Protocol Title: PD-1 inhibition to determine CNS reservoir of HIV-infection
NCT#: NCT03239899
Document Date: 1/09/2022

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Abbreviated Title: HIV CNS PD-1
Protocol Number: 17-N-0145
Date of This Submission/Version1/9/2022/Amendment o

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Total requested accrual
(20) Patients
(0) Healthy Volunteers

Project Uses Ionizing Radiation: □No √ Yes
□□Medically-indicated only
√ Research-related only (*attach RSC/RDRC documentation)*
□□Both

IND/IDE □ No √ Yes
Drug/Device/#_IND 133900_____
Sponsor: __NINDS_____

Durable Power of Attorney √ No □Yes

Multi-institutional Project √ No □Yes
Institution#1_____ FWA #_____
Date of IRB approval _____

Institution#2_____ FWA #_____
Date of IRB approval _____

Data and Safety Monitoring Board □No √Yes

Technology Transfer Agreement No √ Yes

Samples are being stored □No √ Yes

Flesch-Kincaid reading level of consent form: 8.8

Précis:

Objective

In this Phase I, proof-of-concept study, we aim to determine the safety and tolerability of pembrolizumab, an FDA-approved monoclonal antibody against programmed cell death protein (PD)-1, in viremically suppressed human immunodeficiency virus-1 (HIV) positive patients. We are examining the correlation of immune activation and suppression markers in viremically suppressed HIV positive patients with the effects of pembrolizumab on immune restoration function (e.g. CD4 count, HIV viral load) and immune activation (e.g. HIV-specific T-cell responses).

Study Population

HIV is estimated to infect 37.6 million people globally, with 690,000 deaths and 1.5 million new infections occurring yearly. There is no cure. Opportunistic infections and neoplasms contribute to a large portion of mortality and morbidity within the HIV-positive population. Even in well-controlled, viremically suppressed patients, neurologic complications including HIV-associated neurocognitive disorder, continue to contribute to disease morbidity and mortality.

There is evidence that HIV reservoirs contribute to the inability to cure HIV infection. In the brain, macrophages and astrocytes harbor HIV. It is theorized that the brain is a potential reservoir for replication competent HIV. PD-1 expression is elevated in patients with HIV compared to uninfected controls. Upregulated PD-1 expression is associated with higher viral load and increased mortality in infections. PD-1 co-expression on regulatory T-cells has been shown to correlate with disease progression in perinatally-infected HIV-positive children. Drugs targeting the PD-1 pathway in HIV infection have shown upregulation of T-cell responses that are potentially critical to eradication of infection. Pembrolizumab is an attractive option due to its mechanism of action, although it has been rarely used in the HIV population.

Design

In this single-center, single-arm, open label, baseline-versus-treatment phase I clinical trial, twelve patients with HIV-1 infection receive a one-time dose of 200mg pembrolizumab with a baseline study period of 3 weeks, a one-day treatment phase, and a 6-month post treatment phase. Outcome measures are collected every 3 to 6 weeks for the duration of the study.

Outcome Measures

The primary outcome is the safety and tolerability of pembrolizumab, which is measured by clinical exam, laboratory studies and adverse event tabulations using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

In addition, viral and immunologic outcome measures investigating the impact of pembrolizumab on HIV-1 biology and its effects on immune function is measured in the CSF and periphery, including single copy HIV analysis, CD4+ T-cell count, PD-1 lymphocyte expression and T-cell phenotype analysis, T-cell proliferation against HIV-proteins, CSF cytokine analysis and/or CSF antibody profiling (LIPS). These additional studies offer indirect proof of a HIV viral reservoir in the CNS as well as potential efficacy of pembrolizumab in reversing immune exhaustion against latent HIV

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List of Abbreviations

AE	Adverse Event/Adverse Experience
ALT/SGPT	serum glutamic-pyruvic transaminase
AML	Acute Myeloid Leukemia
ART	Antiretroviral Therapy
AST/SGOT	serum glutamic-oxaloacetic transaminase
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events (CTCAE) v5.0
DoH	Declaration of Helsinki
DSMB	Data and Safety Monitoring Board
ELISA	Enzyme-Linked Immunosorbent Assay
FDG	2-deoxy-2(12F) fluoro-D-glucose
G-CSF	granulocyte colony-stimulating factor
HAND	HIV-associated neurocognitive disorder
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HTLV	Human T-Lymphotropic Virus
IEC	Independent or Institutional Ethics Committee
IL-8	interleukin-8
IP-10	interferon y-induced protein 10
irAE	immune-related Adverse Events
IRB	Institutional Review Board
LIPS	Luciferase Immunoprecipitation System
MCP-1	monocyte chemotactic protein 1
MIP-1β	macrophage inflammatory protein-1β
MRI	Magnetic Resonance Imaging
N	Number (refers to number of participants/sample size)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
PBMC	peripheral blood mononuclear cells
PD-1	Programmed Cell Death Protein
PD-L1	Programmed Cell Death Protein Ligand 1
PD-L2	Programmed Cell Death Protein Ligand 2
PET	Positron Emission Tomography
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
SAE	Serious Adverse Event/Serious Adverse Experience
SIV	Simian Immunodeficiency Virus
SMC	Safety Monitoring Committee
SST	Serum Separator Tube

