Immunologic Response to Euphorbia Kansui Extract Powder Prepared as Tea in HIV-Infected Antiretroviral Therapy (ART)-Suppressed Individuals

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Clinical Research Protocol

IMMUNOLOGIC RESPONSE TO *EUPHORBIA KANSUI* EXTRACT POWDER PREPARED AS TEA IN HIV-INFECTED ANTIRETROVIRAL THERAPY (ART)-SUPPRESSED INDIVIDUALS: A DOSE ESCALATION STUDY

Protocol Number:	Kansui.04
Version Date:	October 8, 2018
Investigational Product:	*Euphorbia kansui* extract powder prepared as tea
IND Number:	127153
Development Phase:	Phase 1
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Immunologic response to *Euphorbia kansui* extract powder prepared as tea in HIV-infected antiretroviral therapy (ART)-suppressed individuals: a dose escalation study

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: Kansui.04

Protocol Title: Immunologic response to *Euphorbia kansui* extract powder prepared as tea in HIV-infected antiretroviral therapy (ART)-suppressed individuals: a dose escalation study

Protocol Date: 10/08/2018

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse event
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral therapy
CA-US RNA	Cell-associated unspliced HIV-1 RNA
CBC	Complete blood count
CD38	Cluster of differentiation 38
CD69	Cluster of differentiation 69
CFR	Code of Federal Regulations
CHEM-7	Basic metabolic panel (chemistry-7)
CRF	Case report form
DMC	Data Monitoring Committee
FDA	Food and Drug Administration
GCRC	General Clinical Research Center
GMP	Good Manufacturing Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
HLA-DR	Human Leukocyte Antigen-DR
HPLC	High performance liquid chromatography
ICF	Informed consent form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
LFT	Liver function test
MS	Mass spectrometry
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
PID	Participant identification
PD	Pharmacodynamics
PK	Pharmacokinetics
SAE	Serious adverse experience
SFGH	San Francisco General Hospital
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedures
TCM	Traditional Chinese medicine
TSH	Thyroid stimulating hormone
UCSF	University of California, San Francisco

PROTOCOL SYNOPSIS

TITLE	Immunologic response to *Euphorbia kansui* powder prepared as tea in HIV-infected antiretroviral therapy (ART)-suppressed individuals: a dose escalation study
SPONSOR	Investigator Initiated Study (Dr. Sulggi Lee)
FUNDING ORGANIZATION	1. amfAR HIV Cure Institute 2. Salary support from K23 grant (K23GM112526) 3. Departmental Funds
NUMBER OF SITES	2 (1 coordinating site, UCSF, and 1 enrollment site, University of Utah)
RATIONALE	The key challenge of HIV eradication strategies is persistence of a small pool of resting memory CD4+ T cells that harbor latent replication-competent HIV. One potential strategy to eliminate this reservoir is "shock and kill" in which agents designed to reverse latency (latency reactivating agents, or LRAs) are combined with agents aimed at eliminating these virus-expressing cells. The goal of the current proposal is to evaluate the *in vivo* biological response to an herbal supplement used in traditional Chinese medicine ("kansui)" that has potent in vitro latency reactivating capabilities.
STUDY DESIGN	This is a phase I open label dose escalation study with a randomized, double-blinded, placebo-controlled crossover cohort.
PRIMARY OBJECTIVE	1. To determine the safety and tolerability of a single or 3 consecutive daily 1g doses of *Euphorbia kansui* extract powder, measured and prepared as tea, in HIV+ ART-suppressed individuals. 2. To determine the immunologic effect of a single or 3 consecutive daily 1g doses of *Euphorbia kansui* extract powder, measured and prepared as tea, in HIV+ ART-suppressed individuals.
SECONDARY OBJECTIVES	3. To determine the effect of a single or 3 consecutive daily 1g doses of *Euphorbia kansui* extract powder, measured and prepared as tea, on latent HIV in HIV-infected ART-suppressed individuals.
NUMBER OF PARTICIPANTS	N=8 total (The first N=2 participants will receive 1 dose for a total of 1 g; the final N=6 participants will receive 3 consecutive daily doses for a total of 3 g of *Euphorbia kansui* extract powder prepared as tea during the study period). Following enrollment of the first 2 participants, an interim analysis will be conducted to review all study data to determine if additional participants are needed to accomplish the study purpose, and if so, if enrollment should continue at the same

	or a higher dose (i.e., next cohort).
PARTICIPANT SELECTION CRITERIA	Inclusion Criteria: 1. Confirmed HIV-1 infection in adults aged 18 years or older. 2. Continuous therapy with a DHHS recommended/alternative combination ART for least 36 months (at least 3 agents) at study entry with no regimen changes in the preceding 24 weeks. 3. Maintenance of undetectable plasma HIV-1 RNA (<40 copies/ml) for at least 36 months. Episodes of single HIV plasma RNA 50-500 copies.ml will not exclude participation if subsequent HIV plasma RNA is <40 copies/ml. 4. Two CD4+ T cell counts >350 cells/μl in the six months prior to screening. Exclusion Criteria: 1. Pre-ART viral load <2000 copies/ml (HIV controllers) 2. Based on prior history and/or virologic testing, no alternative ART regimens are available in the event that the current ART regimen is compromised as a result of this study. 3. Recent hospitalization in the last 90 days. 4. Recent infection in the last 90 days requiring systemic antibiotics. 5. Recent vaccination within the last 8 weeks prior to study scree or any study blood draw. 6. Any known prior (lasting >6 months) or current history of liver-related diseases including but not limited to: a. Hepatic cirrhosis of decompensated chronic liver diseases b. Clinically active hepatitis B or C infection as evidenced by clinical jaundice or Grade 2 or higher liver function test abnormalities c. Any hepatic impairment, regardless of the graded liver function test abnormalities. 7. Any known prior (lasting >6 months) or current history of gastrointestinal diseases including but not limited to: a. History of diarrheal illness requiring the use of anti-motility agents including inflammatory bowel disease, chronic diarrhea not otherwise specified. b. History of gastrointestinal bleeding with hemoglobin below 12.5 g/dL. c. History of gastric or duodenal ulcers d. Inflammatory gastrointestinal disease such as Crohn's

	disease or ulcerative colitis 8. Any significant renal disease (eGFR < 90 ml/min) or acute nephritis. 9. Screening hemoglobin below 12.5 g/dL. 10. Screening TSH consistent with hypothyroidism. 11. Significant myocardial disease (current myocarditis or reduced left ventricular ejection fraction below the lower limit of normal) or diagnosed coronary artery disease. 12. Significant respiratory disease requiring oxygen. 13. Diabetes or current hypothyroidism. 14. Participants of reproductive potential or breastfeeding. Women of childbearing potential must have a negative serum pregnancy test at screening. All participants of childbearing potential must agree to use a double-barrier method of contraception throughout the study period and up to 90 days after the last dose of kansui. 15. Exposure to any immunomodulatory drug (including maraviroc) in the16 weeks prior to study. 16. Prior or current use of experiment agents used with the intent to perturb the HIV-1 viral reservoir. 17. History of seizures, psychosis, abnormal electroencephalogram or brain damage with significant persisting neurological deficit 18. Positive test for tuberculosis by either skin test (PPD) or blood interferon-gamma release assay (QuantiFERON). 19. Significant substance use, which in the opinion of the investigator(s), is likely to interfere with the conduct of the study.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	1 g of *Euphorbia kansui* extract powder, measured and reconstituted in 4 fluid ounces of boiled water allowed to cool and administered as tea, taken by mouth daily as either a single or 3 consecutive daily doses.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Cohort 1: no placebo. Cohort 2: a placebo treatment arm will be assigned to patients in a randomized crossover design. The placebo will be decaffeinated black loose leaf tea which produces a tea that is similar in appearance, consistency, and (bitter) taste to kansui extract powder, without any known immune modulating properties or drug-drug interactions (e.g., changes in pH balance that might affect drug absorption) with HIV antiretroviral therapy.

DURATION OF PARTICIPANT PARTICIPATION AND DURATION OF STUDY	Cohort 1: Participants will be on study for up to 45 days. **Screening:** up to 14 days **Treatment:** 1 day (participants to be admitted to the hospital during the day; no overnight stay) **Follow-up:** 31 days after treatment dose day Cohort 2: Participants will be randomized to either of two treatment arms, kansui or placebo, using a crossover design with a 56-day washout period in between treatment arms. Participants will be on study for up to 146 days. **Screening (for each arm):** up to 14 days prior to treatment arm. **Treatment (for each arm):** 3 days (participants to be admitted to the hospital each day; no overnight stays) **Follow-up (for each arm):** 31 days after the initial dose for that treatment arm. The total duration of the study is expected to be 1 year.
CONCOMMITANT MEDICATIONS	Allowed: 1. In the absence of drug-drug interaction information regarding kansui, participants may use their usual medications, as long as they are medically necessary and can be monitored safely during the study period. Prohibited: 1. As stated in the exclusionary criteria above, participants may not have exposure to any immunomodulatory drug (including maraviroc) in the 16 weeks prior to study or during study period. 2. Also as stated in the exclusionary criteria, participants may not have prior or current use of experimental agents used with the intent to perturb the HIV-1 viral reservoir. 3. Participants may not be allowed to take anti-motility agents during the study period in order to accurately assess adverse events related to diarrhea. 4. Participants will not *initiate* new medications that may also cause diarrhea, including proton pump inhibitors, antibiotics, antidepressants, colchicine, digitalis, etc., during the study period. In addition to the above medications, per traditional Chinese medicine practices, participants should also not ingest ice-cold food or beverages, spicy foods, or seafood in the two days prior to, during, and up to two days after kansui treatment.

EFFICACY EVALUATIONS	• CD4+ and CD8+ T cell activation (expression of CD69, CD38, and HLA-DR). • Latent HIV reservoir quantification (cell-associated HIV RNA and plasma HIV RNA levels).
PRIMARY ENDPOINT	• Safety and tolerability of 1 g of *Euphorbia kansui* extract powder, prepared as tea, given as a single or 3 consecutive daily doses; no Grade 2 or greater clinical or laboratory adverse effects
SECONDARY ENDPOINTS	• Immunologic effect on CD4+ and CD8+ T cell activation (CD69, CD38, and HLA-DR expression on CD4+ and CD8+ T cells measured by flow cytometry). • Virologic effect on latent HIV Reservoir (cell-associated HIV RNA and plasma HIV RNA measured by rtPCR).
OTHER EVALUATIONS	With colleagues from the University of Utah Department of Pharmacology and Toxicology, the study team has begun developing an assay to measure levels of ingenol in participant plasma samples. Based on the bioassays performed in Matija Peterlin's lab at UCSF, the abundance of ingenol in *Euphorbia kansui* extract powder is estimated to be approximately 1:100. We will be storing plasma samples collected from this study in order to measure plasma ingenol levels post-dosing with kansui as part of later pharmacokinetic/ pharmacodynamics analyses.
SAFETY EVALUATIONS	Incidence of adverse events Interim analysis following completion of Cohort 1
PLANNED INTERIM ANALYSES	During the study, safety will be monitored by a Safety Monitoring Committee (SMC), which will be led by an HIV specialist. The SMC will review all study procedures before the initiation of the study. They will have the full and final authority to stop the study for any safety concern at any point. Given the unknown safety profile of *Euphorbia kansui*, the first participant in each dosing level will complete 5 days of study prior to any further participants commencing the study. The decision to proceed with further drug administration will be taken by the Principal Investigators, taking into consideration clinical and laboratory adverse events. The decision to proceed with the next dosing level will be taken by the SMC. Specifically, dosing will be stopped and the SMC will be notified if one Grade 4 or two Grade 3 drug related adverse events or laboratory abnormalities are reported, if a participant develops virologic failure (defined as any HIV-1 RNA levels >200 copies/mL or a single HIV-1 RNA level >1000 copies/mL), becomes pregnant or initiates breastfeeding, or is required to initiate one of the prohibited concomitant medications listed in section 7.1. If stopping/pausing criteria are not met, the remainder of the dosing cohort will be enrolled/dosed the subsequent week. The study will proceed to the next dosing level provided the

