Protocol Title: HERV-K Suppression Using Antiretroviral Therapy in Volunteers with Amyotrophic Lateral Sclerosis (ALS)
Abbreviated Title: ART in ALS
Protocol Number: 15-N-0126
Date of this Submission/Version: July 13, 2021 (M)

Total requested accrual
(200) Participants
(0) Healthy Volunteers

Project Uses Ionizing Radiation: ☐ No ☒ Yes
☐ Medically-indicated only
☒ Research-related only
☐ Both

IND/IDE ☒ No ☐Yes (attach FDA documentation)
Drug/Device/#_____
Sponsor: _____

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☒ No ☐ Yes

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☐ No ☒Yes
Agreement type and number _Please refer to Study Application in iRIS____ Expiration Date_____

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of standard consent form: 8.8
Flesch-Kincaid reading level of blood draw only consent form: 7.6

Précis:

Objective

In this Phase I, proof-of-concept study, we aim to determine whether an antiretroviral regimen approved to treat human immunodeficiency virus (HIV) infection would also suppress levels of Human Endogenous Retrovirus-K (HERV-K) found to be activated in a subset of participants with amyotrophic lateral sclerosis (ALS). We propose to measure the of blood levels of HERV-K by quantitative PCR before, during, and after treatment with an antiretroviral regimen. We will evaluate the safety of the antiretroviral regimen for participants with ALS and also explore clinical and neurophysiological outcomes of ALS symptoms, quality of life, and pulmonary function.

Study Population

We will study a subset of ALS participants who have a ratio of HERV-K:RPP30 greater than or equal to 13. About 30% of ALS participants may have detectable levels of HERV-K; about 20% of participants with ALS have a level >1000 copies/ml. To show whether the HERV-K could be suppressed, we will recruit from the approximately 20% of participants with the high levels so that the antiretroviral effect can be determined.

Design

This is an open-label study of a combination antiretroviral therapy for 24 weeks in up to 25 HIV-negative, HTLV-negative ALS participants with high ratio of HERV-K:RPP30. The study duration for each participant will be up to 72 weeks. Participants will be followed regularly for safety, clinical, and neurophysiological outcomes.

Outcome Measures

The primary outcome measure will be the percent decline in blood HERV-K concentration measured by quantitative PCR. Percent decline for a participant is measured by: 100 x (screening visit – week 24 visit measurement) / screening visit. The safety of antiretrovirals in volunteers with ALS as measured by the frequency and type of AEs, the ability to remain on assigned treatment (tolerability), physical examinations, laboratory test results, vital signs, and weight/body mass index (BMI). Efficacy will be explored by measuring the change in mean scores of: the ALS Functional Rating Scale-Revised (ALSFRS-R), the ALS Specific Quality of Life Inventory-Revised (ALSSQOL-R), the ALS Cognitive Behavioral Screen (ALS-CBS), vital capacity and maximal inspiratory pressure as measured by handheld spirometer, electrical impedance myography (EIM), the change in neurofilament levels in blood and/or CSF, and the change in urine p75ECD levels.

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List of Abbreviations

AE	Adverse Event/Adverse Experience
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale-Revised
ALSSQOL-R	ALS Specific Quality of Life Inventory-Revised
ALS-CBS	ALS Cognitive Behavioral Screen
ART	Antiretroviral Therapy
BMI	Body Mass Index
CRF	Case Report Form
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EIM	Electrical impedance myography
EMG	Electromyography
fALS	Familial ALS
FDA	Food and Drug Administration
HERV	Human Endogenous Retrovirus
HIV	Human Immunodeficiency Virus
HTLV	Human T-Lymphotropic Virus
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
LP	Lumbar Puncture
MIP	Maximal Inspiratory Pressure
N	Number (refers to number of participants/sample size)
NCS	Nerve Conduction Study
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NRTI	Nucleoside Reverse Transcriptase Inhibitor
PCR	Polymerase Chain Reaction
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
SAE	Serious Adverse Event/Serious Adverse Experience
sALS	Sporadic ALS
SOD1	Superoxide Dismutase 1
TAF	Tenofovir alafenamide
TARDP	TAR-DNA-binding protein

1. Introduction

ALS

Amyotrophic Lateral Sclerosis (ALS) is a rare, usually fatal degenerative disorder of large motor neurons of the cerebral cortex, brain stem and spinal cord that results in progressive wasting and paralysis of voluntary muscles. It has a prevalence of 3.9 cases/100,000 persons in the United States and the lifetime risk for individuals is 1/600 to 1/1000. ALS affects individuals of nearly all ages, genders, races, and geographic locations.[1] The median age of onset is 55 years. There is a slight male predominance (3:2 men:women) in sporadic ALS. Age and gender are the only risk factors consistently seen in epidemiological studies.[1] Fifty percent of people with ALS will die within three years of onset of symptoms and 90% die within five years.[2] The only available pharmacotherapy for ALS, riluzole, prolongs survival by only 60-90 days.[3]

Essential features of ALS are progressive signs and symptoms of lower motor neuron dysfunction (atrophy, cramps, and fasciculations) associated with corticospinal tract signs (spasticity, enhanced and pathological reflexes) typically in the absence of sensory findings.[4] There is relative sparing of muscles of eye movement and the urinary sphincters. The course is relentless; strength, respiratory function and overall function invariably decrease with time. Hypoxia and pneumonia account for the majority of deaths in patients with ALS.[5]

The majority of ALS cases are sporadic (sALS); 10% are familial (fALS). Mutations in specific genes or loci have been identified in ~40% familial and ~10% of sporadic cases.[6] Those most frequently detected involve C9ORF72, superoxide dismutase-1 (SOD1) and the TAR-DNA-binding protein (TARDBP). C9ORF72 is strongly associated with ALS cases in which motor neuron degeneration is accompanied by frontotemporal dementia.[7] Clusters of cases were also reported in football and soccer players, suggesting the possibility of trauma as a triggering event. Pathophysiological processes implicated in ALS include: toxicity from excess excitation of the motor neuron by transmitters such as glutamate, free radical-mediated oxidative cytotoxicity, neuroinflammation, mitochondrial dysfunction, autoimmune processes, cytoskeletal abnormalities, and aberrant activation of cyclo-oxygenase.

ALS and Viruses

It was also suggested that a virus may be associated with ALS, in part because of the marked similarities in the histopathologic and neuroanatomic findings in ALS and poliomyelitis.[8] Both are motor neuron diseases causing asymmetric-onset weakness that then spreads to other areas and eventually leading to diffuse muscle paralysis. Additionally, post-polio syndrome occurs many years after infection and produces progressive weakness similar to ALS. However, it is improbable that a virus or a post-viral syndrome is the primary cause of ALS. A concrete link to a viral infection has never been documented in ALS; however, the research investigating such a link led to several noteworthy findings.

The first demonstration of a possible viral association with ALS was in 1975 when reverse transcriptase (RT), an enzyme essential for replication of retroviruses (e.g., human immunodeficiency virus [HIV] or Human T-lymphotropic virus [HTLV]), was found post-mortem in two Guamanian ALS patients' brains but not in brains from two control individuals.[9] Subsequent studies showed approximately 53% (n=131) of ALS patients have detectable serum levels of RT compared with 12% (n=152) of controls (Table 1). Interestingly, relatives related by blood to patients with ALS are more likely than unrelated controls to have detectable RT. To date, there are no reported genetic testing results for these unaffected controls with detectable RT levels to see if they share a genetic mutation associated with ALS.

Table 1. Summary of Published Data on ALS and Serum Reverse Transcriptase

Author	Subjects (number)	RT in serum	Screened Retroviruses
Andrews et al. [10]	ALS (56) Controls (58)	59% 5%	HIV, HTLV, HFV, HRV5
Steele et al.	ALS (30) Unrelated controls (44) Related controls (14)	47% 18% 43%	NR
MacGowan	ALS (23) Controls (21)	56% 19%	HIV
McCormick et al.	ALS (22) Controls (14)	50% 7%	HIV HTLV

RT, reverse transcriptase. HTLV, Human T-Lymphotrophic Virus. HFV, Human Foamy Virus. HRV, Human Retrovirus-5. NR, not reported

Human Endogenous Retroviruses (HERV)

The reason for some ALS patients having detectable levels of the RT enzyme in brain and serum is unclear. The studies that have looked for retroviruses have not been able to detect them (Table 1). However, RT may not be produced by an exogenous/acquired retrovirus and could be associated with an endogenous retrovirus instead. When retroviruses infect humans, they incorporate their genetic information into a host's DNA. Through ancestral retroviral infections, and the successive sharing of these retroviral genetic elements with infected persons' progeny, 8-9% of our human genome is now made of retroviral elements. These elements closely resemble viruses and thus are termed human "endogenous" retroviruses (HERV) in contrast to exogenous, infectious retroviruses such as HIV or HTLV. Some of these elements may form viral particles although there is no evidence that these endogenous retroviruses remain infectious or are the primary etiologic agent of any disease. Research studies have shown that some diseases are associated with an increased expression of endogenous retroviral elements, e.g., in rheumatoid arthritis or systemic lupus erythematosus; however, the significance of these findings is uncertain.[11,12]

Of the HERV families, HERV-K and HERV-W have gained the most attention due to the presence of a complete open-reading frame (cORF) and the ability to form virus-like particles. We analyzed serum samples from 190 different participants: 72 with ALS, 53 with frontotemporal dementia (a neurodegenerative condition often associated with ALS), 26 with Alzheimer's dementia, 9 from family members of ALS patients, and 30 healthy controls. All samples were obtained from neurologists at Emory University. We analyzed the samples in a blinded fashion so that we did not know the diagnosis until after determining the results. The methods are described in Section 9.2. Six of 72 samples from patients with

sporadic ALS had very high levels (>1000 RNA copies/ml) of HERV-K *gag* RNA expression as measured by quantitative polymerase chain reaction (PCR). There was no detectable HERV-K in samples from healthy controls or patients with either frontotemporal dementia or Alzheimer's dementia (**Figure 1**). Low level activation was found in first-degree blood relatives of ALS patients similar to the finding reported for reverse transcriptase detection.