1. Introduction and Background

1.1 HIV

In 2020, there were an estimated 37.6 million people living globally with HIV infection[2]. In the last year alone, 690,000 adults and children died from AIDS and 1.5 million people were newly infected with HIV. The introduction of the first antiretroviral drug zidovudine in 1987, followed by combination antiretroviral therapy in 1996, signaled the first change in the HIV/AIDS epidemic and led to a marked shift in disease outcomes with an expected decrease in the incidence of patients affected by HIV-related opportunistic infections and AIDS-related deaths. However, nearly thirty years after the advent of ART, with 73% of people living with HIV on antiretroviral therapy, patients continue to be affected by opportunistic infections, HIV-associated neurocognitive disorders (HAND) and major depressive disorder (MDD). Cognitive disorders, with their non-acute onset and relatively mild initial clinical presentations, can lead to gradual, but ultimately significant, functional deterioration even in those patients who have suppressed virus and are on antiretroviral therapy.

1.2 HIV Persistence and HIV Reservoir

Although control of chronic HIV infection can be achieved with optimum ART[3] there is no cure for the disease. HIV continues to persist in latently infected CD4+ T cells and is integrated into the host cell nucleus until cell death. Tissue reservoirs contribute to chronic persistence where the virus evades the immune system. In well-controlled HIV-positive patients, HIV is usually undetectable in the CSF although non-specific immune activation can be detected indicating continuing response to the underlying infection.[4] While studies from the pre-antiretroviral era clearly show that HIV can infect cells within the central nervous system (CNS), including microglia, astrocytes and macrophages,[5], CNS reservoir remains a site of active investigation. Recent investigations reporting CSF escape detail a rate of approximately 6%, with neurosymptomatic escape associated with concurrent low-level viremia or plasma suppression.[6] In the CHARTER cohort, a longitudinal study of 401 individuals living with HIV-1 on ART, up to 13% of individuals had detectable HIV in CSF.[7, 8]

Currently there are no methods to detect HIV in the brain of living patients and very few autopsy brains are available from HIV-positive patients who are viremically suppressed and on ART at the time of death. These factors limit our ability to conclude whether or not the brain itself is a reservoir for latent HIV infection. An indirect method to detect the CNS viral reservoir would be to monitor immune responses directed against HIV antigens in the CSF and compare to those present in blood.

1.3 The Berlin Patient

In 2008, a patient in Berlin, Germany with HIV-1 infection for 10 years on stable ART developed acute myeloid leukemia (AML) requiring stem cell transplantation.[9] An HLA-identical donor was screened for homozygosity for the CCR5 delta32 allele, which confers resistance to HIV infection with a truncated CCR5 protein that is not expressed on the cell-surface. To date, this Berlin patient is the only known patient with an HIV sterile cure. Although this is the only true HIV cure to date,

a small population of HIV-positive individuals known as “elite controllers” or “long term non-progressors” are able to maintain high CD4+ counts and low HIV-1 RNA viral loads off of ART. Elite controllers typically have strong HIV-specific CD8+ T-cell proliferative and cytotoxic responses against HIV proteins, such as gag or nef, or HIV-infected CD4+ T cells.[10] Although stem cell transplantation has failed to afford any patient other than the Berlin patient with a sterile HIV cure, new methods to enhance HIV specific T-cell responses against Gag and Nef are being developed to target the HIV-reservoir with the goal of developing cure strategies.[11]

Elite controllers have also been shown to have lower levels of macrophage inflammatory protein-1β (MIP-1β), interferon γ-induced protein 10 (IP-10), and monokine induced by interferon-γ (MIG), with CD4+ T-cell counts inversely correlating with plasma cytokine MIP-1β levels.[12] Increased levels CSF cytokine IP-10, along with interleukin-8 (IL-8), monocyte chemotactic protein (MCP)-1, and granulocyte colony-stimulating factor (G-CSF), has been implicated in HIV-associated neurocognitive disorder (HAND).