	stopping/pausing criteria are not met. A University of Utah Department of Medicine faculty member who is not otherwise involved in the study and who will not communicate with the study investigators regarding any aspect of the SMC reports will prepare the monitoring reports. The SMC will review accrual, adverse events summaries, CD4+ T-cell counts and HIV RNA levels/suppression over time, off-study rates and completeness of follow-up, by dose cohort.
STATISTICS **Primary Analysis Plan**	Dr. Lee has expertise in epidemiology and biostatistics. She will be performing the data analysis for the study. Paired analyses using Wilcoxon signed rank tests will be performed to evaluate whether there is a statistically significant change in markers of immune activation (CD69, CD38, and/or HLA-DR) on CD4+ and CD8+ T cells after a 1 or 3 doses of *Euphorbia kansui* extract powder prepared as tea. Multivariate analyses will then be performed to evaluate the association between kansui and measures of immune activation at post-dosing, using linear mixed effects models, which account for within-participant correlation of observations and allows for greater stability in the estimate of the outcome measures within individuals. Similarly, for measures of the HIV reservoir, we will perform negative binomial mixed effects regression (a method to analyze over-dispersed count data) to compare the size of the latent HIV reservoir before and after kansui administration.
Rationale for Number of Participants	A total of 8 participants (2 from dosing cohort 1 and 6 from dosing cohort 2) will contribute to the analysis, with 8 or 25 timepoint measures, respectively. The within-individual variability will be reduced by use of the within-individual control measures, as well as the repeated measurements post-dosing. The primary outcome of the study will be an increase in the % T cell activation (CD38+ HLA-DR+) of CD8+ and CD4+ T cells. Based on our recent experience performing a phase 1 trial of canakinumab (an interleukin-1 beta inhibitor) to *reduce* inflammation in chronic HIV-infected ART-suppressed individuals,[1] we estimate that we will have 80% power to detect a within-individual *increase* of 2.7 points in the % CD8+ T cell activation (standard deviation [SD] at 31 days of 1.6%). We will also assess virologic changes as secondary outcomes in the study. Based on data from our recent HIV latency phase 2b trial of disulfiram,[2] we estimate that we will have 80% power to detect 0.6-0.7 log increases in cell-associated unspliced (CA-US) HIV RNA (SD=0.4 and 0.5 for Day 4 and Day 31, respectively), and a 0.6-0.5 log increases in ultra-sensitive plasma HIV RNA (SD=0.4 and 0.3 for Day 4 and Day 31, respectively).

1 BACKGROUND

The key challenge of HIV eradication strategies is persistence of a small pool of resting memory CD4+ T cells that harbor latent replication-competent HIV, untouched by current antiretroviral therapy (ART).[3] One potential strategy to eliminate this reservoir is a "shock and kill" approach in which agents designed to reverse latency (latency reactivating agents, LRAs) are use to "shock" the virus out of these cells in order for the host immune response, ART, and/or additional immunomodulatory agents can then eliminate virus-expressing cells.[4-7] The goal of the current proposal is to evaluate the *in vivo* biological response to an herbal supplement used in traditional Chinese medicine ("kansui)" that has potent *in vitro* latency reactivating capabilities.

Kansui has been prescribed in Chinese medicine for thousands of years as a cathartic agent. It is typically given as a single dose of 0.5 -1.5 grams, and is used to treat intestinal obstruction, nodular skin lesions, water retention, and epilepsy.[8,9] Kansui extract is made from the *Euphorbia* root and is thought to contain several potentially biologically active components, including a large number of ingenols, which are known to activate and/or interrupt a number of cellular pathways, including some known to be involved in the maintenance of HIV latency.[10-12] Recently, a semi-synthetic version of ingenol from *Euphorbia tirucalli* has been developed as Ingenol B (Ing B).[13] Ing B demonstrates the potent ability to reactivate latent HIV in *ex vivo* and non-human primate models.[11,12,14] Ing B was shown to induce significant immune activation (upregulation of CD38, HLA-DR, CD69 expression on CD4+ T cells) and reactivate virus in both untreated and ART-suppressed SIVmac251-infected rhesus macaques without hypercytokinemia.[14,15] These effects were observed as early as three days in untreated monkeys[15] to approximately 30 days post-Ing B in ART-suppressed monkeys.[14] The ability to induce T cell activation and viral reactivation were even more pronounced when Ing B was administered concomitantly with another LRA called suberanilohydroxamic acid (SAHA).[14]

Kansui contains ingenols that induce immune activation and reactivate latent HIV-1

Several compounds have already been tested to reactivate latent HIV, including histone deacetylase inhibitors such as suberoylanilide hdroxamic acid (SAHA)[16] and protein kinase C (PKC) agonists such as prostratin[17] and bryostatin.[18] More recently, a new semi-synthetic ingenol ester that acts as a PKC agonist, 3-caproyl-ingenol (Ingenol B, or Ing B, patent-pending), has been tested in both J-Lat cell lines and in primary resting CD4+ T cells.[11-13] Ing B induced high levels of viral reactivation beyond those induced by SAHA, TNF-a, phorbol 12-myristate 13-acetate (PMA), or hexamethylene bisacetamide (HMBA) with minimal cellular toxicity.[12] In addition, Ing B was found to act synergistically with a BET bromodomain inhibitor (JQ1) or HMBA *in vitro*. Ing B promotes PKC activation and NF-kB nuclear translocation, and also upregulation of P-TEFb (which plays a critical role in HIV-1 transcriptional elongation). Therefore, ingenols may have multiple functions in promoting latent HIV reactivation.[12] Administration of Ing B has also been shown to increase the circulating viral load in untreated SIVmac251-infected monkeys as well as to reactivate virus from resting CD4+ T cells in ART-treated SIVDeltaB670+SIV17E-Fr-infected monkeys.[15] Among untreated rhesus macaques, Ing B induces immune activation, upregulating CD38, HLA-DR, and

CD69 expression on CD4+ T cells.[15] In all instances, no significant toxicity was observed.

1.1 Overview of Pre-Clinical Studies

1.1.a. Kansui is a potent activator of *in vitro* HIV-1 transcription

Based on *in vitro* bioassays performed in Matija Peterlin's lab, *Euphorbia kansui* extract powder is a potent activator of HIV transcription in latently infected Jurkat cells.[19] From these experiments, 1 μM of Ing B was estimated to possess equivalent bioactivity as 10 μL/mL of *Euphorbia kansui* extract, suggesting a ratio of up to but not greater than 1:100 of Ing B to kansui. Since there are up to 12 different ingenols that could differ in their potency in the *Euphorbia kansui* powder, this range could smaller. Importantly, *Euphorbia kansui* reactivated virus in patient peripheral blood mononuclear cell (PMBC) samples from HIV-infected ART-suppressed individuals (**Figure 1**), and a combination of *Euphorbia kansui* and vorinostat (a histone deacetylase inhibitor and also a latent reactivating agent) or JQ1 (a bromodomain inhibitor and latent reactivating agent) resulted in synergistic reactivation of latent HIV at suboptimal concentrations. Taken together, these results demonstrated that *Euphorbia kansui* functioned as a potent PKC agonist to reactivate latent HIV, which may be later used in combination with another HIV latent reactivating agent (LRA). In addition, Dr. Peterlin's lab tested the stability and potency of the kansui powder over time (stored at room temperature) and has not observed any loss of biological activity (latency reversal). These results have been internally validated in the lab of our clinical trial study collaborator, Dr. Adam Spivak, at the University of Utah. In a separate study published by another group, the researchers

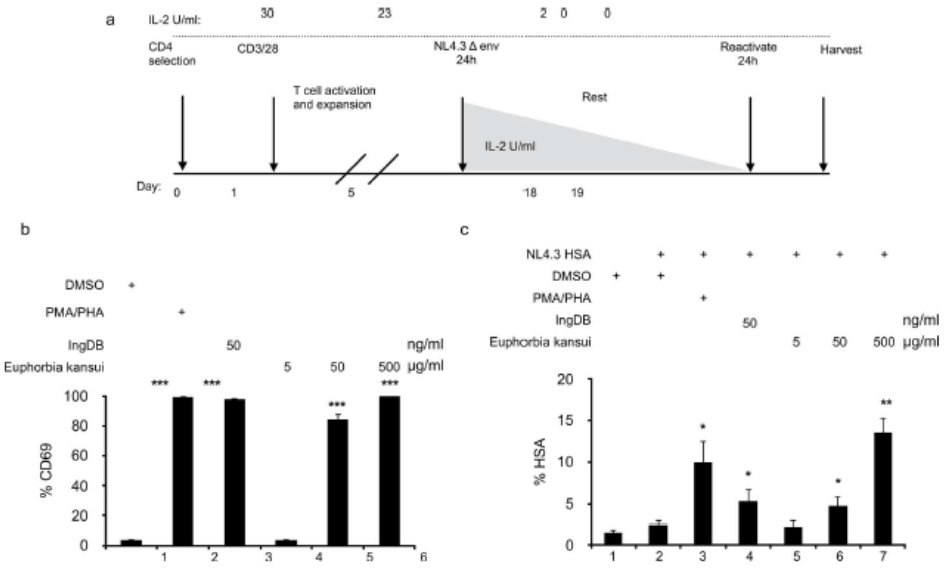

Figure 1. Euphorbia kansui reactivates HIV in a model of HIV latency using resting human CD4+ T cells. (a) Human primary CD4+ T cells were activated and expanded with CD3/CD28 beads and 30 U IL-2/ml for 7 days. Cells were infected with VSV-G-pseudotyped HIV-1 NL4.3 HSA R+E- for 24 hours. Infectious virus was removed and cells were maintained over 12 days with decreasing concentrations of IL-2 to establish latency. Uninfected cells were maintained in the same conditions, for uninfected controls and to determine T cell reactivation. At 12 days post infection, cells were stimulated for 24 hours with DMSO, PMA (2 μg/ml) and PHA (10 ng/ml), IngDB (50 ng/ml) or Euphorbia kansui (5, 50, and 500 μg/ml) (***p<0.001). (b) CD69 expression was measured by flow cytometry. (c) HSA expression was measured by flow cytometry. Data are representative of infections using cells from three healthy donors. Error bars represent standard error of the mean (*p<0.05, **p<0.01). (Cary et. al, *PLoS ONE*, 2016).

identified an ingenol derivative (EK-16A) from *Euphorbia kansui*. They determined that this derivative of *kansui*, EK-16A, was 200-fold more potent than prostratin (a strong protein kinase C agonist) in reactivating HIV-1 from latently infected cell lines and primary HIV+ patient cells.[20] They also found that EK-16A exhibited synergy with other LRAs in reactivating latent HIV-1.