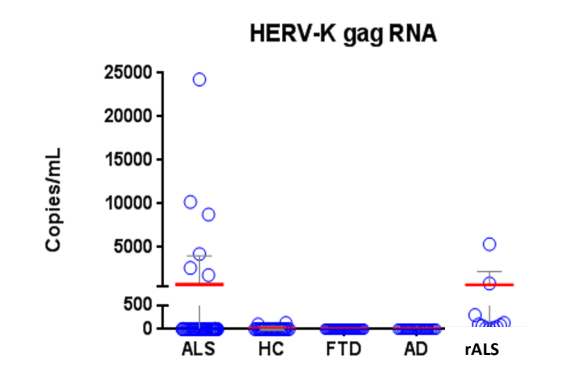

Figure 1: Detection of HERV-K in plasma. Quantitative RT-PCR was used to detect HERV-K transcripts in 140 ul of plasma from patients with ALS (n=72), healthy controls (HC; n=30), frontotemporal dementia (FTD; n=53), Alzheimer's disease (AD; n=29) and healthy first degree relatives of patients with ALS (rALS n=9). Highest levels are seen in a subgroup of ALS patients.

We also confirmed the presence of HERV-K in post-mortem brain samples from ALS patients. Using samples from both patients and healthy controls, we determined that the entire HERV-K genome (**Figure 2A**) and spliced transcripts were expressed in both sets. Initial observations suggested that the 4.5 kb spliced *env* transcript was exclusively expressed in brains from ALS patients (3 of 11). Expression of HERV-K transcripts was further confirmed by quantitative PCR which showed that transcripts for all three genes, *gag*, *pol* and *env*, were elevated in the ALS brain samples (**Figure 2B and C**). There was good correlation between the expression of each of these genes confirming that the entire viral genome is expressed in these patients. This is consistent with our previous observation where HERV-K *pol* RNA transcript was expressed in the brain of sporadic ALS patients but not in those with Parkinson's disease[13]. No particular clinical phenotype was associated with the expression of HERV-K in ALS patients.

To determine the cell types in which HERV-K was expressed, we immunostained brain tissue from ALS patients using an antibody to the HERV-K *env*. We found expression of the *env* protein in 3 of 5 individuals (**Figure 2D and E**), with strong expression in the large pyramidal neurons of the cortex (**Figure 2F**). The expression was exclusively in the neuronal cytoplasm and was concentrated in foci at the cell membrane (**Figure 2F**). Anterior horn neurons in the spinal cord also showed immunostaining for HERV-K *env*. These cells had a dysmorphic appearance (**Figure 2G**). No immunostaining was noted in the glial cells or in the white matter (**Figure 2H**).

It is likely that reverse transcriptase is a less specific finding than the RNA transcripts of HERV-K and thus fewer ALS patients have HERV-K compared to those with reverse transcriptase activity. To date, there is no clear phenotypic differences in those ALS patients with or without elevated levels of HERV-K; however, the sample size of current data is limited to make such conclusions.

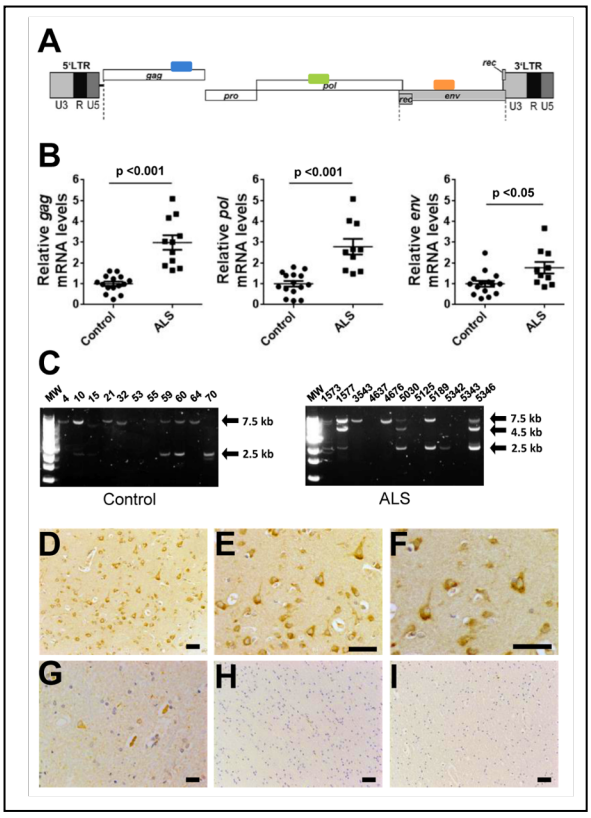

Figure 2: HERV-K expression in brain of ALS patients.. Total RNA was extracted from frozen brain tissue with RNeasy Plus mini kit. The RNA extracts were treated with RNase-free DNase. Reverse transcription was performed with 1µg RNA using Superscript III first stand kit. Quantitative PCR was done using Applied Biosystems Vii 7. (A) HERV-K genome showing regions amplified by PCR

(B) All HERV-K genes were significantly elevated in ALS patients (n=11) compared to controls (n=16). The amount of RNA in brain samples was expressed as relative levels to control samples after normalization with 18s RNA. (C) Full-length HERV-K RNA, 7.5 kb, and spliced products 4.5 kb and 2.5 kb were detected by reverse transcription PCR in brain tissue. 4.5 kb product representing the *env* was present in ALS samples only. (D) Immunostaining for HERV-K *env* in ALS cortical neurons in layer V, (E) in pyramidal neurons, (F) in the cytoplasm with focal accumulation at the cell membrane and in (G) anterior horn neurons in spinal cord. (H) White matter and (I) secondary antibody control show no immunostaining. Scale bar=50 µm

<u>Other markers of HERV-K activity:</u>

We have found that some patients with ALS have antibodies to HERV-K and there is dysregulation of HERV-K in the lymphocytes in these patients. The significance of this immune response is not clear. We are thus validating these assays and developing an ELISA to monitor antibody responses in these patients. We will also develop a cell-based assay to monitor HERV-K expression in peripheral blood lymphocytes. Once developed we will determine the immune response to HERV-K in the context of this current study.

<u>Neurofilament levels in ALS:</u>

Recent studies have shown that phosphorylated heavy and light chain neurofilament levels can be measured in CSF and blood. These levels correlate with disease severity and rate of progression.[14] Neurofilament release in the CSF and blood is an indicator of neuronal injury and even though it may not be specific for ALS, it could be a useful marker for monitoring the effects of treatment in this population.

<u>Urine p75ECD in ALS:</u>

Urine p75ECD has been shown recently to correlate well with ALS disease progression including with the clinical rating scale, the revised ALSFRS.[15]

<u>Human Immunodeficiency Virus and Motor Neuron Disease:</u>

ALS occasionally affects patients with HIV infection with the first case described as early as 1985.[16] To date, at least 30 cases of HIV-associated ALS patients have been reported.[17] With comorbid HIV, there were several unique characteristics of ALS that appear in some cases: younger age at onset, more rapid progression, and elevated CSF protein; however, the significance of these apparent differences is not entirely certain because the number of patients is limited and the phenotypic variability is rather high.

The most striking and likely significant feature of HIV-associated ALS is that some patients improved when treated with antiretroviral therapy. Motor and respiratory strength improved, and in some patients, strength returns to normal and is sustained for years.[18-20] This improvement in ALS symptoms that some patients have when treated with HIV medications is remarkable.

In a small case series, HIV infection was newly diagnosed at the time of ALS symptom presentation for 38% of patients. The severity of immunosuppression was variable, with CD4 counts ranging from 2 to 618 cells/mm^3, and HIV viral load ranged from undetectable to $7.8x10^4$ copies/ml. Because of the somewhat unique clinical characteristics and the potential for a clinical response to antiretroviral therapy (ART), ALS is often named "ALS-like" when it affects a patient with comorbid HIV even though patients meet El Escorial criteria for an ALS diagnosis. Alternatively, some authors have referred to it as "motor neuron disease."

Response to Antiretroviral Therapy:

All patients with HIV-associated ALS who clinically improved or became stabilized were treated with ART. The response to ART typically coincides with reduced plasma HIV viral load and increased CD4 count. However, despite continued ART, normal CD4 counts and an undetectable plasma viral load, three patients experienced a clinical relapse after a period of initial improvement (15 months in one and 24 months in two).[17] Based on a small sample size, prognosis is not related to the age at onset, the initial CD4 cell count or viral load, or the duration of known HIV seropositivity.[17] Corticosteroids and intravenous immunoglobulin were not beneficial. ART is the only class of drugs associated with stabilization or even improvement in ALS patients.

ALS in HIV-infected patients may be primarily from HIV infection of the motor neuron, an unknown complication that mimics ALS, or there may be a mere coincidence. However, the likelihood that the ALS-like presentation in these patients is caused by at least a shared etiology with patients with ALS and without HIV is suggested by the fact that the neurological manifestations of ALS either with or without HIV are indistinguishable in many cases. The patients may be younger and the course of disease without treatment may be worse; however, the clinical phenotype is indistinguishable. A retrovirus as a cause of a subset of ALS cases is supported by the response to ART in 52% of HIV-associated ALS cases. This is quite controversial. HIV does not infect neurons.[21] Thus, selective damage to motor neurons may occur by neurotoxic viral proteins or cytokines produced as a consequence of the viral infection.[22] Autopsy studies failed to connect HIV, or any virus, directly to motor neurons and muscle fiber loss[16,23,24] although the possibility exists that in these rare cases, HIV may transactivate an endogenous retrovirus[25] which would explain the response to ART in these patients.

Treatment of ALS with ART

Two pilot studies of monotherapy in patients with ALS and negative for HIV were unsuccessful. Zidovudine was used for 12 patients with ALS at a dose of 500 mg daily. Only interim results were published with patients completing between 2-10 months of therapy. The authors did not feel there was a significant change in the disease course with zidovudine since 5/12 patients died at the time of publication. They noted that zidovudine was well tolerated among the patients and that serum CK levels decreased during treatment although no other outcome measures were reported.[26] Indinavir was tested in a double-blind placebo-controlled trial[27] powered to measure safety and feasibility. Four of 23 participants receiving indinavir developed nephrolithiasis, and only 12 patients in the treatment group completed the 9-month trial. The authors cite disease progression and inability to travel as the two primary reasons why participants could not complete the trial. In the intention-to-treat analysis, there was a faster rate of

decline in the ALSFRS, a symptom scale used in most ALS clinical trials, in the treatment group compared to the placebo group.