Studies on the Berlin patient and elite controllers show that the HIV antibody profiles of these groups differ from non-controllers and can be used to monitor curative intervention. [13] In the Berlin patient, the only known patient with a functional cure of HIV, there are no HIV directed antibodies against p24, matrix, nucleocapsid, integrase, protease or gp120 proteins. Low level antibody responses continued to be seen 5 years’ post-cure against reverse transcriptase, tat and gp41 in the Berlin patient. LIPS assay in a subset of exceptionally strong elite controllers showed low antibody responses against p24, matrix and gp120.[10] In follow up studies, loss of antibodies to p24 were uniquely associated with the cured state (Figure 1).[13]

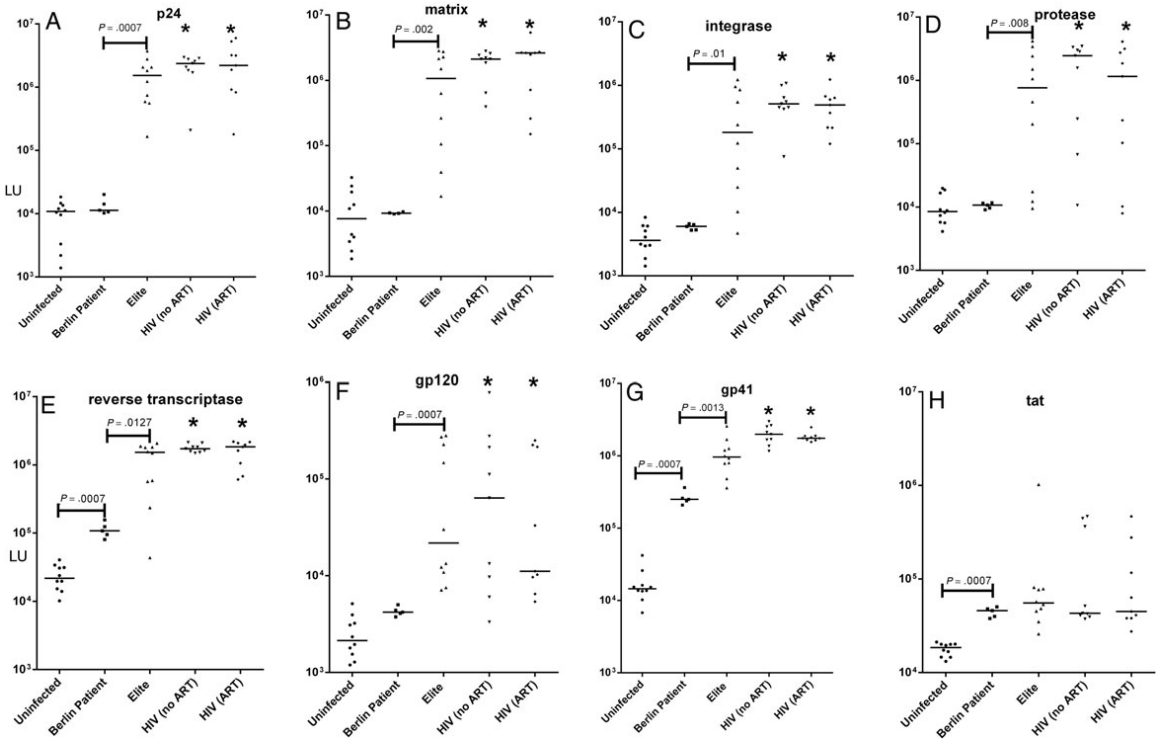

Figure 1: Antibody profiles against HIV antigens in the Berlin Patient and other HIV-infected subjects.

The antibody levels against each of the 9 HIV proteins were determined in the uninfected subjects (n = 10), the 5 serial samples from the Berlin Patient, elite controllers (n = 10), and 9 HIV subjects (n = 9) from before (no ART) and after ART. The antibody levels are plotted on the y-axis using a log10 scale and the median value in each group is shown by a solid horizontal line. Only statistically significant P values between the Berlin Patient and other groups are shown and were calculated using the Mann– Whitney U test. For comparison between the Berlin Patient and HIV (no ART) and HIV (ART) groups, P values less than .008 are denoted by an asterisk. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus. Figure from publication cited in reference #[13].

1.4 Antibody responses to HIV-Tat protein

We have previously measured antibody responses to HIV-Tat protein in the CSF and found that its correlated with viral load in the CSF and the degree of immunosuppression (Figure 2).[14] Measuring antibody responses to Tat could be particularly informative since once the HIV proviral DNA is formed Tat protein is formed unchecked even in the presence of adequate ART. The production of Tat in the brain under such circumstances can be recognized in brain macrophages even when p24 cannot be detected [15].

Indeed, further work from our group showed that – in a cohort of 68 HIV-positive, virally suppressed individuals on ART – tat was detected in 36.8% of collected samples.[16] Exosome analysis revealed that 34.4% of samples were strongly positive for tat protein and /or TAR RNA, with retained tat-transactivation activity in 66.7% of samples.[16] Indeed – tat concentration increased in 4 out of 5 positive individuals after initiation of ART, indicating that tat pathways are not inhibited by current ART, highlighting a potential useful marker of CSF HIV-1 reservoir.[16] .