1.1.b. Non-human primate study

In collaboration with Dr. Dennis Hartigan-O'Connor (University of California, Davis), we have completed a non-human primate study to evaluate the safety of *Euphorbia kansui* prepared as tea, given at increasing dose and frequency to three juvenile SIVmac251-infected rhesus macaques compared to two controls (**Figure 2**). The rhesus macaques studied were part of an NIH-funded study (R01-AI118451, PI:Hartigan-O'Connor). Three juvenile male rhesus macaques (RM) were infected with SIVmac251 by intravenous administration. Two juvenile male RMs were included as "control" animals to receive early ART but no other *kansui* therapy. After 7 days post-infection, the three RM in the treated group began a treatment regimen of once daily subcutaneous injections of tenofovir (TFV, Gilead) 20 mg/kg subcutaneously daily; emtricitabine (FTC, Gilead) 50 mg/kg subcutaneously daily; dolutegravir (DTG, ViiV) 3.25 mg/kg subcutaneously daily for a period of 12 weeks. The three animals assigned to receive kansui were administered kansui at an initial safety dose of 5 mg/kg *Euphorbia kansui* powder prepare as tea was given via oral gavage, a dose equivalent to 1/4 the dose administered in traditional Chinese medicine (~0.25 g, or 5 mg/kg/day = 20 mg x 1 in a typical 4 kg juvenile RM). The animals were then followed for a 2-week washout period. Once safety goals were achieved with this initial dosing, the same treated animals then received *kansui* at 20 mg/kg/day = 80 mg x 1 and followed for another 2 week period. Next, after safety goals were achieved at this second dosing, the same three RM received *kansui* at 200 mg/kg/day = 800 mg x 2 consecutive days and followed for 2 weeks. Finally, after safety goals were achieved at this third dosing, the same three RM received *kansui* at 200 mg/kg/day = 800 mg x 3 consecutive days and followed for 3 weeks. At the end of this fourth dose 2-week washout period, the animals were followed for an additional 2-week follow-up period before undergoing necropsy.

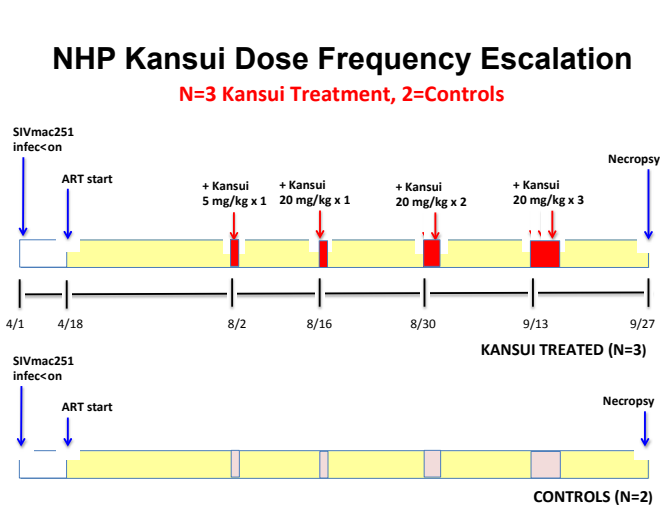

Figure 2. Study design of the non-human primate study. Kansui-treated rhesus macaques received doses as shown above with 2-week washout periods. Control monkeys did not receive placebo but were started on antiretroviral therapy (ART) and followed over the time as the kansui-treated animals.

Given that the animals demonstrated tolerability to an initial safety dose of 5 mg/kg *kansui*, treatment doses of 20 mg/kg *kansui* were administered at increased frequency in 2-week washout period intervals. Even at the highest frequency, 20 mg/kg *kansui* given

for 3 consecutive days, no adverse clinical events (i.e., the expected and anecdotally reported events such as diarrhea). This initial work demonstrates that *Euphorbia kansui* extract powder, administered at weight-based dosing equivalent to that given in traditional Chinese medicine (20 mg/kg) for up to 3 days was well tolerated in rhesus macaques.

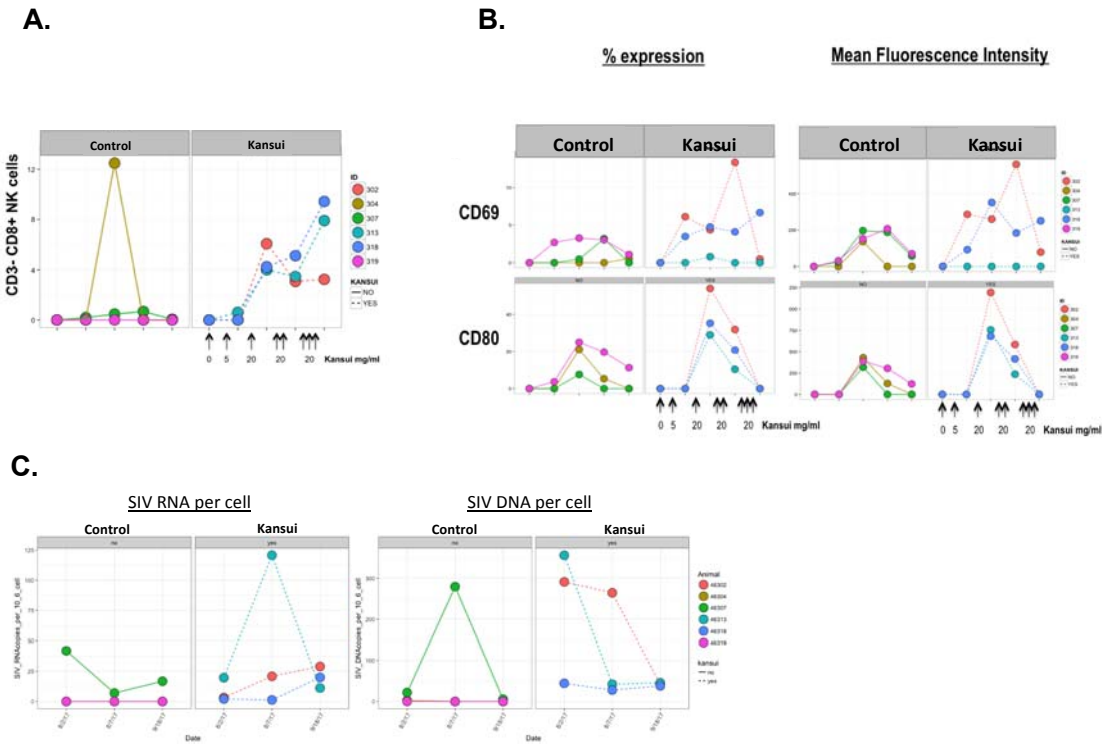

Figure 3. Kansui-treated animals demonstrated increased natural killer (NK) cell expansion (A) and increased activation markers on CD11b^{++}CD11c^{-} monocytes by percent expression and mean fluorescence intensity (B). Two of the three kansui-treated monkeys

In addition, we observed evidence for low levels of biologic efficacy in this small study such as increased natural killer cell expansion (measured as CD3-CD8+ cells) and increased activation markers on CD11b++CD11c- monocytes (**Figure 3**). Most strikingly, we observed a decrease in the size of the viral reservoir (SIV DNA) with evidence of increased SIV transcription (RNA) in two of the three kansui-treated monkeys (measured as cell-associated SIV DNA and RNA, respectively, from PBMCs). We are currently proceeding with performing the virologic assays on the necropsy tissues and performing additional immunophenotyping assays.

1.2 Overview of Clinical Studies

Kansui is well tolerated at doses prescribed in traditional Chinese medicine

Kansui is made by grinding up the root of Euphorbia plants into powdered form and is known to have a bitter taste.[21] Per traditional Chinese medicine practices, kansui is efficacious and well tolerated at doses ranging from 1 gram to 8 grams for adults, given as a single dose, above which mild diarrhea has been reported.[8,9] Mice studies suggest that at extremely high doses of 1.2 grams/kg given orally as a single dose (approximately

84 grams for a 70 kg human) *Euphorbia kansui* extract can induce severe diarrhea with abdominal distension and evidence of acute inflammatory intestinal injury.[22] But these abnormalities were not observed at the lower doses studied (0.3 and 0.6 grams/kg, equivalent to 21 and 42 grams, respectively, for a 70 kg human), only slight changes in intestinal relaxation, secretion, and motility. Other potential side effects shown in mice include mild perturbations in glycolysis and amino acid and lipid metabolism.[23]

Ingenols are one of many diterpenoid forms isolated from the sap of plants of the genus *Euphorbia*.[13] The root of *Euphorbia*, processed into powdered or liquid form, has long been used in traditional Chinese medicine to treat medical conditions such as edema, pleural effusion, urinary retention, and cancer.[8,9,24-26] *Euphorbia kansui* is one of the most commonly used *Euphorbia* in traditional Chinese medicine, which is prescribed as an herbal supplement known as "kan sui."[27,28] Various chemical constituents have been identified in *Euphorbia* plants, including sesquiterpenoids, diterpenoids (which includes ingenanes), triterpenoids, and phenol derivatives.[21] The constituents thought to play a role in latent HIV reactivation include ingenols,[10-12] but euphols[29,30] and other chemical constituents[31] may also be biologically active. Recently, ingenol mebutate, has been FDA-approved for the topical treatment of actinic keratosis.[24]

2 STUDY RATIONALE

Millions HIV-infected individuals are now receiving life-saving antiretroviral therapy.[32] However, mortality remains high, particularly in resource-limited countries.[33] Moreover, chronic HIV-infected individuals demonstrate persistent immune activation despite ART-mediated viral suppression, which is an independent predictor of mortality in this setting.[34] Given the current absence of an effective HIV vaccine, finding a cure for HIV will likely have a large impact on the long-term health of ART-treated HIV-infected individuals, in particular for those living in resource-limited settings.

Kansui is an inexpensive, readily available herbal supplement prescribed for thousands of years in traditional Chinese medicine[8,9] and contains active compounds such as ingenols that have been shown to reverse latency in an animal model. A semi-synthetic form of ingenol has been shown to potently reactivate latent SIV in rhesus macaques and is currently undergoing early drug development.[11-13] A human clinical trial testing a non-pharmacologic agent to treat HIV disease will be challenging unless we can demonstrate evidence of both safety and *in vivo* biologic activity. Though kansui has been studied extensively in traditional Chinese medicine, the herbal supplement has never been evaluated for biologic activity using Western scientific research methods.

Per discussions with the Department of Herbal Medicine at the American College of Traditional Chinese Medicine in San Francisco, kansui administration leads to clinical elimination of feces within two hours after administration of a single 1 gram dose of *Euphorbia kansui* extract powder, measured by weight, boiled with water, and prepared as a tea, suggesting rapid absorption and bioavailability. In mice, *Euphorbia kansui* extract potentiates time of fecal excretion but not overall quantity of fecal mass.[22] This pilot study will provide key preliminary data to test the safety of low doses of *Euphorbia kansui* extract powder (1 gram daily for a single or 3 consecutive doses) prepared as tea

and confirm *in vivo* human biologic activity of kansui. This study will provide important preliminary data for future studies to perform a larger, more comprehensive clinical and pharmacokinetic/pharmacodynamics (PK/PD) study of kansui.

2.1 Risk / Benefit Assessment

The most significant potential benefit of this study is to contribute to the study of an inexpensive, non-pharmacologic agent for the possible use in future HIV cure strategies. The study will generate key preliminary data for investigating an agent that may have similar potency to a semi-synthetic compound currently undergoing early drug development. The collaborations formed as part of this study will provide a foundation for future larger studies evaluating similar botanicals for the use in HIV cure trials. The benefits of this short-term treatment with *Euphorbia kansui* extract powder prepared as tea (up to 3 days of dosing) outweigh the potential risks outlined below.

Kansui-related risks

Kansui is known to induce (and is used for the purpose of causing) a mild diarrhea as a means of eliminating excess water from the body in traditional Chinese medicine.[8,9] Participants will be advised to read labels and check ingredients of foods to be sure to avoid products that may also induce diarrhea. Per traditional Chinese medical literature, common adverse events may include gastrointestinal irritability and headache, usually occurring within 30 minutes to 2 hours after ingestion.[8,9] At very high doses (between 9 to 15 grams of kansui extract powder), kansui can cause dehydration with subsequent light-headedness, hypotension, and tachycardia. These high doses of kansui may also cause diarrhea with mucus and blood, pupillary dilation, convulsions, and unconsciousness. Participants for this study will be monitored closely for 8 hours after each dose of kansui and will be instructed to report any diarrhea with mucus or blood immediately to the study staff.