In a recent study in Australia, called the Lighthouse Study, 35 patients were treated with combination ART, Triumeq (abacavir, dolutegravir and lamivudine) for a period of six months. The patients were enrolled at four different sites. Serum samples from 10 patients were made available to us from one site. We have analyzed these samples for HERV-K using a newly developed digital PCR assay in our lab. The advantage of this assay as compared to the qPCR assay is that it is highly accurate, many of the steps are automated hence it is highly reproducible as well. Data from those 10 patients shows that in 4/10 patients there was a drop in HERV-K levels. These were also the patients with the highest viral load (Figure 3). The clinical significance of this drop in viral load is not clear.

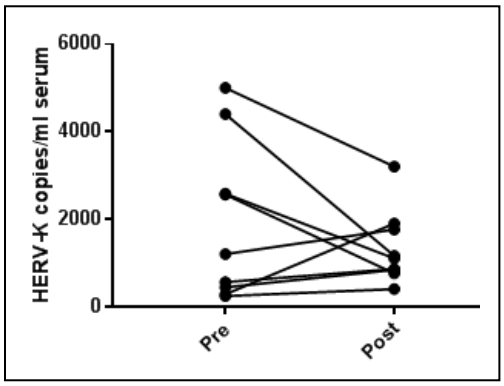

Figure 3: Effect of ART on serum HERV-K levels in ALS patients. HERV-K levels were measured by digital PCR. Four patients with the highest viral load also showed a drop in viral load following treatment with Triumeq for six months.

Rationale Summary

It is well established that a subset of patients with ALS have detectable blood levels of HERV-K viral load, and both the titer levels and proportion of patients with detectable levels are greater in patients with ALS compared to healthy controls and to those with other neurologic diseases. The clinical significance of this HERV-K viral load to disease pathogenesis and phenotype is entirely uncertain. It is also well established that HIV-infected patients who develop an ALS-like syndrome may have lasting improvement in strength and function when treated with antiretroviral therapies. This study will allow an initial clinical investigation to explore the concept that the HERV-K viral load is susceptible to suppression similar to levels of HIV RNA when treated with antiretroviral therapies.

Rationale for Using Antiretroviral Therapy

We have tested approximately 30 antiretroviral therapies for activity against HERV-K. [27] We synthesized the HERV-K genome, pseudotyped it with VSV-glycoprotein, and then infected two different cells lines. The infection was monitored using viral replication by quantitative PCR. We found that the virus could replicate in these cells efficiently. In a separate set of experiments, we infected the cells in the presence of reverse transcriptase inhibitors including abacavir and zidovudine. Some drugs suppressed HERV-K viral loadlevels in a dose-responsive manner. To determine if protease inhibitors could impact HERV-K expression, we transfected HeLa cells with the HERV-K plasmid and then exposed the cells to a panel of protease inhibitors. We found that darunavir had the most potent effect against HERV-K (**Figure 3**). Of note in Figure 3, indinavir has limited activity against HERV-K; this was the agent studied in one of the prior pilot studies of antiretroviral therapy in ALS.

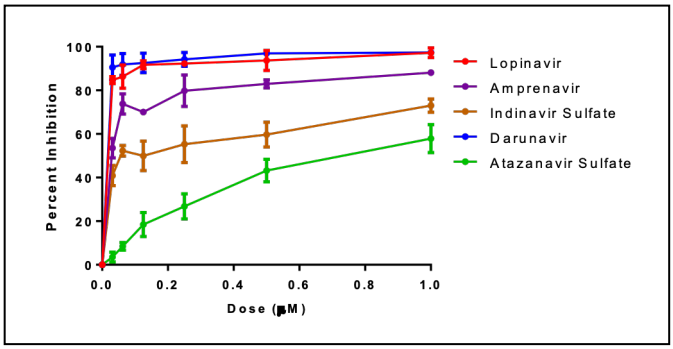

Figure 4. Relative suppression of HERV-K gag RNA transcript expression by protease inhibitors. Darunavir has the most suppressive activity of the comparison drugs.

We calculated the IC_{90} (the drug concentration required for 90% suppression, a standard for determining antiretroviral drug efficacy) for each of the drugs and compared it to that reported in the literature for effects on HIV replication (**Table 2**). It appears that the effects of zidovudine and abacavirare similar against both viruses, while lopinavir has very limited effect; however, the effects of darunavir appeared to be 3-10 fold less potent compared to the effect on HIV although the effects are still in the nM range (**Table 2**). In addition to the protease inhibitor (darunavir boosted with ritonavir) with the highest impact on HERV-K replication, we propose using an integrase inhibitor (dolutegravir) because it penetrates into the CNS, is effective against a broad range of retroviruses (less specific for HIV) and it could prevent infection of new cells by blocking viral integration.

<u>Rationale for tenofovir alafenamide fumerate (TAF)</u>
A recent study shows that the IC50 for tenofovir against HERV-K was 3.9 uM while that of abacavir was 14.6 uM and zidivudine was 15.9uM, suggesting that tenofovir was the most efficacious drug against HERV-K.[28] While the penetration of tenofovir across the blood brain barrier is poor, a new form of the drug, tenofovir alafenamide fumerate (TAF) has several hundred-fold higher concentrations in the brain. We thus propose to use TAF instead of zidovudine, which also has a better side effect profile.

Rationale for dolutegravir
Dolutegravir is a newer integrase inhibitor with similar properties to raltegravir; however it has much better CNS penetration and thus is more appropriate for this study.

Table 2: IC_{90} for Antiretroviral Therapies Targeting HIV and HERV-K

Drugs	HIV IC_{90}	HERV-K IC90
TAF	Unknown	Unknown
Darunavir	2.7nM to 13nM	50± 20nM
Lopinavir	IC_{50} 17nM (no serum) IC_{50} 102nM (50% serum)	0.8μM ±0.2 μM
Dolutegravir	Unknown	Unknown

Rationale for Experimental Therapeutics in ALS

Riluzole, a drug that has multiple mechanisms of action including inhibition of release of glutamate at pre-synaptic terminals, was reported in two controlled studies to extend survival by three months for patients with ALS although without a concomitant improvement in strength. This is currently the only FDA-approved agent for use in ALS. Trials of thalidomide, tamoxifen, dexpramipexole, IGF-1, gabapentin, brain-derived neurotrophic factor (BDNF), xaliproden, topiramate, celecoxib, creatine, lithium, talampanel, and ceftriaxone, among others, were shown to be ineffective in treating ALS.

Because there are no ALS therapies with strong benefit on the disease course, there is a need for trials to explore novel pathways in ALS. This study is an early-phase proof-of-concept trial that may help open pathways for additional novel experimentation in ALS.

24-week Treatment Duration Rationale

We anticipate that suppression of reverse transcriptase to undetectable levels will require treatment for several months. By having a 24-week duration of medications, we expect to observe sustained suppression in HERV-K viral load if it can occur with the ART regimen.

Description of anti-retroviral regimen

Table 3. Antiretroviral regimen

Name	Dose	Frequency	Route of Administration	Food Considerations	Trade name
Darunavir[1]	600 mg	Twice daily	PO	Should be taken with meals	Prezista®
Ritonavir[1]	100mg	Twice daily	PO	Should be taken with meals	Norvir®
Dolutegravir	50 mg	Once daily	PO	May be taken without regard to meals	Tivicay®
TAF	25 mg	Once daily	PO	May be taken without regard to meals	Vemlidy®

[1]Darunavir must always be administered with ritonavir.

Table 3 describes the dose, frequency, route of administration of the combination antiretroviral medication regimen.

Table 4. Alternative formulations for study medications available for patients with dysphagia

Name	Chewable tablet	Oral suspension	Frequency
Darunavir	n/a	6ml of 100 mg/ml solution (600 mg)	Twice daily
Ritonavir	n/a	1.25 ml of 80 mg/ml solution (100 mg)	Twice daily
Dolutegravir	n/a	Dissolved crushed tablet in water	Once daily
TAF	n/a	Dissolved crushed tablet in water	Once daily

Table 4 describes alternative formulations available for patients with dysphagia who cannot tolerate the formulations from Table 3.

Darunavir is an orally-administered protease inhibitor, the most effective class of drugs against HIV. As a protease inhibitor, it prevents the cleavage of protein precursors that are required to produce new viral particles. It was FDA-approved to treat HIV in 2006.

Because darunavir is extensively metabolized by CYP3A it is always combined with ritonavir, another protease inhibitor that inhibits CYP3A. When a single dose of ritonavir-boosted darunavir is given, there is an approximate 14-fold increase in the systemic exposure of darunavir.

Dolutegravir is an orally-administered integrase inhibitor that blocks the integration of viral DNA into host cell chromosomes. Inhibiting integration prevents propogation of viral infection. It was FDA-approved to treat HIV in 2013. No dosage adjustment is necessary for patients with mild-moderate hepatic or renal impairment. Dolutegravir is available in 50mg film-coated tablets.

Darunavir with boosted ritonavir and another integrase inhibitor, raltegravir, were studied extensively in clinical trials. 8-12% of patients with HIV have not had virologic suppression during trials lasting up to 212 weeks. During this time, 66-70% of patients starting either medicine remained on the medicine and on study until completion and remained virologically suppressed (**Table 5**).

Tenofovir alafenamide is a nucleotide reverse transciptase inhibitor that blocks the enzyme required for replication of retroviruses. It was FDA approved to treated HIV in 2016. No dosage adjustments are recommeneded for patients with mild-severe renal impairment or mild hepatic impairment. It is available only as a 25mg tablet to be taken once daily and there is no liquid formulation available.

For antiretroviral therapies, the CNS penetration effectiveness (CPE) ranking is a validated measure of CSF:plasma concentrations for each drug with a score of 4 for the most CNS penetrating and 1 for least. The CPE scores for , darunavir + ritonavir, and dolutegravir are 3 and 4 respectively indicating that all have strong penetration into the CNS.[29] The CPE score for TAF is currently not reported in the literature.

Table 5. Clinical trial results in treatment-naïve, HIV-positive subjects

	Concomitant medications	Study duration (weeks)	Virologic success (lower limit of detection in copies/ml)	Virologic failure	Discontinued study
Darunavir (+ritonavir)[30]	FTC/TDF	192	70% (<50)	12%	18%
Dolutegravir[30]	FTC/TDF	96	81% (<50)	5%	2%
TAF	FTC/EVG/COBI	48	92%	4%	6%

FTC, emtricitabine. TDF, tenofovir. 3TC, lamivudine. IDV, indinavir. EVG, elvitegravir. COBI, cobicistat. NR, not reported

Study Progress Update as of April 11, 2019
Since approval of Amendment C on March 4, 2019, we have pre-screened several potential participants. We have had one enrollment as of April 11, 2019.