A small study of in vivo work with HIV-1 tat demonstrated an induction of PD-L1 expression on APC/dendritic cells through TNF-alpha and TLR-4 mechanisms, potentially offering a new route of exhaustion in chronic non-progressors driven by continued tat transactivation.[17]

1.5 HIV and anti-PD-1

A hallmark of chronic viral illness is the impairment of antigen-specific T-cell responses, with upregulation of programmed cell death protein 1 (PD-1). The presence of inflammatory cytokines and continued expression of tat up-regulate the expression of PD-1 and PD-L1 expression, which in turn downregulates the T-cell effector responses (Figure 2).

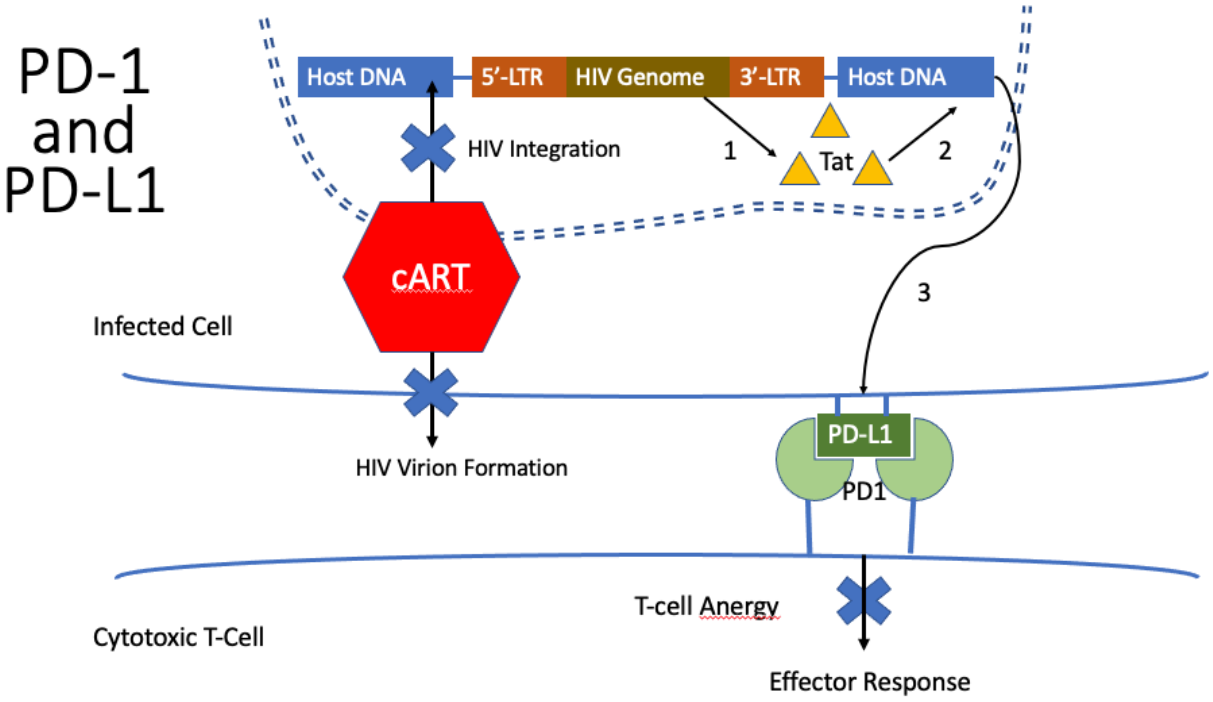

Figure 2: PD-1 CD8+ T-cell Effector Response
This schematic shows the relationship between PD-1 upregulation on CD8+ cytotoxic T-cells, the drive for anergy in the cell through the PD-1 receptor pathway, and the relationship of tat transactivation to the PD-1 pathway even in the presence of optimal cART with suppression of HIV virion.

Upregulated PD-1 expression is associated with higher viral load and increased mortality in infections.[1] PD-1 co-expression on regulatory T-cells has been shown to correlate with disease progression in perinatally-infected HIV-positive children.[18] PD-1 is a member of the B7-CD28 superfamily expressed on activated T cells, NK cells, as well as B-cells, natural killer cells and all antigen-presenting cells.[19] This immune checkpoint is involved in inducing tolerance as well as modulating the magnitude of the antigen-specific immune response to infection.[19]

Animal studies in SIV infected lymphoid tissue and in HIV blood have shown that checkpoint inhibitors PD-1 and TGIT remain elevated on CD8+ T-cells, correlating with disease progression.[20] Furthermore, checkpoint inhibition remained high even in the presence of viral suppression, and check-point antibody blockade restored viral-specific T-cells responses against SIV and/or HIV.[20]

PD-1 further modulates T-cell activity via interaction with its ligand PD-L1, and blocking this interaction has been proven to enhance T-cell specific responses for viral control.[21] In one study, increased frequencies of PD-1+CD8+ T cells correlated with HIV progression and antibody blockade of PD-L1 restored viral-specific CD8+ T cell effector responses, suggesting novel curative HIV targets to reverse T-cell exhaustion.[20] This T-cell response was noted to be particularly robust in a subset of patients against HIV gag protein. Case studies have shown transient increases in HIV-specific responses, increased inflammatory cytokines, as well as potential for decrease in

cell-associated proviral HIV-DNA in individual patients treated with anti-PD1 therapy for oncologic indications [22, 23] This indicates that clinical trials are needed to determine if an anti-PD1 approach in HIV is able to target the CSF HIV-1 reservoir.