Virologic risk

Given that the purpose of studying kansui in this study is to evaluate whether it can reactivate HIV from latently infected cells, there is a risk that taking kansui for up to 3 days could lead to transient loss of viral suppression and subsequent development of resistance to the participant's current antiretroviral therapy. However, given the short duration of kansui administration and the predicted small increases in plasma viremia as a result of kansui, we believe that these risks will be low. Nonetheless, as per protocol with other currently studied latent reversing agents (LRAs), we will perform frequent plasma HIV RNA monitoring (screening, every dosing day, each 24-hour follow-up, and days 31 and 132) and even more frequent monitoring of the research-based reservoir measurements of cell-associated HIV DNA and RNA (see section 9.1 below). In addition, we will only enroll participants for whom alternative ART regimens are available in the event that their previously effective regimens are compromised. Finally kansui treatment will be discontinued if the participant has any two consecutive plasma HIV RNA levels levels >200 copies/mL, or any single plasma HIV RNA level >1000 copies/mL. These values would be above the levels predicted to be seen with HIV reactivation with most current latent reactivating agents and are safety stopping criteria currently used for other HIV latent reactivating agent trials, e.g., the AIDS Clinical Trials

Group (ACTG 5336) trial, evaluating the role of Sirolimus on immune activation and the latent reservoir in chronic HIV-infected, ART-suppressed individuals.

Reproductive risks

Given the unknown reproductive risks associated with kansui as tested scientifically, and its use in traditional Chinese medicine as an abortifacient,[8] women of childbearing potential must have a negative serum pregnancy test in the medical workup for study entry and excluded from participation if pregnant. All participants must agree to use two forms of contraception throughout the study period and up to 90 days after the last dose of kansui (to account for the male sperm cycle). Women (or female partners of male study participants) must not breastfeed during the study period and up to 90 days after the last dose of kansui.

Confidentiality

As with any study collecting personal information in research records, this information could lead to loss of confidentiality. In particular, any study that collects genetic data poses the potential risk of loss of confidentiality that might be relevant to the study participant, relatives of the participant, and/or people with similar genetic background as the participant. Given that this particular study will not perform whole genome sequencing (DNA) data, but rather select RNA sequencing information, the risk of this occurring in this current study is minimal. However, in order to safeguard against this potential risk, genetic information pertaining to the study will not placed in the participant's medical record. All samples will be labeled with de-identified codes, and the informed consent includes language to inform the participant of these risks, per NHGRI guidelines (http://www.genome.gov/10000921#update).

Potential discomforts

Potential discomforts of taking kansui include the bitter taste of the extract powder. Participants will be offered room temperature beverages or snack to alleviate discomfort due to the bitter taste. In addition, the requirement that participants avoid ice-cold beverages and ice-cold foods, may cause discomfort. The dietary restrictions will be for 48 hours prior to the first dose of kansui, during the days of kansui dosing, and up to 48 hours after the last dose of kansui.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to assess the safety and tolerability of up to 3 consecutive daily doses of 1 g *Euphorbia kansui* extract powder prepared as tea in HIV-infected ART-suppressed participants.

3.2 Secondary Objectives

The secondary objective is to determine whether 1 g of *Euphorbia kansui* extract powder prepared as tea, administered up to 3 consecutive daily doses, induces a biologic effect,

measured as immune activation (expression of CD69, CD38, and/or HLA-DR) in CD4+ and CD8+ T cells.

A third objective is to determine whether 1 g of *Euphorbia kansui* extract powder prepared as tea, administered up to 3 consecutive daily doses, reactivates latent HIV, measured as cell-associated HIV RNA and plasma HIV RNA levels.

4 STUDY DESIGN

4.1 Study Overview

Prior trials evaluating latent reactivating agents have demonstrated that a "priming" period appears to yield greater latency reversal.[5,7] Therefore, we will perform this multi-center, phase I clinical study of kansui in eight HIV-infected ART-suppressed participants as a dose escalation trial with a double-blinded, randomized, placebo-controlled crossover cohort. The first two participants will complete a kansui-only treatment phase in which they will be given a single, open label 1g dose of *Euphorbia kansui* extract powder prepared as tea. The second group of six participants will complete the study twice (once on a decaffeinated black tea placebo treatment arm and once with the kansui treatment arm) in a randomized, double-blinded, placebo-controlled crossover design. Which treatment arm occurs first will be randomly assigned and will include a 56-day washout period between the two phases. A second screening visit will occur immediately following the washout period on Day 88. The second screening visit will occur 14-days prior to starting the second treatment assignment on Day 102; the second treatment phase will therefore begin on Day 88 and end on Day 101 (See Study Events Table). As indicated by the dose-escalation design of the study, participants in the kansui treatment phase for group 2 will receive 3 consecutive daily 1g doses of *Euphorbia kansui* extract powder served as tea.

We propose using a crossover double-blinded design due to our experience in performing *in vivo* studies of latent HIV reactivation. We have previously demonstrated statistically significant inter-individual differences in baseline values of the virologic and immunologic outcomes proposed in our study.[2] For these reasons, we have applied a similar crossover study design in another recent study which allowed the ability to detect even small differences in HIV RNA levels after intervention.[35] Therefore, due to the small detectable changes that most HIV latent reactivating agents (LRAs) produce at the doses tested in these trials[2,5,36,37] and the possibility of small detected changes from our trial of *kansui*, a within-subject control component to the study will allow us the greatest ability to accurately detect treatment-related differences in our study outcome measures.[38]

Subjects will initiate dosing on separate days. All safety data from each cohort (through week two of observation) will be collated and presented to an SMC before we escalate to the next dose level, as described below. We hypothesize that 1 g of kansui powdered, measured and prepared as tea, given as a single or 3 consecutive daily doses will be safe and well tolerated. We also hypothesize that kansui given as a single or 3 consecutive daily doses may induce T cell activation (CD69, CD38, and/or HLA-DR expression on CD4+ and CD8+ T cells) and latent HIV reactivation (cell-associated HIV RNA and plasma HIV RNA).

Participants will be screened for eligibility. Individuals meeting inclusion criteria without meeting exclusion criteria will be enrolled in the study in sequential dosing cohorts as below. The following treatment regimens will be used:

- Cohort 1: Kansui Phase of (1 g) x 1 dose (the first N = 2 participants)
- Cohort 2: Kansui Phase of (1 g) x 3 consecutive daily doses that is either preceded or followed by a Placebo Phase (the final N = 6 participants)

Study participants will be followed for 31 days following each treatment phase. There will also be a 56-day washout period in between the two treatment phases. The total duration of the study is expected to be up to 45 days for Cohort 1 and up to 146 days for Cohort 2 (See Study Events Table).

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

There is not a primary efficacy endpoint aim for this phase I safety and tolerability feasibility study, though we will be reporting changes in measures of T cell activation and latent HIV.

5.2 Safety Evaluations

Incidence and nature of adverse events

5.3 Other Evaluations (include only if applicable)

PBMCs will be collected and stored for future gene expression analyses. Plasma samples will be stored for future PK analyses

6 PARTICIPANT SELECTION

6.1 Study Population

We will perform a dose-escalation, proof-of-concept study of *Euphorbia kansui* extract powder prepared as tea in chronically HIV-infected individuals with long-term ART suppression. A total of 8 ART-suppressed patients will be recruited from the University of Utah for participation in this trial. The University of Utah Principle Investigator will oversee the recruitment of potential participants who meet inclusion criteria. Dr. Spivak will identify and recruit potential participants through his own clinic panel in Clinic 1A and through a cohort of patients already participating in an ongoing study at the University of Utah entitled, "Analysis of Latent HIV-1 Infection in Peripheral Blood Lymphocytes of Aviremic HIV-1 Infected Patients and Explorations of Therapeutic Misconception."

Participants will include HIV-positive adults ≥ 18 years old with long-term virologic suppression on stable ART, with a plasma HIV RNA below the assay limit of detection, CD4+ cell count >350 cells/mm^3, and adequate organ function (per criteria listed below).

6.2 Inclusion Criteria

1. Confirmed HIV-1 infection in adults aged 18 years or older.
2. Continuous therapy with a DHHS recommended/alternative combination ART for least 36 months (at least 3 agents) at study entry with no regimen changes in the preceding 24 weeks.
3. Maintenance of undetectable plasma HIV-1 RNA (<40 copies/ml) for at least 36 months. Episodes of single HIV plasma RNA 50-500 copies/ml will not exclude participation if subsequent HIV plasma RNA is <40 copies/ml.
4. Two CD4+ T cell counts >350 cells/μl in the six months prior to screening.
5. Written informed consent obtained from participant and ability for participant to comply with the requirements of the study.

6.3 Exclusion Criteria

1. Pre-ART viral load <2000 copies/ml (HIV controllers).
2. Based on prior history and/or virologic testing, no alternative ART regimens are available in the event that the current ART regimen is compromised as a result of this study.
3. Recent hospitalization in the last 90 days.
4. Recent infection in the last 90 days requiring systemic antibiotics.
5. Recent vaccination within the last 8 weeks prior to study scree or any study blood draw.
6. Any known prior (lasting >6 months) or current history of liver-related diseases including but not limited to:
    a. Hepatic cirrhosis of decompensated chronic liver diseases
    b. Clinically active hepatitis B or C infection as evidenced by clinical jaundice or Grade 2 or higher liver function test abnormalities
    c. Any hepatic impairment, regardless of the graded liver function test abnormalities.
7. Any known prior (lasting >6 months) or current history of gastrointestinal diseases including but not limited to:
    a. History of diarrheal illness requiring the use of anti-motility agents including inflammatory bowel disease, chronic diarrhea not otherwise specified.
    b. History of gastrointestinal bleeding with hemoglobin below 12.5 g/dL.
    c. History of gastric or duodenal ulcers
    d. Inflammatory gastrointestinal disease such as Crohn's disease or ulcerative colitis
8. Any significant renal disease (eGFR < 90 ml/min) or acute nephritis.
9. Screening hemoglobin below 12.5 g/dL.

10. Screening TSH consistent with hypothyroidism.
11. Significant myocardial disease (current myocarditis or reduced left ventricular ejection fraction below the lower limit of normal) or diagnosed coronary artery disease.
12. Significant respiratory disease requiring oxygen.
13. Diabetes or current hypothyroidism.
14. Participants of reproductive potential or breastfeeding. Women of childbearing potential must have a negative serum pregnancy test at screening. All participants of childbearing potential must agree to use a double-barrier method of contraception throughout the study period and up to 90 days after the last dose of kansui.
15. Exposure to any immunomodulatory drug (including maraviroc) in the16 weeks prior to study.
16. Prior or current use of experiment agents used with the intent to perturb the HIV-1 viral reservoir.
17. History of seizures, psychosis, abnormal electroencephalogram or brain damage with significant persisting neurological deficit
18. Positive test for tuberculosis by either skin test (PPD) or blood interferon-gamma release assay (QuantiFERON).
19. Significant substance use, which in the opinion of the investigator(s), is likely to interfere with the conduct of the study
20. Anticipated conflict or inability to attend and complete all protocol-scheduled study visits and assessments, especially for participants in Cohort 2 where treatment phases are separated by a 56-day washout

7 CONCURRENT MEDICATIONS

Participants may continue to take their usual medications, as long as they are medically necessary and can be monitored during the study period.