2. Study Objectives

2.1. Primary Objectives

The primary objective is to assess whether an antiretroviral regimen of darunavir + ritonavir, dolutegravir, and TAF for 24 weeks will suppress blood levels of HERV-K RNA below the limit of detection (1000 copies/ml) in patients with HERV-K-positive ALS.

2.2. Secondary Objectives

The secondary objectives of this study are:

1. To assess the safety of an antiretroviral regimen of darunavir + ritonavir, dolutegravir, and TAF in patients with ALS.
2. The percent decline in blood HERV-K concentration: 100% x (screening visit – week 24 visit measurement)/screening visit measurement.

2.3. Exploratory Objectives:

The exploratory objectives will guide decisions on future studies of antiretroviral therapy in ALS patients.

- Evaluate the efficacy of ART on clinical biomarkers of ALS that measure symptoms, neurophysiologic outcomes, respiratory function, and quality of life.
- Evaluate whether known genetic variability associated with ALS accounts for variable suppression of HERV-K viral load in blood levels by ART.
- Evaluate effect of ART on HERV-K antibody levels in blood and/or CSF and effects on intracellular HERV-K expression in peripheral blood lymphocytes. These assays are currently under development in our laboratory and will be applied to stored samples once available.

3. Subjects

3.1. Description of study populations

Participants may be included with a diagnosis of clinically probable, laboratory-supported clinically probable, or clinically definite ALS. By including participants with probable ALS rather than only definite ALS, we will allow participants to enroll earlier in the course of illness. This is a common inclusion method for ALS clinical trials because the median time from symptom onset to clinically-definite ALS is over 12 months. More than 25% of ALS patients die without meeting the El Escorial criteria for clinically definite ALS.[31]

Participants will be recruited from a small subset of ALS patients who have elevated levels of the HERV-K:RPP30 greater than or equal to 13 at the time of screening. By using this small, specific population for our study, we will limit the generalizability of our results. However, this is a proof-of-concept study and using this limited population with the highest HERV-K levels will afford the best opportunity to test the concept that antiretrovirals can suppress the HERV-K levels.

Accrual ceiling: 200
Target number of completers: up to 25 for a goal of 20
Withdrawals/dropouts will be replaced as defined in Section 10.

3.2. Inclusion Criteria

Subjects must meet *all* of the following inclusion criteria to be eligible to participate in this study:

1. Age 18 years or older at the time of the screening visit.
2. Able to provide informed consent and comply with study procedures.

3. ALS diagnosed as probable, laboratory-supported probable or definite according to the World Federation of Neurology El Escorial revised criteria[32] as determined by a neurologist with neuromuscular subspecialty training.
4. A ratio of HERV-K:RPP30 greater than or equal to 13 measured by quantitative PCR at the screening visit.
5. Duration of disease less than 2 years, or if greater than 2 years, disease progression at a rate that in the judgement of the investigator would allow for completion of the study.
6. If taking riluzole or edaravone, must be on a stable dose for at least 30 days prior to the screening visit, or stopped taking riluzole or edaravone at least 30 days prior to the screening visit.
7. Subject has a competent caregiver who can and will be responsible for administering study drug. If there is no caregiver, another qualified individual must be available to do this.
8. Subject has established care with a neurologist and will maintain this clinical care throughout the study.
9. Subject has had neuroimaging within the last 24 months for participants enrolling at the NIH Clinical Center.

3.3. Exclusion Criteria

A participant will be excluded if he or she has any of the following:

1. Dependence on daytime mechanical ventilation (invasive or non-invasive, including Continuous Positive Airway Pressure (CPAP) or Bilevel Positive Airway Pressure (BiPap) at the time of the screening visit.
2. Participation in any other investigational drug trial or using investigational drug (within 4 weeks prior to the Day 0 visit and thereafter).
3. History of severe sulfonamide allergy (i.e. anaphylaxis).
4. History of positive test or positive result at screening for HIV or HTLV-1.
5. Participants must not be able to become pregnant (e.g., post-menopausal for at least one year, surgically sterile, or using adequate methods of contraception) or breastfeed for the duration of the study. Adequate methods of contraception include: implanted contraception, intrauterine device in place for at least 3 months, or barrier method in conjunction with spermicide. Participants of childbearing potential must have a negative pregnancy test at screening and be non-lactating.
6. Presence of any of the following clinical conditions at the time of screening:
    a. Drug abuse or alcoholism
    b. Unstable medical disease (such as unstable angina or chronic obstructive pulmonary disease), or active infectious disease (such as Hepatitis C or tuberculosis), or current malignancy
    c. Unstable psychiatric illness defined as psychosis or untreated major depression within 90 days of the screening visit
    d. Dementia
    e. Diabetes mellitus
    f. Hemophilia
7. Use of contraindicated medications: amiodarone, dronedarone, lovastatin, simvastatin, rifampin, rifapentine, rifabutin, cisapride, pimozide, midazolam, triazolam, dihydroergotamine, ergonovine, ergotamine, methylergonovine, St. John's wort, alfuzosin, salmeterol, sildenafil for pulmonary arterial hypertension, oxcarbazepine, phenobarbital, phenytoin or dofetilide.
8. Safety Laboratory Criteria at the screening visit:
    a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than 3.0 times the upper limit of normal.

b. Serum creatinine, serum phosphorous, total bilirubin, triglycerides, amylase, or lipase greater than 2.0 times the upper limit of normal.
c. Estimated glomerular filtration rate <60mg/dl.
d. Platelet concentration of <100,000/ µl.
e. PT and PTT >1.2 times the upper limit of normal for participants enrolling at the NIH Clinical Center.
f. Hemoglobin <10mg/dL.
g. Positive Hepatitis B Surface Antigen and Hepatitis C Virus Antigen

4. Study Design and Methods

4.1. Study Overview

This is a single-center, single-arm, open-label, baseline-versus-treatment study of darunavir, dolutegravir, ritonavir, and TAF in ALS patients. Up to 25 patients with ALS and with a ratio of HERV-K:RPP30 greater than or equal to 13 will receive the antiretroviral regimen for 24 weeks. The goal is to have 20 participants complete the study through week 24. The treatment phase will be followed by a post-treatment follow-up for an additional 24 weeks. The study duration for each participant could be up to 72 weeks (i.e. when 24 weeks elapses between initial blood draw and the screening visit). Study evaluations will be completed as outlined in Section 4.5. For participant convenience, some of the visits may be completed as an inpatient or outside of NIH.

4.2. Recruitment

Participants will be recruited from physician referral, self-referral, and through advertisement. Referring providers may share the contact details with the potential participant who then may contact the coordinator directly.

Potential participants will be pre-screened for this study. Available records may be reviewed to evaluate eligibility for this study. We anticipate the following documentation to be available for pre-screening review: demographic information, the ALS diagnosis category, medications, and comorbid medical conditions. We anticipate that some patients may have been screened for HIV and HTLV during their diagnostic workup for ALS and these results may be available during the pre-screening review.

Potential participants who meet eligibility criteria based on the pre-screening review will be offered enrollment onto this study.

4.3. Screening

After signing on to the Blood Draw Only Consent, participants will undergo a venipuncture with removal of up to 20 ml of whole blood to check the HERV-K:RPP30 ratio. Subjects may have research blood drawn as outpatients at the NIH's CRC in Bethesda, MD or at an offsite location for convenience of the participant. The offsite location will provide venipuncture as a fee-for-service and will not be considered an outside research institution. Tubes and pre-paid shipping materials would be provided if blood samples are being drawn locally and will be transported to the NIH Clinical Center lab of Dr. Avindra Nath, where an assay will be performed to measure the HERV-K:RPP30 ratio. This bloodwork will be done for research purposes only. If blood is collected outside of NIH, the participant will be signed on to the Blood Draw Only Consent via telephone (See section 14.2 for the consenting process). If participant has had their

HERV-K:RPP30 ratio measured as part of another study, those results may be used in place of this screening process.

Participants who are still eligible after the results are available will sign the consent for the full study and complete their screening at NIH. The screening visit will take place at a maximum of 24 weeks from the date of the screening HERV-K blood draw. During the screening and enrollment visit, participants will review and sign the study consent before any procedures are performed. Vital signs (blood pressure, pulse rate, temperature), height, and weight will be measured. A comprehensive medical history will be taken including details of medical illness, vaccinations, current medications, allergies, social and family histories, and participation in other research trials. Each participant will have a general and neurologic examination. Prior neuroimaging will be reviewed. Vital capacity and maximal inspiratory pressure will be measured with a handheld spirometer. Laboratory evaluations will include tests for research and safety labs as detailed in Section 4.5. The ALSFRS-R, ALSSQOL-R, optional ALS-CBS, lumbar puncture and neurophysiologic measurements will be completed.

During times that participant participation in the Clinical Center is restricted or when participant travel to the Clinical Center is not safe, for example during times of pandemics, protocol screening and study visits will continue offsite. The study team will use the most recent exam by the participant's community neurologist as the baseline neurological exam, vital signs, and weight. Safety and research labs as outlined in Section 4.5 may be collected offsite. The labs will be collected at a location that will provide venipuncture as a fee-for-service and will not be considered an outside research institution. NIH will cover the cost of the blood draw and laboratory tests. The study team will arrange a telehealth or telephone visit with the participant to get informed consent, a medical history, and administer the ALSFRS-R, ALSSQOL-R, and optional ALS-CBS. Participants that enroll offsite will continue the study offsite through Week 48. Vital capacity, maximal inspiratory pressure, neurophysiologic measurements, lumbar puncture will be omitted for these participants.

An assessment of all inclusion and exclusion criteria will be completed using an eligibility criteria and eligibility will be documented in the participant's NIH Clinical Center chart.

4.4. Study Visits

Day 0

The study medications will be prescribed by the NIH Clinical Center pharmacy. These will be: darunavir 600 mg, ritonavir 100 mg, dolutegravir 50mg, and TAF 25 mg. Darunavir and ritonavir will be taken twice daily. Dolutegravir and TAF will be taken once daily. No titration will be needed at the beginning of medication administration, and no tapering is needed at the end of medication administration.

If participants are not local, the medications may be mailed to the participant once all screening studies have resulted.