1.6 HIV and Pembrolizumab

Although there are now several anti-PD1 monoclonal antibodies that are FDA approved for varying cancer treatments, Pembrolizumab was the first anti-PD-1 agent to be approved by the FDA as a fully humanized, highly selective, IgG4-kappa monoclonal antibody against PD-1.[19] Original trials have supported the use of pembrolizumab in melanoma treatment trials among other cancers [24], and it has more recently been expanded as off-label in infections such as BK encephalitis and CNS JC virus reactivation.[25]

An open-label, non-randomized, phase 1 multicenter study evaluated the safety of 200mg IV pembrolizumab every 3 weeks for up to 35 doses in 30 participants with HIV and advanced cancers in three CD4 count defined cohorts.[26] In this study, pembrolizumab was found to have a similar safety profile in patients with cancer, HIV on ART and CD4 count greater than 100 as compared to the non-HIV patients with cancer. One participant with pretreatment KS herpesvirus viremia developed a B-cell lymphoproliferation after anti-PD1 therapy and died, suggesting a potential association. Most adverse events at least possibly related to pembrolizumab were low grade, and 20% were grade 3. Immune-related adverse events (irAEs) included hypothyroidism, pneumonitis, rash, elevated liver enzymes, and musculoskeletal event. HIV was controlled in all participants.

A small, phase I safety study of PD-L1 inhibition, the ligand to PD-1 and not the receptor, in HIV positive patients (N=8) measured HIV gag-specific CD8+ T cell responses correlated to HIV RNA and CD4+ counts before and after treatment.[27, 28] There were no major adverse treatment-related adverse events in this study, but was discontinued early due to retinal toxicity in animal studies. A case of hypophysitis was also seen after a few months in this study and could not be excluded as being related to study drug.[28]

A phase ½, double-blind, placebo-controlled, ascending multiple dose study of anti-PD1 antibody cemiplimab was halted early after two treatment related adverse events after enrollment of 5 participants.[29] Stopping criteria was stringent. One participant experienced sub-clinical hyperthyroidism after 1 dose and a second (with baseline grade 1 elevation in AST/ALT) experienced an asymptomatic grade 3 elevation in AST/ALT. Both participants returned to baseline function without intervention, but the study was halted on the advice of their DSMB.

Since 2003, there have been six interventional clinical trials looking at checkpoint inhibitors in otherwise healthy, aviremic, on ART individuals living with HIV (Table 1). Two of these trials are at the NIH and have enrolled half of their target cohorts, and only one trial targets the CNS reservoir (NCT03239899). All but one trial has targeted the PD-1 pathway. Most safety events self-resolved or resolved with treatment in the trials that were halted for reasons of safety.

Table 1: Checkpoint Trials In HIV

Organization	Status	Trial Number	Target	Description	Outcome

NINDS	Recruiting	NCT03239899	PD1	PD-1 Inhibition to Determine CNS Reservoir of HIV-Infection	Ongoing, no concerning AEs to date
NIAID	Recruiting	NCT03367754	PD1	A Single Dose of Pembrolizumab in HIV-Infected People	Ongoing, no concerning AEs to date
ACTG	Completed	NCT03787095	PD1	Safety and Immunotherapeutic Activity of an Anti-PD-1 Antibody (Cemiplimab) in HIV-1-infected Participants on Suppressive cART	Halted for reasons of safety – grade 2 hyperthyroidism and grade 3 ALT/AST elevation
AbbVie	Recruiting	NCT04223804	PD1	A Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of ABBV-181 (Budigalimab) in Adult Participants With HIV-1	Ongoing, no data to date
ACTG	Completed	NCT02028403	PD-L1	Safety and Immune Response of BMS-936559 in HIV-Infected People Taking Combination Antiretroviral Therapy	Halted for reasons of safety in animal study – retinal findings in macaques. At 36 week follow up, asymptomatic hypophysitis in one participant with eventual return to baseline
BMS	Completed	NCT03407105	CTLA-4	A Dose-Escalation Study of MDX-010 Administered Monthly as Immunotherapy in Subjects Infected With Human Immunodeficiency Virus (HIV)	Completed. N=24. One grade 2 facial palsy. No AEs greater than grade 2.