7.1 Allowed Medications and Treatments

Participants may not have exposure to any immunomodulatory drug (including maraviroc) in the 16 weeks prior to study or during study period and may not currently be taking experimental latent HIV reversing agents.

In the absence of drug-drug interaction information with kansui, only concomitant medications that are medically necessary will be continued with appropriate monitoring. In addition, the following medications are prohibited during the study and administration will be considered a protocol violation.

- Participants may not be take anti-motility agents during the study period. This is to allow accurate assessment of diarrhea-related adverse events.

- Participants may not initiate new medications that may cause diarrhea during the 31-day study period. These may include but are not limited to:
    - Proton pump inhibitors
    - Antibiotics
    - Antidepressants
    - Colchicine
    - Digitalis

In addition to the above medications, per traditional Chinese medicine practices, participants should also not ingest ice-cold food or beverages, spicy foods, or seafood in the two days prior to, during, and up to two days after kansui treatment.

8 STUDY TREATMENTS

8.1 Method of Assigning Participants to Treatment Groups

Cohort 1: NA

Cohort 2: Participants will be randomized to either kansui versus placebo treatment first using a random number generator. Whichever treatment the participant receives first, they will receive the other treatment (placebo or kansui) for their second treatment staring at Day 102 (See Study Events Table in Section 9 below).

8.2 Blinding

Cohort 1: NA
Cohort 2: The order in which participants in Cohort 2 complete the two treatment phases (i.e., kansui and placebo) will be unknown to both the participants and the PI/study team. Both the kansui and placebo will be prepared and delivered to the Center for Clinical & Translational Science (CCTS) in identical, HDPE vials (described later in section 8.3.3). Upon arrival at the CCTS, the HDPE vial will be received by a CCTS study nurse. At the appropriate time, the CCTS study nurse will prepare the tea (either kansui or placebo) in the med room of the CCTS, out of sight of the study participant, study coordinator, and study site PI to maintain double blinding. Following preparation the study nurse will administer the tea (kansui/placebo) to the participant following protocol section 8.4.3. The University of Utah Investigational Drug Services ("UU IDS") will randomize each participant in Cohort 2 according to the methods described in Protocol section 8.1. In the event that participant experiences an adverse event that requires the identity of the study drug be revealed, the PI will be able to contact UU IDS to break the blind. In the event that a participant blind is broken, the study PIs will determine the impact upon the un-blinded subject's continued participation in the study and if a replacement subject is needed on a case-by-case basis.

8.3 Formulation of Test and Control Products

8.3.1 Formulation of Test Product

For the current pilot study, we will use the same standardized batch of *Euphorbia kansui* extract powder studied in Dr. Matija Peterlin's laboratory at UCSF to perform the preliminary experiments described above. The *Euphorbia kansui* extract powder was purchased from Baoji F.S. Biological Development Company, and is Good Manufacturing Practice (GMP)-certified. The *Euphorbia kansui* root powder will be provided by the study investigator at UCSF and shipped in bulk to the University of Utah Health Investigational Drug Service (UUH IDS) Pharmacy. The *Euphorbia kansui* root extract powder is stored at the UUH IDS Pharmacy in a re-sealable plastic bag at room temperature, away from moisture and light exposure. Since the test product is in powder form, UUH IDS will prepare individual 1 g doses of *Euphorbia kansui* extract powder provided by the Department of Herbal Medicine at the American College of Traditional Chinese Medicine at UCSF. The details regarding these procedures can be found in the Standard Operating Protocol (SOP), *Euphorbia kansui* Extract Tea Preparation Guidance, for kansui preparation and administration. The University of Utah Health Investigational Drug Service Pharmacy is located at the Huntsman Cancer Hospital, 1950 Circle of Hope, Suite 2110 in Salt Lake City, UT 84112.

8.3.2 Formulation of Control Product

Placebo tea will be prepared using Twinings English Breakfast 100% pure black decaffenated tea. One packet of tea will be prepared and administered as for kansui tea (see section 8.4.3). Briefly, four ounces of boiled water will be cooled to 170-185° F and added to the tea bag in a cup. The tea will be stirred for approximately 30 seconds using a wooden stirring stick and allowed to cool for at least 5 minutes, reaching a temperature of less than or equal to 100° F, before administration. The placebo will be prepared in a separate room such that study participants will not observe differences in tea preparation and packaging.

8.3.3 Packaging and Labeling

The University of Utah Health Investigational Drug Service (UUH IDS) Pharmacy will prepare individual 1 g doses *Euphorbia kansui* extract powder or placebo using a Ohaus® Pharmacy Scale w/ Internal Calibration. As kansui (the root of Euphorbia kansui) is a Chinese herb with known safety issues, all laboratory and clinical personnel who handle kansui extract powder must use proper personal protection equipment (specifically - facemask, labcoat, and gloves) to prevent potential allergic reactions (e.g., contact dermatitis). Preparations will be packaged in white HDPE (high density polyethylene plastic) vials with screw caps and a tight, leakproof inner seal. Each study preparation bottle will be labeled with the required FDA warning statement, the protocol number, study participant number, a visit number, and directions for patient use.

Hunstman Cancer Hospital IDS

1950 Cirlce of Hope Salt Lake City UT 84112 801-585-0272

RX: xxxxx-x Presriber name

Date Subject ID: xxxxxxx Protocol#: Kansui

Subject full name:

Mix 1 gram of powder in 4 ounces of warm water and ingest by mouth as directed. Euphorbia kansui extract powder or placebo.

Qty: 1 gram

Rt: oral Do not use after: "expiration date" Refills: 0

Caution: Drug limited by Federal (United States) Law to investigational use only

8.4 Supply of Study Drug at the Site

8.4.1 Dosage/Dosage Regimen

One gram of *Euphorbia kansui* extract powder or placebo, reconstituted in 4 fluid ounces of warm water as tea, will be taken orally once daily for up to 3 days total. Participants enrolled in cohort 1will be administered 1 g of *Euphorbia kansui* extract powder prepared as tea for a total of 1 day and participants in cohort 2 will be administered 1 g of *Euphorbia kansui* extract powder prepared as tea or placebo for 3 consecutive days during the study period.

8.4.2 Dispensing

All handling of the trial products, storage, reconstitution, individual dose preparation, and drug accountability will be done by the study investigators, delegated pharmacy staff, or delegated member of the study team.

8.4.3 Administration Instructions

Study Investigators will instruct study staff on how to prepare the *Euphorbia kansui* extract powder as a tea, per traditional Chinese medicine practice as outlined in the *Euphorbia kansui* Extract Tea Preparation Guidance SOP.

Study staff will dispense and observe study participant take 1 vial (1 g) of *Euphorbia kansui* extract powder in 4 mL of water prepared as follows:

a. One gram of *Euphorbia kansui* extract from the labeled vial will be place into a 6-ounce Styrofoam or paper cup.

c. Four ounces of boiled water cooled to 170-185° F will be added to the powder in cup. Research staff will measure the water temperature using a designated thermometer for tea use only.

d. The tea will be stirred for approximately 30 seconds using a wooden stirring stick.

e. The tea will be allowed to cool for at least 5 minutes, reaching a temperature of less than or equal to 100° F, before administration.

f. All contents in the cup will be checked to ensure that the powder and water have been stirred appropriately and fully consumed. Research staff will record the time of dose (Time 0) on the treatment flowsheet.

g. Specific restrictions will be followed: no ice-cold beverages below <40°F) but graham crackers and room temperature juice will be allowed after the kansui dose (up to three servings of each), but other food & beverages will be restricted until AFTER 2+hr post-dose draw.

Study staff will then observe administration and side effects of participants on the day(s) of kansui treatment.

Study staff will similarly dispense and observe study participant take 1 packet of 100% pure decaffeinated black tea in 4 mL of water prepared as follows:

a. One packet of Twinings English breakfast 100% pure black decaffeinated tea from the labeled vial will be place into a 6-ounce Styrofoam or paper cup.

c. Four ounces of boiled water cooled to 170-185° F will be added to the tea in cup. Research staff will measure the water temperature using a designated thermometer for tea use only.

d. The tea will be stirred for approximately 30 seconds using a wooden stirring stick.

e. The tea will be allowed to cool for at least 5 minutes, reaching a temperature of less than or equal to 100° F, before administration.

f. All contents in the cup will be checked to ensure that the tea and water have been stirred appropriately and fully consumed. Research staff will record the time of dose (Time 0) on the treatment flowsheet.

g. Specific restrictions will be followed: no ice-cold beverages below <40°F) but graham crackers and room temperature juice will be allowed after the kansui dose (up to three servings of each), but other food & beverages will be restricted until AFTER 2+hr post-dose draw.

Study staff will then observe administration and side effects of participants on the day(s) of placebo administration as above for kansui treatment.

8.5 Supply of Study Drug at the Site

UUH IDS will package the study drug in HDPE vials and arrange for courier delivery directly from the UUH IDS Pharmacy to the University of Utah Center for Clinical and Translational Science (CCTS) located 421 Wakara Way, Suite 360 in Salt Lake City, UT 84108.

8.5.1 Storage

Euphorbia kansui extract powder storage will be in a cool, dry place, away from strong light, heat, or moisture. The shelf life is 2 years when properly stored with its original packaging. For this study, the vials of pre-measured 1 g doses of *Euphorbia kansui* extract powder will be stored in a locked cabinet in the UUH IDS Pharmacy. Entrance to the pharmacy is secure by electronic badge access, limited to IDS pharmacy personnel and managers. Temperature is controlled by air conditioning and monitored between 15-25 °C. A fully automated temperature monitoring system provides continuous temperature monitoring, recording, and notification. Temperature monitoring hardware accurately measure temperature and continually sends information to a web-based software system, which is accessible by IDS pharmacy personnel. Temperature records can be produced by authorized users for investigational refrigerator, freezer, or ambient temperature sensor through the temperature monitoring software system. If the temperature begins to go out of range, then the temperature monitoring software system will send a message to the on-call IDS pharmacist, who will respond within 15-30 minutes and take appropriate action to preserve the quality of the investigational product.

The UUH IDS will dispense study drug to the CCTS and maintain the distribution records. The CCTS Clinical Services Center (CSC) bionutritionist will administer study drug to participants and maintain the administration records.

8.6 Study Drug Accountability

An accurate and current accounting of the dispensing of study drug for each participant will be maintained on an ongoing basis by the study investigators. The number of study drug dispensed will be recorded on the Investigational Drug Accountability Record. The study investigators and CSC bionutrtionist will verify these documents throughout the course of the study.

8.7 Measures of Treatment Compliance

Study participants will be monitored in the CCTS for directly observed therapy on the day(s) when *Euphorbia kansui* extract powder prepared as tea or placebo is administered. They will be receiving clinical and laboratory assessments on those days for up to 6 hours post-treatment.

9 STUDY PROCEDURES AND GUIDELINES

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the participant.

