuous 12-lead ECG will be performed on Groups A3 and A4 only.
[g] Performed between the Screening Visit and predose assessments.
[h] Symptom-directed physical examination at all timepoints other than Screening.

[i]PK urine sampling for males only

Appendix 7: Protocol Amendment(s)– Summary of Changes

Protocol Amendment 2 (Protocol Version 3)

PURPOSE: To update wording related to the appearance and blinding requirements of CPT31 and placebo solutions.

MODIFICATIONS TO PROTOCOL:
General Revisions:
Section 3.5: Wording revised as no systemic exposure specifically defined in section 3.7
Section 5.2: Correction/clarification of timings
Section 5.4: Updates to appearance of CPT31 and placebo solution information and potential for required study blinding actions/processes.

ADDITIONAL RISK TO THE SUBJECT: There are no additional risks to the subjects.

Protocol Amendment 3 (Protocol Version 4)

PURPOSE: To update urine PK assessment to males only.

MODIFICATIONS TO PROTOCOL:
General Revisions:
Section 7 and 7.1.1: Wording updated to state urine samples for males only (for CPT-31 assay)
Appendix 6: Urine sampling information for PK updated to detail males only.

ADDITIONAL RISK TO THE SUBJECT: There are no additional risks to the subjects.