On-treatment phase

Participants will have on-treatment phase visits at Weeks 1, 4, 8, 12, 16, 20 and 24. If the participant elects to not travel to NIH for their study visit for Weeks 1, 4, 8, 16, or 20, vital signs, weight, and physical exam will be omitted. Safety and research labs as outlined in Section 4.5 may be collected offsite and the study team will arrange a phone call with the participant to assess the interim history within the study visit window. The labs will be collected at a location that will provide venipuncture as a fee-for-service and will

not be considered an outside research institution. NIH will cover the cost of the blood draw and laboratory tests. During the on-treatment phase, the following procedures will be conducted for NIH visits: vital signs, weight, physical examination, and laboratory evaluations for safety monitoring and research as indicated in Section 4.5.

At each on-treatment phase visit, participants will talk with a member of the NIH study team about adherence to study medications. Subjects will be asked about their health and the use of any concomitant medications since screening or the previous study visit. They will also be asked if they are experiencing any AEs. Subjects experiencing any AEs or potential toxicity may be evaluated during unscheduled study visits.

At each NIH visit when participants are taking study drugs, participants will be asked to bring the study medications and empty study medication bottles.

If participant is unable to travel for Weeks 12 and 24 safety and research labs as outlined in Section 4.5 may be collected offsite and the study team will arrange a phone call with the participant to assess the interim history within the study visit window. The labs will be collected at a location that will provide venipuncture as a fee-for-service and will not be considered an outside research institution. NIH will cover the cost of the blood draw and laboratory tests. ALSFRS-R, ALSSQOL-R, ALS-CBS will be done as indicated in Section 4.5. Vital capacity, maximal inspiratory pressure, neurophysiologic measurements, lumbar puncture, and research urine will be omitted. This will only be done if participant is not able to travel or initially enrolled offsite in order for the study to meet its objectives.

Final study medication visit

The final study medication visit will be at Week 24 and will include: vital capacity, maximal inspiratory pressure, ALSFRS-R, ALSSQOL-R, ALS-CBS, neurophysiologic measurements, lumbar puncture, and the Week 24 lab work if completed at NIH.

If participant is unable to travel for the final study medication visit, safety and research labs as outlined in Section 4.5 may be collected offsite and the study team will arrange a telehealth or telephone visit with the participant to assess the interim history and administer the ALSFRS-R, ALSSQOL-R, and ALS-CBS as outlined in Section 4.5. The labs will be collected at a location that will provide venipuncture as a fee-for-service and will not be considered an outside research institution. NIH will cover the cost of the blood draw and laboratory tests. Vital capacity, maximal inspiratory pressure, neurophysiologic measurements, lumbar puncture, and research urine will be omitted if participant is unable to travel to NIH or initially enrolled offsite.

The final study medication visit procedures may be completed over multiple days for participant convenience. Subjects will return any unused medications.

Follow-up and Final Study Visits

The Week 28 visit will include ALSFRS-R and blood collection for research. This visit may be done either at the Clinical Center or offsite.

Participants will return to clinic at Week 36 for an additional visit after medication administration is completed for comprehensive evaluations detailed in Section 4.5. The Week 36 visit will include vital signs, weight, medical history, physical examination, vital capacity, maximal inspiratory pressure, ALSFRS-

R, neurophysiologic measurements, and laboratory evaluations for safety and research as indicated in Section 4.5.

If participant is unable to travel for the Week 36, safety and research labs as outlined in Section 4.5 may be collected offsite. The labs will be collected at a location that will provide venipuncture as a fee-for-service and will not be considered an outside research institution. NIH will cover the cost of the blood draw and laboratory tests. The study team will arrange a telehealth or telephone visit with the participant to assess the interim history and administer the ALSFRS-R. Vital capacity, maximal inspiratory pressure, neurophysiologic measurements will be omitted if participant is unable to travel to NIH or initially enrolled offsite.

Week 48, the final study visit, will include an optional ALSFRS-R assessment completed by telephone.

The window periods for the visit schedules are as follows:

- Day 0 (no window)
- Week 1 (+/- 3 days)
- Weeks 4, 8, 12, 16, 20, 24 (+/- 7 days)
- Week 28, 36, 48 (+/- 10 days)

Recontact of Subjects After Week 48

Follow up visits after Week 48 may be allowed to follow participants clinically for evaluation and/or resolution of AEs.

4.5. Research Procedures

History and Physical

All participants who appear to be eligible based on the screening assessments and laboratory test results will undergo a physical exam and have their medical history collected to determine their eligibility for the study. In addition, participants will have physical exams performed at NIH visits on weeks 1, 4, 8, 12, 16, 20, 24, and 36. For offsite visits, the participant's interval history will be assessed over the phone. A targeted clinical physical exam will be performed for individuals as necessary to evaluate AEs.

Drug Accountability and Compliance

At each NIH visit when participants are taking study drugs (Weeks 1-24), participants will be asked to bring the study medications and empty study medication bottles. Participants and caregivers will be asked about compliance with the study drug schedule at each study visit.

Specimen Collection

a) Acute care, hepatic and mineral panels, and CBC with differential will be performed to monitor clinical status, document drug-related benefits, and detect any potential drug-related toxicities. These tests will be carried out during screening and every subsequent study visit.

b) Creatine Kinase, Amylase, and Lipase

These parameters will be measured at screening and at weeks 1, 12, 24, and 36.

c) Hemoglobin A1c, Fasting Lipid Profile, and Urinalysis

These parameters will be measured at screening and at weeks 12, 24, and 36.

d) HIV and HTLV Testing

HIV and HTLV antibodies will be measured by blood draw at screening. Previous HIV and HTLV testing results from within one year of the screening visit are acceptable.

e) Hepatitis B and C Testing

These parameters will be measured at the screening visit.

f) Serum/Urine Pregnancy Test

The pregnancy test will be measured by a standard assay at every scheduled study visit for women of childbearing potential.

g) Research Samples

Research blood and HERV-K:RPP30 ratios will be collected at screening, unless collected as part of another study, and every subsequent study visit. HERV-K:RPP30 ratios will be measured by quantitative PCR in the laboratory of Dr. Avindra Nath. Levels of urine p75ECD will be measured from research urine collected at screening and Weeks 12, 24, 28, and 36 if the participant is seen at the Clinical Center.

h) ALS genetics panel

If the participant is able to come to the Clinical Center, somatic mutation analysis will be performed. Whole exome and/or whole genome sequencing including testing for hexanucleotide repeat expansion in C9ORF72 will be performed in the laboratory of Dr. Bryan Traynor or a CLIA certified laboratory at the participants first study visit at the Clinical Center.

i) PT, PTT

These parameters will be measured at the screening visit.

Nerve conduction studies (NCS) and Electromyography (EMG) (Optional)

NCS and EMG are standard diagnostic tests that have been used for many decades to diagnose disorders of muscle, nerves, and nerve roots. Nerve conduction studies are performed using an electrical stimulator to activate the nerve and recording the nerve response in wire leads taped to the skin. Needle EMG is performed by using a specialized EMG needle to record electrical signals within the muscle. It will be an optional procedure at screening that may be performed for questions of ALS diagnosis by the neurologist performing the neurophysiology evaluation.

Muscle ultrasound (Optional)

Muscle ultrasound is performed to gain a better understanding of the muscle architecture and pathology. The test is performed by placing an ultrasound probe covered with ultrasound gel on the skin. An image of the muscle or nerve can be seen and evaluated. Muscle ultrasound may be done in select cases. It will be an optional procedure at screening that may be performed for questions of ALS diagnosis by the neurologist performing the neurophysiology evaluation.

Electrical impedance myography (EIM)

EIM is a device (Myolex, Inc) that uses a high frequency, low intensity electrical current via a surface electrode to measure the consequent voltage. This produces measurements of resistance, reactance and a calculated phase angle. This is used to follow muscle pathology but is not a diagnostic test. It will be

measured at screening and weeks 12, 24, and 36 if the participant is seen at the Clinical Center. The quantitative data generated from the EIM will be used as an exploratory outcome measure.

Vital Capacity and Maximal Inspiratory Pressure
Forced Vital Capacity (FVC) and maximal inspiratory pressure (MIP) will be measured. The FVC and MIP will be calculated as the percentage of the predicted vital capacity for a participant's height, gender, and age. The FVC and MIP will be completed at screening and weeks 12, 24, and 36 if the participant is seen at the Clinical Center.

Questionnaires

a) ALSFRS-R

The ALSFRS-R is a validated, reproducible, and reliable functional measure that assesses participant function across four domains: bulbar, fine motor, gross motor, and respiratory. It can be performed both in person and by telephone and is highly correlated with survival. The ALSFRS-R takes approximately 5 minutes to complete and will be measured at screening and weeks 12, 24, 28, 36, and optionally at week 48.

b) ALSSQOL-R

The Amyotrophic Lateral Sclerosis Specific Quality of Life Instrument-Revised (ALSSQOL-R) is a 50-item inventory that measures overall quality of life and six specific domains for patients with ALS. The test is completed by the patient with ALS and takes approximately 15-20 minutes to complete and will be completed at screening and week 24.

c) ALS-CBS

The Amyotrophic Lateral Sclerosis Cognitive Behavioral Screen is a validated screening tool that attempts to identify ALS patients at risk for cognitive and/or behavioral impairment by evaluating four areas: initiation and retrieval, concentration, attention, and tracking-monitoring. The questionnaire will take approximately 5 minutes to complete. It will be an optional measurement at screening and week 24.

Lumbar puncture
A lumbar puncture will be completed during the screening evaluations as well as within the time frame of the Week 24 visit if the participant is seen at the Clinical Center. The procedure may be completed under fluoroscopy or at bedside. Up to 30 cc of CSF will be collected with each LP. CSF tests will include cell count, glucose, and protein, and research specimens will be stored for future testing (for example, neurofilament levels).

4.6. End of Participation

Participants will continue care with their providers after this study.

Early Termination Visit
Participants requiring discontinuation as well as those who elect to discontinue study drugs prior to treatment completion for medical or personal reasons should have an end of treatment visit scheduled as soon as possible after study drugs are discontinued. Additionally, participants will be given the option of being followed as outlined in Section 4.5 for safety or research labs until the end of the participant's planned final study visit.

Any participant who chooses to be followed after discontinuation of study drugs will be followed closely for resolution of active laboratory abnormalities or AEs which are considered related to the study agents. Participants who discontinue treatment early and opt not to be followed will be removed from the study after it is deemed safe to do so (i.e., abnormal test results return to a safe range).

Sharing of Information with Participants

Results of genetic testing will not be disclosed to participants unless it may have a direct medical or reproductive implication for the participant or family. In that case, genetic counseling will be provided to participants to discuss these implications in more detail.