Drug safety data on pembrolizumab is extensive in the cancer population, but it should be noted that the populations in which it is most studied are patients with metastatic lung cancer or metastatic melanoma who have previously received multiple chemotherapeutic and/or immunosuppressive drugs. This population is more at risk for adverse events due to the serious nature of their disease and multi-organ involvement of their metastatic cancers. Also, prior therapies and chronic illness complicate the adverse event reporting in these patients. The safety data that is available suggests that risks are not increased in participants living with HIV receiving pembrolizumab compared to the general population.

1.7 Pembrolizumab

Pembrolizumab (Keytruda) is FDA approved for the treatment of 15 different oncologic indications as of January 2020.[30] It is a humanized IgG4 kappa monoclonal antibody that blocks the interaction between PD-1 and its ligands PD-L1 and PD-L2 with an approximate molecular weight of 149 kDa, manufactured by Merck. It has a terminal half-life of 28 days and steady-state concentrations are reached after repeat dosing with an every 3-week regimen of doses between 2 to 10mg/kg every 3 weeks in solid tumours.[30]

1.8 Pembrolizumab and Pharmacokinetics

Based on patients who received doses of 1 to 10mg/kg every 2 weeks or 2 to 10mg/kg every 3 weeks, the clearance of drug was 0.22L/day and the mean elimination half-life was 26 days. Steady state concentrations were reached by 18 weeks with repeat dosing every 3 weeks. Renal impairment and mild hepatic impairment had no clinically important effect on the clearance of pembrolizumab. Updated guidance recommends a flat dose of 200mg every 3 weeks for most indications.[30]

1.9 Pembrolizumab and Safety

Table 1: Immune-mediated adverse reactions by solid tumor type with adverse time to onset.[31]

Adverse Reaction	**Melanoma**	**Average time to adverse reaction onset after starting treatment**	**AE Grade 2**	**AE Grade ≥3**	**Non-Small Cell Lung Cancer**	**Average time to adverse reaction onset after starting treatment**	**AE Grade 2**	**AE Grade ≥3**
Hypo-thyroidism	8.1%	3.3 months (range 5 days to 18.9 months)		0.1%	6.9% (14.6% in HNSCC Trial 4)	4.2 months (20 days to 11.2 months)	5.5%	0.2% (0.5% HNSCC trial 4)
Hyper-thyroidism	3.3%	1.4 months (range 1 day to 21.9 months)	0.6%	0.1%	1.8%	1.8 months (2 days to 3.4 months)	0.7%	0.3%
Pneumonitis	2.0%	4.3 months (range 2 days to 19.3 months)			3.5%	1.7 months (range 4 days to 12.9 months)	1.1%	1.9%
Colitis	2%	3.4 months (range 10 days to 9.7 months)	0.5%	1.2%	0.7%	1.6 months (range 28 days to 2.2 months)	0.2%	0.4%
Hepatitis	1.0%	26 days (range 8 days to 21.4 months)	0.1%	0.8%	0%			
Hypophysitis	0.8%	3.3 months (range 1 day to 7.2 months)	0.3%	0.4%	0.2% (n=1)	3.7 months		0.2%
Nephritis and Renal Dysfunction	0.4%	5.1 months (range 12 days to 12.8 months)	0.2%	0.3%	0%			

Type 1 Diabetes Mellitus	0.1% (n=3) (all trials 1-3 combined melanoma and NCSLC)							
Other immune-mediated: including cardiac toxicities	<1%				<1%			
Immune Arthritis	1.6%							
Infusion Reaction	0.1% (all trials 1-3 combined melanoma and NCSLC)							
Total Adverse Events	19.4%		1.7%	2.9%	14.1% (or 21.8% including trial 4 data)		7.5%	3.0% (or 3.3% including trial 4 data)

With repeat dosing for solid tumor immunotherapy, side effects were overall well tolerated in the majority of cancer patients but immune mediated adverse reactions could be seen (Table 1).[31, 32] The major serious adverse reactions that can occur secondary to exposure and repeat exposure to pembrolizumab are autoimmune in nature (Table 1). There has also been a reported case of reactivated tuberculosis following pembrolizumab treatment.[33] Patients receiving anti-CTLA 4 and/or anti-PD-1 therapy have developed cardiotoxic side effects, with one patient receiving pembrolizumab experiencing cardiac arrest attributed to a Takotsubo cardiomyopathy.[34]

In patients with metastatic melanoma, the following laboratory abnormalities were increased from baseline with 2mg/kg every 3 weeks dosing of pembrolizumab (Table 2)[31, 32].