Eligible participants will receive a single dose for either a single or 3 consecutive days (Cohort 1: day 1; Cohort 2: days 1, 2, 3). Extensive sampling will occur before, during, and after the last dose. A full schedule of events for each cohort is provided below:

Study Events Table Cohort 1

Parameter	Day -14[a]	Day -7[a]	Day 1	Day 1	Day 1	Day 1	Day 2	Day 31	Early Withdrawal Visit (if applicable)
	Screen	Baseline	0 hrs[b]	0.5 hrs[b]	2 hrs[b]	4 hrs[b]	24-hr post-dose Follow-up		
Informed consent	X								
Questionnaires	X	X	X				X	X	X
Demographics	X								
Medical history, medications, AE	X	X	X				X	X	X
Vital signs	X		X		X	X	X	X	X
Physical exam (either Complete / Targeted)[c]	X		X				X	X	X
Study kansui administration			X						
Complete blood count w/diff	X		X	X	X	X	X	X	X
Comprehensive Metabolic Panel	X		X				X	X	X
Quantiferon test (QFT)	X								X
Pregnancy test (female participants)	X							X	X
Thyroid-stimulating hormone (TSH)	X								
Hep B Ag	X								
HCV Ab	X								
CD4+/CD8+ T cell counts	X	X	X				X	X	X
HIV RNA: standard assay	X	X	X				X	X	X
Immune activation (CD69, CD38, HLA-DR)	X	X	X	X	X	X	X	X	X
HIV reservoir measures	X	X	X	X	X	X	X	X	X
PBMC and plasma cryopreservation for future studies (PK, transcriptome analyses)	X	X	X	X	X	X	X	X	X

[a] *Days -14, -7, 4 and 31 represent the time from receiving the first dose of Euphorbia kansui extract powder prepared as tea on the Day 1 Visit.*

[b] *Hours 0, 0.5, 2, and 4 represent time from receiving Euphorbia kansui extract powder prepared as tea dose on that dosing day.*

[c] *The complete physical exam must occur at Screening. In the event of an Early Withdrawal Visit a complete physical exam is to be performed. A targeted physical exam is to be completed for all other time points.*

Cohort 2. Participants in cohort 2 will be randomly assigned to either *kansui* or placebo first. The study will include a 56-day washout period between the two treatments (*kansui* and placebo). On study Day 88 a "second screening" visit for the second treatment assignment will occur; the second treatment phase will begin on study Day 102 and end on Day 132.

Study Events Table Cohort 2 (Treatment Phase 1)

Parameter	Day -14[a]	Day -7[a]	Day 1	Day 1	Day 1	Day 1	Day 2	Day 3	Day 3	Day 3	Day 3	Day 4	Day 31	Day 32-87
	Tx #1 Screening	Baseline	Tx #1 0 hrs[b]	0.5 hrs[b]	2 hrs[b]	4 hrs[b]	0 hrs[b]	0 hrs[b]	0.5 hrs[b]	2 hrs[b]	4 hrs[b]	24-hr post-dose follow-up		56-Day Washout Period
Informed consent	X													*No Study Procedures During Washout*
Questionnaires	X	X	X				X	X				X	X	
Demographics	X													
Medical history, medications, AE	X	X	X				X	X				X	X	
Vital signs	X		X		X	X	X	X		X	X	X	X	
Physical exam (either Complete / Targeted)[c]	X		X				X	X				X	X	
Study kansui administration			X				X	X						
Complete blood count w/diff	X		X	X	X	X	X	X	X	X	X	X	X	
Comprehensive Metabolic Panel	X		X				X	X				X	X	
Quantiferon test (QFT)	X													
Pregnancy test (female participants)	X												X	
TSH	X													
Hep B Ag	X													
HCV Ab	X													
CD4+/CD8+ T cell counts	X	X	X				X	X				X	X	
HIV RNA: standard assay	X	X	X				X	X				X	X	
Immune activation (CD69, CD38, HLA-DR)	X	X	X	X	X	X	X	X	X	X	X	X	X	
HIV reservoir measures	X	X	X	X	X	X	X	X	X	X	X	X	X	
PBMC and plasma cryopreservation for future studies (PK, transcriptome analyses)	X	X	X	X	X	X	X	X	X	X	X	X	X	

[a] *Days -14, -7, 4, 31, 88, 102-105, and 132 represent the time from receiving the first dose of treatment on the Day 1 Visit.*

[b] *Hours 0, 0.5, 2, and 4 represent time from receiving treatment (kansui or placebo) on that dosing day.*

[c] *The complete physical exam must occur at screening visits (Day -14 and Day 88). In the event of an Early Withdrawal Visit a complete physical exam is to be performed. A targeted physical exam is to be completed for all other time points.*

Study Events Table Cohort 2 (Treatment Phase 2)

Parameter	Day 88	Day 102	Day 102	Day 102	Day 102	Day 103	Day 104	Day 104	Day 104	Day 104	Day 105	Day 132	Early Withdrawal Visit (if applicable)
	Tx #2 Screening	Tx #1 0 hrs[a]	0.5 hrs[a]	2 hrs[a]	4 hrs[a]	0 hrs[a]	0 hrs[a]	0.5 hrs[a]	2 hrs[a]	4 hrs[a]	24-hr post-dose follow-up		
Informed consent													
Questionnaires	X	X				X	X				X	X	X
Demographics													
Medical history, medications, AE	X	X				X	X				X	X	X
Vital signs	X	X		X	X	X	X		X	X	X	X	X
Physical exam (either Complete / Targeted)[c]	X	X				X	X				X	X	X
Study kansui administration		X				X	X						
Complete blood count w/diff	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Metabolic Panel	X	X				X	X				X	X	X
Quantiferon test (QFT)	X												X
Pregnancy test (female participants)	X											X	X
TSH													
Hep B Ag													
HCV Ab													
CD4+/CD8+ T cell counts	X	X				X	X				X	X	X
HIV RNA: standard assay	X	X				X	X				X	X	X
Immune activation (CD69, CD38, HLA-DR)	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV reservoir measures	X	X	X	X	X	X	X	X	X	X	X	X	X
PBMC and plasma cryopreservation for future studies (PK, transcriptome analyses)	X	X	X	X	X	X	X	X	X	X	X	X	X

[a] *Hours 0, 0.5, 2, and 4 represent time from receiving treatment (kansui or placebo) on that dosing day.*

9.1 Evaluations by Visit

A full schedule of events by visit is show above.

9.1.1 Screening Procedures

1. Participants will be consented by the study investigator, or properly trained and delegated coordinator, before any procedures take place. Eligibility will be confirmed and medical history and concurrent medications ascertained.
2. A complete physical exam and vital signs, including blood pressure, pulse, temperature, respiratory rate, and weight will be performed.
3. A detailed interview regarding past and current medical history, medications, substance abuse will be obtained.
4. Participants will complete the Baseline Questionnaires, which includes questions regarding general and HIV-specific medical history, previous and current medications or therapies, medication adherence, previous and intercurrent illnesses/conditions, hospitalizations, and minor lifestyle questions.
5. The full screening blood tests will be obtained:
    a. Complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count)
    b. Comprehensive metabolic panel (serum sodium, potassium, chloride, bicarbonate, random glucose, blood urea nitrogen, creatinine)
    c. Liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, total bilirubin, direct bilirubin)
    d. Thyroid panel (thyroid stimulating hormone [TSH]) (Please note that for Cohort 2: TSH collected at the Screening Visit for Treatment Phase 1 but NOT collected for Treatment Phase 2)
    e. Hepatitis B surface antigen and Hepatitis C antibody (Please note that for Cohort 2: Hep B and HCV Ag is collected at the Screening Visit for Treatment Phase 1 but NOT collected for Treatment Phase 2)
    f. Quantiferon (QFT)
    g. Plasma β-hCG (females only)
    h. CD4+ and CD8+ T cell counts and plasma HIV RNA by clinical assay
6. Blood will also be collected for:
    a. Immunophenotyping (CD69, CD38, HLA-DR expression on CD4+ and CD8+ T cells)
    b. Latent HIV reservoir quantification (cell-associated HIV RNA, plasma HIV RNA). Given the potential for diurnal effect on the measures of HIV in resting cells, the first blood draw on this day will occur between 7:30 to 9:30 am.
    c. Transcriptome analysis (RNA-seq)

9.1.2 Baseline Procedures

1. For eligible participants, a baseline visit will occur within 2 weeks of the screening visit and prior to day 1.
2. Concomitant medications and adverse events will be reviewed.
3. Baseline questionnaire will be re-administered.
4. Blood will be collected for:
    a. CD4+ and CD8+ T cell counts and plasma HIV RNA by clinical assay
    b. Latent HIV reservoir quantification (cell-associated HIV RNA, plasma HIV RNA. Given the potential for diurnal effect on the measures of HIV in resting cells, the first blood draw on this day will occur between 7:30 to 9:30 am.
    c. PMBC (transcriptomics analyses) and plasma (PK analyses) storage

9.1.3 Dosing Day Procedures

1. For eligible participants, Day 1 will occur within 2 weeks of the screening visit. The 0.5, 2, and 4 hour visits noted below will occur within 30 minute windows. Participants will be free to leave the clinic in between the 0, 0.5, 2, and 4 hour visits.
2. 0 hours (prior to study drug administration that day)
    a. History including concomitant medications and adverse event record
    b. Follow-up questionnaires will be administered
    c. Targeted physical examination & vital signs
    d. Full blood examination (CBC, CMP)
    e. HIV RNA: standard assay
    f. Immune activation (CD69, CD38, HLA-DR expression on CD4+ and CD8+ T cells)
    g. HIV reservoir: Given the potential for diurnal effect on the measures of HIV in resting cells, the first blood draw on this day will occur between 7:30 to 9:30 am.
        i. Plasma HIV RNA (single copy assay)
        ii. Cell associated HIV RNA (unspliced RNA) in CD4+ T-cells.
    h. PBMC and plasma storage as below
3. 0.5 hours (after study drug administration that day)
    a. CBC with differential
    b. Immune activation
    c. PBMC and plasma cryopreservation
4. 2 hours (after study drug administration that day)

a. Vital signs
b. CBC with differential
c. Immune activation
d. PBMC and plasma cryopreservation

5. 4 hours (after study drug administration that day)
    a. Vital signs
    b. CBC with differential
    c. Immune activation
    d. PBMC and plasma cryopreservation

9.1.4 Post-Dosing Days Procedures

1. No dosing will occur at the 24-hour follow-up visit following the last dosing day, nor on Day 31.
2. A history including concomitant medications, adverse event record and assessment of adherence antiretroviral therapy.
3. A physical assessment will occur as needed.
4. Vital signs will be obtained.
5. Follow-up Questionnaire will be re-administered.
6. Full blood examination including CBC, CHEM-7, and LFTs will be performed.
7. Samples will be collected for immunophenotyping (CD69, CD38, HLA-DR expression on CD4+ and CD8+ T cells), and for quantification of the latent HIV reservoir (plasma HIV RNA, cell associated HIV RNA in CD4+ T-cells). PBMCs and plasmas will be collected for future transcriptomics and PK analyses, respectively.

9.1.5 Early Withdrawal Visit

1. Record any Adverse Experiences and/or Review participant diary for adverse experiences and exclusionary medication use.
2. Record changes to concomitant medications.
3. Follow-up Questionnaire will be re-administered.
4. Perform complete physical examination, including recording of vital signs.
5. Collect blood for clinical laboratory tests (CBC, CHEM-7, LFTs)
6. Collect samples for immunophenotyping (CD69, CD38, HLA-DR expression on CD4+ and CD8+ T cells), and for quantification of the latent HIV reservoir (plasma HIV RNA, cell associated HIV RNA in CD4+ T-cells.
7. PBMCs and plasmas will be collected for future transcriptomics and PK analyses, respectively.

9.2 Clinical Assessments

9.2.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening/ Baseline, Study Treatment Day(s), at the 24-hour post dosing follow-up visits on Day 2 (for Cohort 1) and Days 4 and 105 (for Cohort 2), and Day 31 and 132. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.2.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

9.2.3 Medical History

Relevant medical history, including history of current disease, other pertinent medical history, and information regarding underlying diseases will be recorded at Screening. Updated medical history including medication reconciliation and medication adverse effects will be recorded at Baseline, Study Treatment Day(s), at the 24-hour post dosing follow-up visits on Day 2 (for Cohort 1) and Days 4 and 105 (for Cohort 2), and Day 31 and 132.