Results of other procedures will be shared with the participant and with the participant's ALS physician if follow up is necessary. Should there be a clinical need to communicate urgently with the participant or his/her physician, this will be pursued via telephone communication in addition to the written communication.

5. Management of Data and Samples

5.1. Storage

All collected samples, including blood, urine, and CSF will be coded and stored in secured freezers on the NIH campus. NIH samples will be transferred and retained at the end of the study under a repository protocol after the appropriate IRB approval is received. Only study investigators will have access to the stored samples and data. Any loss or destruction of samples will be reported to the IRB.

5.2. Data

This protocol is not subject to the Genomic Data Sharing (GDS) policy. It does not generate "large-scale" human genomic data as defined in the NIH Supplemental Information to the GDS Policy.
Data and samples may be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained in the original consent form.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6. Additional Considerations

6.1. Research with investigational drugs or devices

This protocol is a clinical investigation of drug products that are lawfully marketed in the United States and are exempt from the requirements of IND as described in 21 CFR 312.2(b). The investigation is not

intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drugs; the drugs that are undergoing investigation, are lawfully marketed as a prescription drug products; the investigation is not intended to support a significant change in the advertising for the product; the investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of these drug products; the investigation is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and the investigation is conducted in compliance with the requirements of 312.7.

6.2. Gene Therapy

This protocol does not involve gene therapy.

7. Risks and Discomforts

Risks associated with use of darunavir

Based on clinical trials,[33] the most commonly reported adverse drug reactions with darunavir in combination with ritonavir are: diarrhea (16%), nausea (7%), headache (6%), and abdominal pain (6%). Drug-induced hepatitis was reported with darunavir at a rate of 0.5% of patients receiving combination darunavir + ritonavir; pre-existing liver dysfunction is a risk factor. Severe skin reactions were reported in 0.4% of patients in clinical trials receiving darunavir (Stevens-Johnson Syndrome was diagnosed in 0.1% of cases). Rash of mild-to-moderate severity occurred in 10.3% of participants with a discontinuation rate of 0.5%.

Darunavir has a sulfonamide moiety and must be used with caution in patients with a known sulfonamide allergy; the incidence and severity of rash were similar in participants with and without a history of sulfonamide allergy.

Other rare, serious side effects include increased bleeding in patients with hemophilia, diabetes mellitus/hyperglycemia, and increased cholesterol.

In a clinical trial of darunavir versus lopinavir (both combined with ritonavir, emtricitabine, and tenofovir), 5% of participants discontinued darunavir because of AE or death in comparison with 13% of subjects discontinuing lopinavir.

Risks associated with use of dolutegravir

Based on clinical trials,[30] the most commonly-reported adverse drug symptoms with dolutegravir are: insomnia (3%), headache (2%), and fatigue (2%). The most commonly-reported adverse drug-related laboratory abnormalities are: elevated CK (7%), increased lipase (7%), increased liver function tests (4-5%), hyperglycemia (6%), increased bilirubin (3%), and neutropenia (4%).

The most serious complications associated with dolutegravir are potentially life-threatening and fatal skin reactions including Stevens-Johnson syndrome and toxic epidermal necrolysis. Patients with underlying hepatitis B or C may have worsening development of transaminase elevations.

Other rare (<2%) significant side effects include: abdominal pain and discomfort, myositis, pruritic, hepatitis, renal impairment, and suicidal ideation/behavior.

In a clinical trial of dolutegravir versus efavirenz (both combined with emtricitabine and tenofovir), 4% of participants discontinued dolutegravir because of AE or death compared with 14% of subjects discontinuing efavirenz.

Risks associated with use of ritonavir

Note: The dose of ritonavir used for clinical trials as monotherapy is 600 mg twice daily. The dose used for this study is 100 mg twice daily. Potential risks are presented here for the full dose because the risks for the 100 mg dose are described in clinical trial information together with the other protease inhibitors with which it is always prescribed (i.e., the risks of darunavir include the risk of ritonavir).

Potentially fatal skin reactions have occurred with ritonavir.[34] Pancreatitis has occurred that was life-threatening and fatalities were reported. PR interval prolongation with 2nd and 3rd degree heart blocks have occurred. Hepatic reactions included significant elevations in liver function tests (>5x ULN) and hepatitis, with pre-existing liver disease being a risk factor.

Other rare (<2%) significant side effects include: diabetes mellitus/hyperglycemia, fat redistribution, myopathy and abnormal bleeding in patients with hemophilia.

Common (>5%) side effects with ritonavir include diarrhea (68%), nausea (57%), fatigue (46%), vomiting (32%), abdominal pain (26%), arthralgia (18%), dizziness (15%), elevated triglycerides (9%), myalgia (9%), flatulence (8%), urticaria (8%), elevated liver function tests (8%), blurred vision (6%), and edema (6%).

Risks associated with TAF

Most common adverse reactions (incidence ≥5%; all grades) were headache, abdominal pain, fatigue, cough, nausea and back pain. Cases of acute renal failure and Fanconi syndrome have been reported with the use of tenofovir prodrugs. Tenofovir but not TAF has been associated with rare cases of lactic acidosis and hepatotoxicity with steatosis.

Risks of medication interactions with the study drugs

The study drugs, particularly the protease inhibitors, have the potential for significant interactions with a number of medications. A list of significant interactions is found at: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. The document contains tables for drug-drug interactions with the protease inhibitors darunavir and ritonavir, reverse transcriptase inhibitor tenofovir, and the integrase inhibitor dolutegravir.

Risks of blood draw

There may be some discomfort and bruising at the site of venipuncture. There is a very small risk of fainting. Infection in the area of needle entry is rare.

Risks of Nerve conduction studies (optional)

The risks of the electrical stimulation using commercial isolated stimulators are low. Most subjects find electrical nerve shocks to be mildly uncomfortable; it is usually perceived as a brief stinging or tapping sensation. The intensities required for nerve conduction studies are usually well tolerated. Risk is minimized by using the lowest intensity possible to obtain the nerve response.

Risks of Electromyography (optional)

The risks of EMG are low, and consist of local discomfort, bleeding and bruising at the site of needle insertion. Needle puncture carries a small risk of infection. Sterile needles are used to minimize this risk.

Risks of Nerve and muscle ultrasonography (optional)
There are no known medical risks and minimal discomfort associated with this test.

Risks of Electrical Impedance Myography
EIM device is placed on the muscle and a high frequency current is delivered to the skin. There is no sensation related to the application of the high frequency impulse. The only discomforts would be pressure of placing the EIM on the skin.

Risks of vital capacity and maximal inspiratory pressure testing
There is minimal risk associated with vital capacity and maximal inspiratory pressure testing. Participants may feel short of breath or lightheaded during testing.

Risks of ALS questionnaires (ALSFRS-R, ALSQOL-R, ALS-CBS)
Some patients may feel frustrated or stressed when answering questions. There are no other risks with the questionnaires.

Risks of lumbar puncture
Subjects may experience a brief pain or tingling sensation in their legs during the procedure if the needle brushes against a nerve. If this happens, the needle will be adjusted. Following the LP, some people have a mild backache at the site of needle insertion. About one-third of people have a headache for a few days following the procedure. Usually the headache is not severe and improves without treatment other than mild pain relievers. Headaches lasting longer than seven days develop in 1 in 50 to 200 LPs and usually improve gradually over two weeks. In rare cases, headaches have persisted longer. Prolonged headaches may be due to persistent leakage of CSF from the area of the LP and may require a “blood patch.” For a blood patch, venous blood will be removed from the patient and then injected into the area of the back where the lumbar puncture was performed to seal off the CSF leak. We will not perform an LP if neuroimaging suggests that the patient is at increased risk of herniation. If the LP is done under fluoroscopy, there is also a small amount of radiation exposure. Conscious sedation is associated with a small risk of respiratory depression and an even smaller risk of cardiac arrhythmias. Patients will be monitored closely for possible side effects and treated with medications or intubation, if necessary. The LP will be performed by trained clinicians and every precaution will be taken to minimize the risks associated with this procedure. Lumbar puncture may be done in the radiology department under fluoroscopic guidance for patient medical or scheduling needs. The radiation exposure during this study is 0.049 rem, which is well below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

Risks of genetic testing
Genetic testing can provide information about how illness is passed on within a family. This knowledge may affect participants’ emotional wellbeing. They might feel differently about their life if they learned that they or their children were at increased risk of a disease, especially if there were no treatments. Their children, brothers or sisters may find out that they are at risk for health problems because of information found out about the participants, which might affect the participants’ relationships with family members. Other family members may also be affected by uncovering risks they have but did not want to know about. This information can cause stress, anxiety, or depression.

Some genetic testing can also determine if people are directly related. These tests sometimes show that people were adopted or that their biological parent is someone other than their legal parent. If these facts were not known previously, they could be troubling. Genetic counseling is available at the NIH to help participants understand the nature and implications of genetic findings.

Because of the emotional risk, some participants do not want to know the results of genetic testing. It is our policy to not disclose the results of genetic testing unless it may have direct medical or reproductive implications for participants or the participants' families.

Results of genetic testing will not be shared with participants unless they have direct implications for participants' health (are clinically actionable). Participants will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. If participants state that they would like to obtain the results, confirmatory testing will be conducted at a CLIA-certified laboratory either at NIH or elsewhere which will be paid for by the IC. If the research finding is verified by the CLIA-certified laboratory, the participant will be offered the opportunity to receive genetic counseling from an NIH genetic counselor (at our expense) to explain the results. Participants have the option to receive a referral to a local genetic healthcare provider (at their expense). This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Genetic information will be kept confidential to the extent possible. The results of genetic testing will be kept in a locked and secured manner at the NIH.

Problems, such as with insurance or employment discrimination, may occur if they disclose information about themselves or agree to have their research records released. We will not release any information about participants to any physician, insurance company or employer unless they sign a document allowing release of the information.

8. Subject Safety Monitoring

The Principal Investigator (PI) and study personnel will be responsible for monitoring individual subjects during participation in study procedures.

Parameters to be monitored

Previous experience demonstrated a low human toxicity profile for darunavir, ritonavir, dolutegravir, and TAF. The most frequently and consistently experienced adverse reactions reported are headache, insomnia, abdominal pain or cramping, hyperglycemia, increased amylase and lipase, abnormalities in liver function tests and elevated CK levels. Hematologic abnormalities such as decreased absolute neutrophil count and thrombocytopenia are also expected adverse events.