Table 2: Laboratory abnormalities in patients with metastatic melanoma in >20% of patients after receiving repeat dosage pembrolizumab[31, 32]

Laboratory Test	**All Grades Adverse Event (%)**	**Adverse Event Grade ≥ 3 (%)**
Chemistry		
Hyperglycemia	45	4.2
Hyponatremia	28	4.6
Hypoalbuminemia	27	2.4
Hypertriglyceridemia	43	2.6
Increased AST	27	2.6
Increased ALT	23	3.1
Hypocalcemia	24	
Hematology		
Anemia	35	3.8
Lymphopenia	33	6

Common clinical abnormalities in patients include fatigue, gastrointestinal side effects, fever, joint aches, and itching/rashes (Table 3).

Table 3: Clinical Adverse reactions in patients with NSCLC in ≥ 10% of patients after receiving repeat dosage pembrolizumab.[31]

Clinical Symptom	**All Grades Adverse Event (%)**	**Adverse Event Grade 3 (%) (no higher AEs)**
Fatigue	44	4
Pyrexia	12	1
Peripheral Edema	10	0
Decreased Appetite	25	1
Dyspnea	23	4
Cough	29	<1
Nausea	18	1
Diarrhea	15	1
Constipation	15	<1

Vomiting	12	1
Arthralgia	15	1
Back Pain	10	2
Rash	18	<1
Pruritis	12	0

1.10 Pembrolizumab and Drug Dosage

Pembrolizumab is FDA approved for intravenous infusion over 30 minutes at a dose of 200mg every 3 weeks for most of its 15 oncologic indications.{(FDA), #450} A pooled analysis from three trials of pembrolizumab in melanoma that evidenced a flat-exposure-toxicity relationship across the dose range of 1 to 10mg/kg every 2 to 3 weeks identified no new major safety concerns.{Barone, 2017 #453}

1.11 Pembrolizumab and Fetal Toxicity

Pembrolizumab is an abortifactant. Animal models link the PD-1 pathways to maintenance of pregnancy through immune tolerance pathways. Although mouse models have not shown fetal malformations, there was an increase in fetal loss in mice where PD-1 was blockaded. Fetal exposure to pembrolizumab is hypothesized to increase the risk of the fetus to immune mediated disorders. Patients of reproductive potential should be advised to use two forms of highly effective contraception during drug administration and for at least 4 months following the last drug dose.{(FDA), #450}

1.12 Pembrolizumab and Breastfeeding

It is not known if pembrolizumab is excreted in breast milk. Nursing mothers are advised to discontinue pembrolizumab.{(FDA), #450}

1.13 Pembrolizumab and the Blood Brain Barrier

There is no literature on the specifics of pembrolizumab and trafficking across the blood brain barrier. Although it is likely that peripheral antibody is trafficked into the CNS through adsorptive transcytosis, receptor mediated transcytosis and disrupted tight junctions, inherent mechanisms also exist to remove pembrolizumab, an Fc-containing mAb, from CNS space and therefore the CSF bioavailability would be expected to be distinct from the periphery.{Chacko, 2013 #454} Unpublished work from our group (Figure 3) shows that CSF PD-1+ CD8+ and CSF PD-1+CD4+ T-cell levels recover in a manner distinct from the peripheral PD-1+CD8+, PD-1+CD4+ T-cell populations, suggesting that while peripheral administration of pembrolizumab decreases CSF as well as peripheral PD1+ T-cell surface expression on CD4+ and CD8+ T-lymphocytes, the CNS immune space is independently regulated and distinct from the peripheral space.