9.2.4 Physical Examination

A complete physical examination will be performed by a study physician at Screening. Targeted physical examination will be performed on Study Treatment Day(s), at the 24-hour post dosing follow-up visits on Day 2 (for Cohort 1) and Days 4 and 105 (for Cohort 2), and Day 31 and 132.

9.2.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at Baseline/Screening, Study Treatment Day(s), at the 24-hour post dosing follow-up visits on Day 2 (for Cohort 1) and Days 4 and 105 (for Cohort 2), and Day 31 and 132.

9.2.6 Questionnaires

Study questionnaires were developed in collaboration between UCSF and UCLA investigators for use in clinical trials evaluating the safety and efficacy of HIV cures. The questionnaires consist of a detailed interview, including medical history, current and previous medications/therapies, medication adherence, previous and intercurrent illnesses/conditions, hospitalizations, lifestyle, side effects, adverse events, etc. There is a baseline and follow-up version, both of which are completed electronically through the REDCap web application. The baseline/screening questionnaires require approximately 45-60 minutes to complete and the follow-up questionnaires require approximately 15-30 minutes.

9.2.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF). Criteria for participant management, dose interruptions, modifications, and discontinuation of treatment will be mandated only for toxicities attributable to kansui.

9.3 Clinical Laboratory Measurements

9.3.1 Basic Laboratory Measures

Blood will be obtained and sent to the San Francisco General Hospital clinical laboratory for complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), blood chemistry profile (serum sodium, potassium, chloride, bicarbonate, random glucose, blood urea nitrogen, creatinine), and liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, total bilirubin, direct bilirubin). At screening, additional laboratory measures will include Hepatitis B surface antigen and Hepatitis C antibody, thyroid panel (thyroid stimulating hormone [TSH]), quantiferon (QFT), and plasma β-hCG (females only).

9.4 Pharmacokinetic Measurements

Blood for determination of serum concentrations of ingenol (the component in kansui powder that has been shown to reactivate latent HIV *in vitro* and in animal models) will be collected at 0.5, 2, 4, and 24 hours after each dose of kansui, with the exception of the Day 2 and Day 103 visits for Cohort 2, which will be collected at 24 hours post-dose. For example, for cohort 1, blood will be collected for PK measurement on day 1 (hours 0.5, 2, 6) and day 2 (hour 0). For cohort 2, blood will be collected for PK measurement on days 1 and 102 (hours 0.5 2, 4), days 2 and 103 (hours 0), and days 3 and 104 (hours 0, 0.5, 2, 4). Serum concentrations of ingenol will also be collected at the 24-hour post dosing follow-up visits on Day 2 (for Cohort 1) and Days 4 and 105 (for Cohort 2), and Day 31 and 132 for all dosing cohorts.

9.5 Research Laboratory Measurements

9.5.1 HIV Clinical Laboratory Assay Measures

Blood will be obtained and sent to San Francisco General Hospital clinical laboratory for real-time determination of CD4+ and CD8+ T cell counts and plasma HIV RNA (viral load).

9.5.2 HIV Immunologic and Virologic Research Assay Measures

Blood will also be collected before, during, and after dosing to measure parameters of immune activation (CD69, CD38, and HLA-DR expression on CD4+ and CD8+ T cells) and to quantify the latent HIV reservoir (cell-associated HIV RNA [unspliced RNA] and plasma HIV RNA [single copy assay]). Given the potential for diurnal effect on the measures of HIV in resting cells, the blood draw for the 0-hour (zero hour) time point

(prior to first dose of kansui) will occur between 7:30 to 9:30 am on the screening, baseline, and treatment day(s).

9.5.3 Transcriptome Analyses

We will measure the host and viral transcriptome pre- and post-dosing by performing RNA sequencing (RNA-seq) on isolated CD4+ T-cells and applying validated bioinformatic pipelines for gene expression analysis. Transcriptomic profiling of longitudinal samples will enable characterization of the effects of kansui (which contains ingenols) on host gene expression. In addition, consideration of the gene expression data within the context of the aforementioned virologic measurements will allow us to identify specific host transcriptomic correlates of viral reactivation. We hypothesize that the expression of particular genes (NFkB pathway components, cell-cycle regulators, cell-intrinsic immune factors) will be associated with the degree of viral reactivation.

9.6 Optional Tissue Banking

We are interested in collecting plasma and peripheral blood mononuclear cells taken from peripheral whole blood to be used in future studies on HIV eradication, including immunologic studies to evaluate markers of inflammation and viral transcription. Provided samples will be shipped from the University of Utah to be stored and maintained as part of the UCSF SCOPE Cohort at the UCSF AIDS Specimen Bank (ASB), which specializes in biospecimen storage and processing. Specimens will be stored in Oyster Point at 612 Forbes Boulevard in South San Francisco, CA.

Specimens stored for future use will not contain any identifiable information. Samples will be coded with a 4-digit PID code and include the collection date and specimen type (i.e., PBMC, plasma, serum, etc.). This code will be kept confidential and only the Principle Investigators will have access to the key linking specimens to study participants. The Principle Investigator will not give participant names or other identifying information to researchers who want to use specimens stored in the tissue bank, but will only provide non-identifying information, like age and disease history.

Future studies may be performed by scientists based at the University of Utah, the University of California, San Francisco and/or by scientists based at other institutions, such as other universities, the NIH and commercial laboratories. The types of studies which may be performed using tissue bank specimens include:

- tests for infections that are common in HIV-infected patients, such as cytomegalovirus or human herpesvirus 8
- genetic testing
- new tests to measure the amount of “latent” HIV reservoir.

Genetic information (genotype data) and medical record data (phenotype data) may be shared broadly in a coded form for future genetic research or analysis. Non-identifiable medical information (diagnoses, blood pressure, age if less than 85) may be provided to other scientists or companies not at the University of Utah or UCSF, including a public government health research database. Research results from studies conducted from the

tissue bank will not be returned to participants, included in patient medical records, or provided to their local study doctors.

Researchers that wish to obtain tissue samples from the bank are required to complete a SCOPE sample request form, which is reviewed and approved by the SCOPE study PI, Dr. Steve Deeks, MD, and the primary study coordinator, Becky Hoh. Dr. Deeks is a Professor of Medicine in Residence at the University of California, San Francisco (UCSF) and a faculty member in the Division of HIV, Infectious Diseases and Global Medicine at Zuckerberg San Francisco General Hospital. Dr. Deeks has been engaged in HIV research and clinical care since 1993.

Specimens will be kept until they are used up or destroyed.

Providing the specimen for use in future research is optional for participants. Should a participant choose to withdraw their sample from the tissue bank they can have their blood sample removed by contacting the Principle Investigator using contact information contained in the consent and authorization form.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the participant, for the occurrence of AEs during each participant visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The participant may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The participant is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the participant's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

10.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity

- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the participant or require intervention to prevent one of the outcomes listed.

10.2.1 Serious Adverse Experience Reporting

The study site will document all SAEs that occur (whether or not related to study drug) per the University of Utah IRB Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

10.3 Monitoring

During the study, safety will be monitored by a Safety Monitoring Committee (SMC), which will be led by a qualified University of Utah faculty member in the Department of Medicine with expertise in HIV clinical research (see Section 13 below). The SMC will review all study procedures before the initiation of the study. They will have the full and final authority to stop the study for any safety concern at any point. The Principal Investigator at the University of Utah will be the primary medical monitor responsible for study adverse event monitoring and will report adverse events and unanticipated problems to the University of Utah IRB and the SMC. All participants will be followed for possible adverse events and unanticipated problems throughout the study period.

Participants will be monitored in an outpatient clinic during the first 4-6 hours post-dose on dosing days. At each visit, participants will be assessed for any new symptoms, and a study coordinator will obtain vital signs. The study will require 48-hour reporting of all laboratory values, signs/symptoms, and serious adverse events (SAEs). The study will have intensive Phase I safety monitoring, which will include regular and frequent team review (every week for the first 2 months of the study; then every two weeks thereafter) of all reported events. In addition, for any Grade 3 or 4 events, sites will be instructed to contact the study team by email.

- Grade 1 or 2 Toxicity: Participants who develop a Grade 1 or 2 AE or toxicity may continue study treatment. If a participant chooses to discontinue study treatment, the site should notify the kansui protocol core team as noted above, and encourage the participant to complete any remaining study visits.

- Grade 3 or 4 Toxicity: Participants experiencing Grade 3 or 4 AEs requiring permanent discontinuation of study treatment should be followed closely for resolution of the AE to Grade ≤ 2 and the protocol core team must be notified. Participants discontinuing study treatment should be encouraged to complete any remaining study visits.

Dr. Adam Spivak will be contacted directly to report medical concerns or questions regarding safety.

Phone: (443) 854-1582

10.4 Criteria for Discontinuation

Safety will be monitored by the dedicated SMC, led by a qualified HIV specialist in the University of Utah Department of Medicine (see Section 13 below). The SMC will have full and final authority to stop the study at any point for safety concerns. Given the unknown safety profile of *Euphorbia kansui*, the first participant in each dosing level will complete 5 days of study prior to any further participants commencing the study. The decision to proceed with further drug administration will be taken by the Principal Investigator, taking into consideration clinical and laboratory adverse events. The decision to proceed with the next dosing level will be taken by the SMC. Specifically, dosing will be paused and the SMC will be consulted if one Grade 4 or two Grade 3 drug related adverse events or laboratory abnormalities are reported.

In addition, since there is currently insufficient data demonstrating the effect of kansui on antiretroviral therapy or other concomitant medications (and vice versa), we will evaluate serum HIV-1 RNA levels using standard clinical assays within each dosing cohort prior to dose escalation. HIV RNA measures will be collected on the initial dosing day(s) (Day 1 for Cohort 1, Days 1-3 and 102-104 for Cohort 2), at the 24-hour post dosing follow-up visits on Day 2 (for Cohort 1) and Days 4 and 105 (for Cohort 2), and on Day 31 and 132. This is to safeguard against the potential risk of virologic failure and/or the development of resistance due to sub-therapeutic antiretroviral therapy drug levels in study participants.

Specifically, safety discontinuation criteria will include:

1. Any serious adverse event assessed as at least possibly related to study treatment:
    a. Any Grade 4 drug-related adverse event or laboratory abnormality
    b. Two Grade 3 drug-related adverse event or laboratory abnormality
2. Virologic failure (defined as any two consecutive HIV-1 RNA levels >200 copies/mL or a single HIV-1 RNA level >1000 copies/mL). These values would be above the levels predicted to be seen with HIV reactivation with most current latent reactivating agents and are safety stopping criteria currently used for other HIV latent reactivating agent trials, e.g., the AIDS Clinical Trials Group (ACTG 5336) trial, evaluating the role of Sirolimus on immune activation and the latent reservoir in chronic HIV-infected, ART-suppressed individuals.
3. Pregnancy or breastfeeding
4. Requirement for prohibited concomitant medications (see section 7.1)
5. Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity section of the protocol

The specific adverse events that will be solicited include: diarrhea, gastrointestinal irritability, gastrointestinal bleeding, headache, and symptoms of dehydration (light-

headedness, tachycardia). For the most likely expected reported adverse event, diarrhea, we will strictly adhere to established toxicity grading used by the National Cancer Institute for chemotherapy-induced diarrhea.[39,40]

Grade	Description
1	Increase of fewer than four stools per day over baseline
2	Increase of four to six stools per day over baseline
3	Increase of seven or more stools per day over baseline; incontinence; hospitalization indicated
4	Life-threatening consequences; urgent intervention indicated
5	Death

** Based on information from National Cancer Institute, 2010.*

If stopping/pausing criteria are not met, the remainder of the dosing cohort will be enrolled/dosed the subsequent week. The study will proceed to the next dosing level provided the stopping/pausing criteria are not met. A University of Utah Department of Medicine faculty member who is not otherwise involved in the study and who will not communicate with the study investigators regarding any aspect of the SMC reports will prepare the monitoring reports. The SMC will review accrual, adverse events summaries, CD4+ T-cell counts and HIV RNA levels/suppression over time, off-study rates and completeness of follow-up, by dose cohort.