In particular, given the rare risk of severe skin and hypersensitivity reactions (Stevens-Johnson syndrome and toxic epidermal necrolysis; rash with fever, fatigue, malaise, conjunctivitis or other constitutional symptoms), the patient will be instructed to immediately discontinue all the study medications and contact the study investigators should a severe skin reaction or hypersensitivity symptoms occur.

Additionally, female participants will be instructed to contact the investigator immediately if she believes that she is pregnant. Testing will be performed to verify that the participant is pregnant. If the pregnancy test is positive, the participant would be instructed to immediately stop taking the study drug.

Medical events typical for the clinical course of ALS (i.e., typical neurological signs/symptoms that a patient experienced during pre-treatment baseline and their worsening as would be expected from the natural history of ALS) can be regarded as expected events in the course of the current trial.
Because the clinical course of ALS is known to be a steady decline in neurologic and respiratory function, we expect participants to have certain ALS-specific events that will not be attributed to study drugs. Examples of these events include:

1. Increased motor weakness, either defined by changes in neurophysiological measurements, by participant's self report, or by physical examination. Careful attention to the pattern of weakness will be important because ALS causes both upper and lower motor neuron patterns of weakness.
2. Dyspnea. Dyspnea is a known complication of ALS and may be noted by a decline in vital capacity or participant's self report.
3. Dysphagia. Dysphagia is also a known complication of ALS and is typically reported by patients. Dysphagia may be noticed because of the pill burden during study drug administration. Participants who may not be able to tolerate the pill burden may opt for alternative study drug administration methods as described in Table 4.
4. Cognitive changes. 10-15% of patients with ALS reach criteria for frontotemporal dementia at some point in their disease course.[35] Milder forms of cognitive impairment may develop during the course of the study. If cognitive impairment progresses to the point that the participant would not be able to safely remain in the study or would not be able to continue to provide informed consent, the participant may be removed from the study.

The participants will be instructed to contact the study investigators and/or seek medical care if they have any questions or concerns. Participants will be instructed to contact the investigator immediately should they manifest any new signs or symptoms or change in their condition during the period extending from the time of consent up to and including the final study visit at Week 36. After this period of time, participants should report to the investigator only AEs they perceive as possibly related to their participation in this study.

Definitions of AEs and SAEs and assessments of AE severity and causality are listed in Appendix A.

Laboratory monitoring will occur according to the schedule described in Section 4.5. In the event of unexplained clinically significant abnormal laboratory test values, the tests should be repeated immediately and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded.

Toxicity tables/ criteria to be used
The Investigator will grade the severity of each AE according to the "Division of Aids Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.1, July 2017 which can be found at:
http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf

Some grade 1 lab parameters on the DAIDS Toxicity Table [Fibrinogen, Calcium (low), Phosphate (low), Uric Acid (males only, elevated)] fall within the NIH lab reference range for normal values. These normal values will not be reported as grade 1 adverse events. The grade 1 values for these tests will be reported as follows:

- Fibrinogen: 100 - 176 mg/dL
- Phosphate (low): There is no grade 1 reportable value
- Calcium (low): 1.95 - 2.04 mmol/L
- Uric Acid (males): 8.7 – 10.0 mg/dL

Criteria for stopping procedures in an individual

A participant *must* permanently discontinue study drug treatment for any of the following reasons:

1. The participant becomes pregnant (pregnancy test will be performed for female patients with child bearing potential during each study visit as indicated in Section 4.5). If a patient becomes pregnant, treatment must be *immediately* discontinued. The pregnancy must be reported to the IRB immediately. Information about the participant, the participant's pregnancy, and the outcome of the pregnancy will also be collected.
2. The participant desires to discontinue study drug treatment under this protocol.
3. If the participant develops a severe skin or hypersensitivity reaction to a study drug(s) he will be instructed to immediately discontinue all study drugs and contact the study investigators who will coordinate the management.
4. Laboratory safety criteria may be used as a stopping criterion when the abnormal laboratory result continues after one week of cessation of study drugs. Participants may resume the study drugs if the laboratory abnormality resolves within one week.
   a. ALT, AST, amylase, or lipase more than three times the upper limit of normal.
   b. Hemoglobin reduction of >25% of baseline value or Hemoglobin <7.5mg/dl.
   c. Absolute granulocyte count reduction of >50% of baseline value or absolute granulocyte count of <1000 cells/mm^3
   d. Any other highly abnormal laboratory finding that is deemed critical by an investigator.
5. The participant has greater than 30% weight loss compared to weight at baseline.
6. The participant has rapid muscle weakness beyond what is expected for the natural course of ALS.

There will be no dose reductions in the study drugs due to intolerance or toxicity, and all four study drugs will be held/discontinued even if the AE is likely attributable to only one study drug.

The reasons for discontinuation of the study drugs must be recorded in the participant's Clinical Center patient file. If discontinuation was because of an AE, the patient will be followed until the event is resolved or stabilized. If the discontinuation was because of pregnancy, the participant will be followed until after the birth.

Criteria for individual subject withdrawal from the study

Participants have the right to withdraw from the study at any time and for any reason. The investigator also has the right to withdraw participants from the study in the event of an intercurrent illness, AEs, treatment failure, protocol violations, administrative reasons, or other reasons.

9. Outcome Measures

9.1. Primary outcome measures

The primary outcome measure will be the percent decline in blood HERV-K concentration measured by quantitative PCR. Percent decline for a patient is measured by: 100 x (screening visit – week 24 visit measurement) / screening visit.

9.2. Secondary outcome measures

1. The safety of antiretrovirals in volunteers with ALS as measured by the frequency and type of AEs, the ability to remain on assigned treatment (tolerability), physical examinations, laboratory test results, vital signs, and weight/body mass index (BMI).
2. The percent decline in blood HERV-K concentration: 100% x (screening visit – week 24 visit measurement)/screening visit measurement.

9.3. Exploratory outcome measures

Efficacy will be explored by measuring the change in mean scores of:

a. ALSFRS-R
b. ALSSQOL-R
c. ALS-CBS
d. vital capacity and maximal inspiratory pressure
e. EIM
f. neurofilament levels.
g. urine p75ECD

Variability in HERV-K viral loads suppression with ART will be evaluated by genetic exome sequencing surveying mutations in specific genes or loci associated with ALS.

10. Statistical Analysis

10.1. Analysis of data/ study outcomes

The design of this clinical trial is one-group pre-test and post-test design. Thus, each participant will have a pre-treatment measurement (screening) and a post-treatment measurement.

Planned analyses

A one-sample t-test with significance level of 0.05 will be used to test the null hypothesis: the Mean reduction percent is zero. For each patient, the reduction percent is calculated as 100 x (screening visit – week 24 visit measurement) / screening visit. The secondary and exploratory outcomes will be summarized using descriptive statistics without conducting statistical inference.

Analysis cohorts

Suppression analysis data set. The analysis set for antiviral activity analyses will include participants who were enrolled into the study and received at least 24 weeks of study drug.

Safety analysis data set. The analysis set for safety analyses will include participants who received at least one dose of study drugs.

Efficacy analysis data set. The analysis set for efficacy analyses will include participants who received at least 24 weeks of study drug. Efficacy analyses will be limited to descriptions of any changes that may occur in results of: vital capacity, maximal inspiratory pressure, ALSFRS-R, ALSSQOL-R, ALS-CBS, EIM, and neurofilament levels or urine p75 levels.

Missing data plan

Missing data can impact interpretation of the study data. Values for missing data will not be imputed. Any participant with missing data due to premature discontinuation of the study drugs (see Section 10.5 below) will be considered a failure at the time points on, or following, the date of discontinuation.

Where appropriate, safety data for participants not completing the study will be included in summary statistics. For example, if a participant received study drugs, the participant will be included in a summary of adverse event. If safety laboratory results for a participant are missing for any reason at a time point, the participant will be excluded from the calculation of summary statistics for that time point. If the participant is missing a pre-dose value, then the participant will be excluded from the calculation of summary statistics for the pre-dose value and the change from pre-dose values.

10.2. Power analysis

Accrual number request

Sample size:

Pilot data on ARV in ALS patients indicates that in those patients with greater than 1000 HERV-K copies per ml serum at screening (n=5), there is an average percent decline in serum concentration of 38% with a standard deviation of 50%. Only individuals with greater than 1000 copies per ml at screening will be recruited into the current study. The average percent decline of 38% is close to the estimated minimally biologically meaningful percent decline of 50%.

To calculate the number of subjects needed, we will power using an inflated standard deviation, due to the issues with using standard deviation from a small pilot to power a study. Browne (1995) indicates that using an 80% percent UCL on standard deviation will provide a sample size sufficient to achieve planned power in at least 80% percent of trials, and we will use that adjustment here.[36] Therefore, the resulting conservative standard deviation is 76%. We estimated that untreated, these individuals will have a 0% decline from baseline.

Achieving 85% power for this test using our minimally relevant difference of 50% decline and SD of 76% would require data from 23 subjects. 20 subjects would allow us to detect a decline of 54% (Table 6).

Table 6. One-sample two-sided t-test power calculations

Effect Size (Percent Decline)	Standard Deviation	Alpha	Power	N
50%	76%	0.05	0.85	23
54%	76%	0.05	0.85	20

We anticipate screening 200 subjects in order to obtain 20 participants who meet eligibility criteria. This will include participants who begin study drug and who discontinue study drugs without the meeting the

primary outcome before Week 24 (and who will be replaced) and subjects who are screened but do not enroll in the treatment phase of the study.

Dropouts
Participants who withdraw voluntarily (not due to toxicity or meeting the primary outcome) after initiation of the study but before week 24 will be considered dropouts. These participants should have a follow-up visit as close as possible to the date of the last dose of the study. These participants will be given the option of being followed as outlined in Section 4.5 for safety or research labs until the end of the participant's planned final study visit.

Patients who voluntarily stop study drugs before 24 weeks will be considered non-completers. They will have the option to remain in the study and continue the protocol-specific evaluations.

11. Human Subjects Protection

11.1. Subject selection

The investigational nature and objectives of this trial, the procedures and treatments involved, as well as the attendant risks, discomforts, and potential benefits will be carefully explained to the patient. A signed consent form will be obtained by the PI or an associate investigator. It will be carefully explained to patients that they may withdraw from the study at any time, for any reason.

All adult ALS patients fulfilling all inclusion criteria and for whom none of the exclusion criteria are applicable, irrespective of gender or race, are included in the trial. Patients 18 years and older will be included in the trial as long as they are willing and able to comply with all the aspects of testing and treatment.

11.2. Justification for the exclusion of children

Children less than 18 years old are excluded from the trial. Since ALS is exceptionally rare in the second decade, pediatric cases might not be representative of the typical pathophysiology of the condition.

11.3. Justification for the exclusion of those who lack consent capacity

Due to the complex nature of this clinical trial, and the off-label use of the study medications, those who are cannot provide informed consent will not be allowed to participate in this trial.

11.4. Justification for sensitive procedures

Genetic testing will be completed as part of this protocol. Approximately 40% of familial ALS and 10% of sporadic ALS cases are associated with a known genetic mutation. It is likely that other genetic mutations account for even larger percentages of familial and sporadic ALS cases. Patients with a C9ORF72 mutation are more likely to have associated frontotemporal dementia; however, there are few other phenotypic differences between cases with genetic mutations. Therefore, it will be difficult to look for genetic differences that could account for the variability in suppression of the HERV-K RNA without assessing for the known genetic mutations.

11.5. Safeguards for vulnerable populations and sensitive procedures

Because there are no adequate and well-controlled studies in pregnant women for darunavir, dolutegravir, and TAF, participants able to become pregnant will be required to use appropriate methods of contraception for the trial duration and all participants able to become pregnant will be evaluated with a pregnancy test at each study visit. Male participants will not be required to use contraception during the study. Women 55 and over who have not had a period for one year will be considered menopausal and will not need pregnancy testing or contraception. Women under age 55 will undergo pregnancy testing and will be required to use appropriate methods of contraception for the trial duration, unless there is a history of hysterectomy, bilateral oophorectomy, or medically-documented ovarian failure. Adequate methods of contraception include: implanted contraception, intrauterine device in place for at least 3 months, or barrier method in conjunction with spermicide. Women of childbearing potential must have a negative pregnancy test at screening and be non-lactating.

12. Anticipated Benefit

There is no expected direct benefit to participants in this study. The study will likely yield generalizable knowledge about how antiretroviral medications affect HERV-K levels in a specific subset of patients with ALS.

13. Consent Documents and Process

13.1. Consent procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review both of the written consent forms and ask questions regarding this study prior to signing.

13.1.1. Telephone Consent

Telephone or telehealth consent may be done for participants having the screening blood collected outside of NIH or for continued enrollment onto the Standard Consent when participant participation at the Clinical Center is restricted.

For participants having the screening blood collected outside of NIH, the participant will be provided with a cover letter (Appendix B) and a copy of the Blood Draw Only Consent form. The study team will also send a copy of the Standard Consent form without the signature page as a reference to the rest of the study if the participant qualifies. The Standard Consent form with signature page will be sent to participants continuing their enrollment but restricted from traveling to the Clinical Center to complete their screening visit.

Once the consent forms are received, the study team will arrange for a telephone call or telehealth visit with an Investigator authorized to obtain consent and the participant to review the study and the consent form and to answer any questions. Once the participant agrees to participate, the participant will sign and date their copy of the consent form. The Investigator will document the consent process. The participant will return their signed copy to the Investigator. Once the copy with the participant signature is received, the Investigator will sign and date the consent form and send a copy to the participant. The telephone consent process will be documented.

13.2. Consent documents

The Blood Draw Only and Standard consent forms contain all required elements. The Blood Draw Only consent allows for only the screening blood draw from consenting participants.

14. Data and Safety Monitoring

14.1. Data and safety monitor

This study will be monitored by an Independent Monitoring Committee (IMC). The IMC will be Drs. Camilo Toro and Mary Wright. Camilo Toro, MD is a neurologist with the Undiagnosed Diseases Network (National Human Genome Research Institute) who has extensive expertise in the evaluation of neuromuscular disorders including ALS. Mary Wright, MD, MPH is an infectious diseases physician with National Institutes of Allergy and Infectious Diseases who has extensive experience with antiretroviral therapies.

14.2. Data and safety monitoring plan

The IMC will review the study prior to initiation, twice a year thereafter, and at the end of the study. The IMC may convene additional reviews as necessary. The IMC will review the study data to evaluate the safety, study progress, and conduct of the study. All serious adverse events and all unanticipated problems will be reported by the PI to the IMC at the same time they are submitted to the IRB. The PI will notify the IMC at the time pausing criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written IMC summary reports with recommendations to the IRB.

14.3. Criteria for stopping the study or suspending enrollment or procedures

Enrollment in the study will be halted if a study drug-related SAE occurs in more than one patient, or AEs at a higher frequency than expected based on the available data.

15. Quality Assurance

15.1. Quality assurance monitor

The NINDS Quality Assurance (QA) Audit Committee will perform QA monitoring for this study.

15.2. Quality assurance plan

This protocol will undergo random review by the NINDS Quality Assurance (QA) Audit Committee as outlined in the NINDS QA Standard Operating Procedure. The purpose of the QA audit is to assess compliance with applicable regulatory requirements, good clinical practice guidelines, NINDS/NIH policies, as well as to provide recommendations for improving the management of clinical research data. The protocol will be audited according to the decision algorithm as described in the NINDS SOP. As such, this protocol is classified as “more than minimal risk” and thus will be audited with a target frequency of once during the first year following IRB approval, and then approximately every three years thereafter.

16. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801.

17. Alternatives to Participation

This is not a treatment protocol and the only alternative is the off-label use of the study medications outside of the study.

18. Privacy

All research activities will be conducted in as private a setting as possible.

19. Confidentiality

Participant confidentiality and privacy is strictly held in trust by the participating investigators and the study team. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

The study monitor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

19.1. For research data and investigator medical records

Each participant will receive a unique study ID number at the time of enrollment. A master list correlating the unique numbers with personal health information such as names, addresses, and contact information will exist but will only be accessible to investigators on this protocol in order to minimize the risk of compromising confidentiality. These data will be kept in password-protected computers on the NIH campus.

19.2. For stored samples

Data and samples will be stored in secured areas. Samples kept at NIH will be stored in a coded fashion in a locked room in the SINS lab.

19.3. Special precautions

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

19.4. For genetic samples

Genetic samples will be stripped of identifiers and will be coded ("de-identified") or unlinked from an identifying code ("anonymized"). Genetic samples will be stored in secured freezers on the NIH campus. Only study investigators will have access to the stored samples and data/results. Access to the "key" of the code is in password protected databases where access is limited to the study team. The Key will not be provided to collaborators or third parties but will remain at NIH. The data/results of the genetic tests will not pose a high risk in the identification of participants or their families as the data will be coded. Any loss or destruction of samples will be reported to the IRB. Samples may be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained in the original consent form. See Section 5.2 Data for additional information regarding the sharing of genetic data.

20. Conflict of Interest

20.1. Distribution of NIH guidelines

NIH guidelines on conflict-of-interest were distributed to all investigators.

20.2. Conflict of interest

There are no conflicts-of-interest to report.

21. Technology Transfer

Technology transfer agreement(s) for this protocol are described in the Data and Sample Sharing spreadsheet. See Study Application in iRIS.

22. Research and Travel Compensation

Patients will not be compensated for their participation in this study. Participants will be reimbursed for travel and subsistence will be offered consistent with NIH guidelines. Participants will not receive financial compensation from any commercial product developed from this research.

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24. Consent Forms

Blood Draw Only Consent
Standard

25. Appendix A. Adverse Event definitions

Definition of Adverse Event (AE)

An AE "is *any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen*." An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses.

Severity of Adverse events

Grade refers to the severity of the AE. The DAIDS toxicity table displays Grades 1 through 4 with unique clinical descriptions of severity for each AE based on this general guideline:
Grade 1 Mild AE
Grade 2 Moderate AE
Grade 3 Severe AE
Grade 4 Potentially life-threatening AE (SAE)

Grade 1: Symptoms causing no or minimal interference with usual social & functional activities	Grade 2: Symptoms causing greater than minimal interference with usual social & functional activities	Grade 3: Symptoms causing inability to perform usual social & functional activities	Grade 4: Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

Definition of Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence or effect at any dose, that:

- results in death,
- is life threatening,
- results in persistent or significant disability/incapacity,
- requires in-patient hospitalization[‡] or prolongation of existing hospitalization,
- results in cancer,
- is a congenital anomaly/birth defect in the offspring of a study participant,
- is deemed, by the investigator, an important or serious medical event that may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above should be considered serious (i.e., intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.)

Assessment of causality

Every effort should be made by the investigator to explain each AE and assess its causal relationship, if any, to the study medication. The degree of certainty with which an adverse event can be attributed to the study medication (or alternative causes, e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of one or more of the following:

- Reaction of similar nature previously observed with this type of medication.
- The event reported in the literature for similar types of medication.
- Subject's underlying clinical state or other medical conditions (defined further in Section 8.1).

- Concomitant agents and/or therapies.

The causal relationship, if any, to study medication will be defined by one of the following terms:

Assessment	Definition
Definitely	There is suspicion of a relationship between study medication and AE (without determining the extent of probability); there are no other more likely causes and study medication is suspected to have contributed to the AE.
Probable	AE cannot be reasonably explained by other factors (i.e., clinical condition, environmental/toxic factors or other treatments).
Possible	AE can be reasonably explained by other factors (as mentioned above).
Unlikely	AE occurs within an unusual time frame of administration of study medication and can also be reasonably explained by other factors (as mentioned above).
Unrelated	There is no suspicion that there is a relationship between study medication and AE; there are other more likely causes and study medication is not suspected to have contributed to the AE.

26. Appendix B. Cover Letter for Telephone Consent

Dear _,

Thank you for your interest in our study: 15-N-0126 “HERV-K Suppression Using Antiretroviral Therapy in Volunteers with Amyotrophic Lateral Sclerosis (ALS).”

Per our previous conversation we are sending you the Blood Draw Only Consent. This is the form that, once signed, gives us permission to collect your blood for research purposes. Please do not sign this form until we talk on the telephone. We are also sending you a copy of the Standard Consent. This form does not have a signature page and is a reference for the entire study.

We are scheduled to discuss these documents on _ at _. Please take the time before then to review the consent forms so that you may gather your thoughts and questions for us to discuss prior to you signing.

A member of the study team will contact you on the number you have provided us at the scheduled time above.

If you have any questions, you may contact

Amanda Wiebold, RN
301-594-5194
amanda.wiebold@nih.gov

Sincerely,
Amanda Wiebold, RN
Research Nurse Specialist
NINDS Section of Infections of the Nervous System