11 DISCONTINUATION AND REPLACEMENT OF PARTICIPANTS

11.1 Early Discontinuation of Study Drug

A participant may be discontinued from study treatment at any time if the participant or the investigator feels that it is not in the participant's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Participant withdrawal of consent
- Participant is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the participant to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test or breastfeeding (females)

If a participant is withdrawn from treatment due to an adverse event, the participant will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All participants who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents (refer to early termination procedures).

11.2 Withdrawal of Participants from the Study

A participant may be withdrawn from the study at any time if the participant, the investigator, or the Sponsor feels that it is not in the participant's best interest to continue.

11.3 Replacement of Participants

Participants who withdraw from the study treatment before last day of study drug dosing (i.e., Day 1 for Cohort 1, Day 104 for Cohort 2) will be replaced by another enrolled participant to maintain total study numbers.

The CRF will document the reason for the withdrawal and date of withdrawal. Date of withdrawal will be documented as the date of last study drug treatment, not the date that the decision to withdraw treatment was made.

Participants will be followed after withdrawal from the study for 3 calendar days after cessation of treatment. All adverse events during that period will be reported.

12 PROTOCOL VIOLATIONS

A protocol violation occurs when the participant or the study investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, participant safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Principal Investigator will determine if a protocol violation will result in withdrawal of a participant.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

13 DATA SAFETY MONITORING

The study will require 48-hour reporting by sites of all laboratory values, signs/ symptoms, and SAEs. The study will have intensive Phase I safety monitoring, which will include regular and frequent team review (every week for the first 2 months of the study; then every two weeks thereafter) of all reported events. In addition, for any Grade 3 or 4 events, sites will be instructed to contact the study team by email.

Safety monitoring will include team review of adverse events (including all reported signs/symptoms, laboratory abnormalities, diagnoses, and SAEs) and team assessment as to the possible relationship of adverse events to the study treatment. The review will also include assessment any participants with an unconfirmed CD4+ T cell or HIV RNA measurement indicating a potential safety endpoint. Regular team monitoring will also assess early study discontinuations and visit/sample completeness. In addition, study accrual and baseline characteristics of study participants will be reviewed periodically during accrual. After a dose cohort has completed accrual and sufficient follow-up time is available, the team will evaluate whether to dose-escalate and open the next dose cohort to accrual, as described below.

Safety Monitoring Committee (SMC). We have established an independent Safety Monitoring Committee (SMC) for this study. As safety concerns for kansui pertain primarily to gastrointestinal symptoms (expected to be mild) and perhaps control of HIV infection, these areas are represented on the SMC by well-recognized experts: Dr. Andrew Pavia (Professor of Medicine, University of Utah, expert in epidemiology, infectious disease, HIV, and clinical research), Dr. Ma Somsouk (Assistant Professor of Medicine, UCSF, expert in clinical gastroenterology), Rick Hecht (Professor of Medicine, UCSF, and an expert in complementary medicine) and Annie Luetkemeyer (Associate Professor of Medicine, UCSF, and expert on HIV management and clinical trials).

The committee will be co-chaired by Dr. Pavia at the University of Utah and Dr. Hecht at UCSF, who both have experience in the regulatory aspects of clinical trials and will independently deliberate using study data.

The SMC will meet after completion of each dose cohort to review safety data. The meeting will occur no earlier that two weeks after the last dose. The decision to move to the next dose cohort will be made by the SMC, after consultation with the study investigators.

A study data coordinator will produce administrative reports regularly describing study progress including the following: (1) accrual, (2) demographics, (3) study subject status, (4) laboratory data, and (5) number and type of serious AEs. Reviews will be communicated to the University of Utah and UCSF Committee on Human Research (CHR), study sponsor, and/or federal agencies, as appropriate. The SMC will have access to treatment assignment. The study will be discontinued if the SMC determines that it is in the best interest of the subjects.

Grade 1 or 2 AEs will not result in any change to the study plans. If there is evidence for a Grade 3 AE that is not caused by the study drug, the study will continue as planned. On

the other hand, Grade 3 AEs thought to be possibly caused by the intervention and any Grade 4 AEs will result in a hold on any future enrollments until a decision to proceed or to stop the study is made by the SMC

14 STATISTICAL METHODS AND CONSIDERATIONS

14.1 Data Sets Analyzed

Dr. Lee will be performing the data analysis for the study. Paired analyses using Wilcoxon signed rank tests will be performed to evaluate whether there is a statistically significant change in markers of immune activation (CD69, CD38, and/or HLA-DR) on CD4+ and CD8+ T cells after a 1, 2, or 3 doses of *Euphorbia kansui* extract powder prepared as tea compared to baseline (reference will be the average of the three baseline measures). Multivariate analyses will then be performed to evaluate the association between kansui and measures of immune activation at post-dosing, using linear mixed effects models, which account for within-participant correlation of observations and allows for greater stability in the estimate of the outcome measures within individuals. Similarly, for measures of the HIV reservoir, we will perform linear mixed effects regression to compare CA-US RNA levels before and after kansui and use negative binomial mixed effects regression (a method to analyze over-dispersed count data) to compare plasma HIV RNA levels before and after kansui administration (reference will be the average of three baseline measures).

14.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized by dose level: race, gender, age, height and weight.

14.3 Analysis of Primary Endpoint

The safety and tolerability data will be summarized by dosing cohort. Adverse events will be tabulated by dosing cohort and will include the number of participants for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug. We may be underpowered to perform Fisher's exact tests to compare crude rates of adverse events between dosing cohorts or to perform adequate Cochran-Armitage trend tests for dose-toxicity.

14.4 Analysis of Secondary Endpoints

We will perform Wilcoxon rank sum tests to compare levels of immune activation (CD69, CD38, and/or HLA-DR expression on CD4+ and CD8+ T cells) between dosing cohorts and Wilcoxon signed rank tests to compare measures within individuals. We will perform linear mixed effects modeling to compare the effect of *Euphorbia kansui* extract powder prepared as tea on immune activation over time and account for repeated measures within individuals, compared to pre-treatment (using the average of three baseline measures). Similarly, we will perform linear mixed effects regression modeling to assess changes in CA-US RNA levels and use negative binomial mixed effects

modeling to changes in plasma HIV RNA levels, before and after kansui administration (the reference will be the average of three baseline measures).

14.5 Sample Size

A total of 8 participants (2 in dosing cohort 1 and 6 in dosing cohort 2) will contribute to the analysis, with 8 or 25 timepoint measures, respectively. The within-individual variability will be reduced by repeated measurements post-dose. Based on existing data from our study population regarding the percent CD38+HLA-DR+ CD4+ T cells (standard deviation of 4.9%) in our study population and a paired, one sample, repeated measures analysis, we will have greater than 80% power to detect a 2.7% change in CD4+ T cell activation levels post-dose. Similarly, we will have greater than 80% power to detect a 17% change in cell-associated HIV-1 RNA and a 0.37% change in plasma HIV-1 RNA.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each participant treated with the study drug.

Case report forms (CRFs) will be provided for each participant. Study personnel will enter data from source documents corresponding to a participant's visit into the protocol-specific paper CRF when the information corresponding to that visit is available. Participants will not be identified by name in the study database or on any study documents to be collected by the study investigators, but will be identified by a four-digit patient identification number (PID). If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Principal Investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Principal Investigator. A copy of the CRF will remain at the study site at the completion of the study.

15.2 Data Management Procedures

All data for our proposed study will be managed by the UCSF Data Coordinating Center, which is housed in the Department of Epidemiology and Biostatistics. This center currently serves 25 multicenter cohort and randomized trials throughout the world. Data management for this study will be directed by Dr. Jeffrey Martin, who has worked on similar studies for the past five years.

The UCSF Department of Epidemiology and Biostatistics complies with federal, state, University, and campus electronic information security requirements through a combination of physical, technical, procedural, and management controls. At the procedural level, all Coordinating Center (CC) personnel sign a confidentiality agreement

and undergo security awareness training for HIPAA and the handling of sensitive data. All employees of the University of California, San Francisco were required to obtain Security Awareness Training and implement appropriate security measures. New employees who use computers must take this training. Remote users of the data also receive training from the Data Management Group prior to gaining access to data systems.

15.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

The network at UCSF CC is privately maintained, hardware firewalled and none of the workstations or database servers can be directly addressed from outside the Local Area Network. Website communications are encrypted using an SSL certificate. Network OS is Windows Active Directory. Remote access is via SSL-VPN. A network administrator and server administrators support the network and servers and the Data Systems Services Group (developers/database administrators) support the database and web applications. The support team is paged 24/7 when servers or critical data center equipment experiences issues.

All study data is housed at the UCSF CC in a secure server room. The building is locked outside of normal business hours. All system servers are located in a limited access suite fitted with an Access Control System. Within the locked suite is a locked server room fitted with an additional secure door. Only critical Information Systems staff possesses the access code required to enter the room. All who enter the system server room must sign a server room access log in accordance with UCSF CC IT Security SOPs.

Study database access is controlled via two-factor password security. Development workstation access is controlled via Microsoft logon. Once a workstation is accessible, access to the study data on the SQL server via any development application requires appropriate logon-specific permission assigned in SQL Security Manager. Communication between study servers and client machines on the UCSF network are encrypted using an SSL certificate. All servers are protected from viruses by McAfee VirusScan. This software automatically checks for virus signature file updates from a McAfee FTP site once an hour, and if necessary directly updates itself. All anti-virus software is monitored and network personnel notified in the event that the software stops functioning on a given server.

All study data are stored on SQL servers managed by the UCSF Coordinating Center. All servers are housed in a secure server room.

Web site access: The study web sites are protected by two hardware-based firewalls to shape incoming and outgoing traffic. Access to the study management web site is restricted to approved personnel only. Approved personnel gain access to the system using a multi-layered authentication scheme. A log of all personnel with level of access is kept and updated regularly. Once a clinic site user accesses the system they are only permitted to view data received from their site, with the exception of official aggregate reports. Users are not permitted to view or alter another clinic's data.

Data transmission from web server to client: The UCSF CC currently utilizes Secure Socket Layer (SSL) protocol which protects all data transmission sent over the Internet between the CC IIS Web Server and every client machine which accesses our study web sites.

System Backup

Back-ups: All department workstations and servers are automatically backed-up every night. Back-up systems are monitored daily.

Failover Site: As part of the nightly database maintenance procedures, all SQL databases are backed up to a "failover" site at our co-location facility. This site has copies of the study databases as well as all associated systems required to carry on a study in the event of a disaster at the primary location.

Off-site Storage: Network back-ups are written to tape and sent to an off-site vendor every two weeks, with tapes being rotated every two months.

Recovery/File Restores: If important files or data from the network are accidentally deleted, the IT staff can locate the items and restore data within an hour in most cases.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each participant must be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that participant. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued.

15.6 Participant Confidentiality

In order to maintain participant confidentiality, only a site number, participant number and participant initials will identify all study participants on CRFs and other documentation submitted to the Sponsor.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the study investigators. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB of the participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB unconditional approval statement will be transmitted by the Investigator prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new

information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Participants (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each participant prior to entering the participant into the trial. Information should be given in both oral and written form and participants must be given ample opportunity to inquire about details of the study. A copy of the signed consent form will be given to the participant, and the original will be maintained with the participant's records.

16.4 Publications

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of participants.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).

7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to participants or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/participants.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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