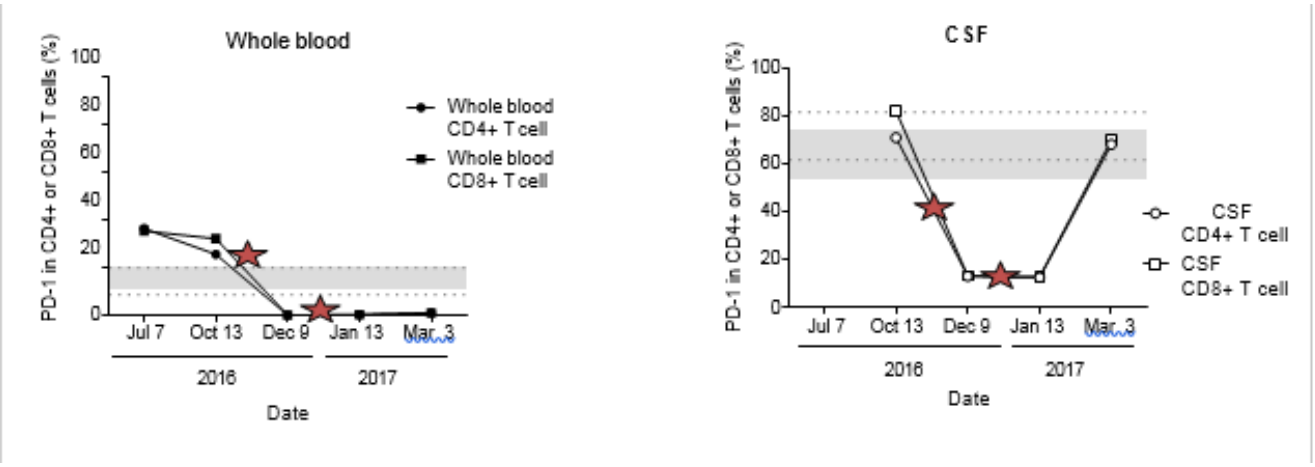

Figure 3: PD-1+ CD4+ and PD-1+CD8+ T-cell response in response to peripheral pembrolizumab administration in the CSF compared to peripheral blood.
This timeline represents the course of PD-1+ T-cell suppression (CD4+ and CD8+ T-cells) demonstrated by flow cytometry in one patient with HIV-PML who was treated with systemic 2mg/kg pembrolizumab at two timepoints (red stars). Peripheral suppression continued when CSF PD-1+ expression began to rebound which is consistent with our groups larger population of non-HIV PML patients treated with anti-PD-1 therapies (data not shown).

1.14 PD-1, Pembrolizumab, and latent HIV reservoirs

Reservoirs in HIV contribute to disease morbidity and provide a source of virus that is not able to be effectively targeted by current antiretroviral therapy, eliminating the possibility of a disease cure. Although HIV has been identified in the CNS, limitations on current studies have not allowed for conclusive evidence of a CNS reservoir. Targeted immune therapies against check-point inhibitors such as PD-1 will potentially release anergic T-cells, increasing the host adaptive and innate immune responses against the latent sites of HIV infection. Evidence of this response in HIV infection, and the potential efficacy as well as safety profile of pembrolizumab, will allow for further targeted therapies for curative intervention. We hypothesize that the number of adverse events related to a one-time dose of pembrolizumab in HIV infected patients will be less than reported rates in the cancer population. This would offer evidence of an equivalent or improved safety profile in the HIV-population compared to the FDA-approved drug indicated cancer populations.

1.15 Results at the time of quadrennial review

Since the approval of this protocol, 13 patients have enrolled. To date, 6 of the enrolled patients have completed participation in the study. Some preliminary study results were presented at the Conference on Retroviruses and Opportunistic Infections (CROI) in 2021:

- https://www.croiconference.org/abstract/effects-of-pd-1-blockade-on-hiv-reservoirs-in-blood-during-art/
- https://www.croiconference.org/abstract/pembrolizumab-treatment-is-associated-with-decreased-cell-associated-hiv-dna-in-csf/

A full interim analysis of the first six participants is currently underway and those results are pending.

2. Study Objectives

2.1 Primary objectives

a. The primary goal of the study is to determine the safety of a single dose of pembrolizumab in HIV-positive patients.

2.2 Secondary objectives

a. To examine if pembrolizumab changes the CSF expression of HIV-specific antibody responses and/or CSF cytokine profiles
b. To determine the effect of pembrolizumab on CSF PD-1 expression

2.3 Exploratory objectives

a. To determine if laboratory correlates of active HIV-1 replication, such as viral RNA and CD4+ T-cell counts, correlate with the effects of pembrolizumab on markers of immune activation
b. To explore the effect of pembrolizumab on ^{18}FDG-PET/CT.
c. To explore the effect of pembrolizumab on MRI brain studies
d. To determine the effect of pembrolizumab on HIV-specific T-cell responses

3. Subjects

3.1 Description of study populations

The study population will consist of 12 subjects with a diagnosis of HIV-1 infection. We anticipate enrolling up to 20 patients to allow for up to 8 dropouts. Participants who withdraw voluntarily, not due to toxicity, after initiation of the baseline phase but before receiving drug are considered dropouts. Patients who voluntarily drop out after receiving study drug are considered non-completers. Patients who no longer wish to participate in the research portion of the study after receiving pembrolizumab are offered the option of remaining in the study to continue safety evaluations. Patients who withdraw are removed from any further study or safety procedures.

3.2 Inclusion criteria

- ☐ 18 years or older
- ☐ Diagnosis of HIV-1 infection, with positive HIV 1 antibody testing
- ☐ HIV RNA ≤40 copies/mL in plasma in the last 12 or greater months
- ☐ CD4 count above 350 cells/uL
- ☐ Antiretroviral therapy for 12 months prior to trial
- ☐ Fully vaccinated against SARS-CoV-2. Fully vaccinated is defined as:
  - Two weeks out from the second dose of a two-dose vaccine series (Moderna, Pfizer-BioNTech); or
  - Two weeks out from a single-dose vaccine (Johnson & Johnson/Janssen)

- Patient must be willing and able to comply with all the aspects of trial design and follow-up.
- Patients must be able to provide informed consent
- Women of childbearing potential must agree to use contraception (defined as two forms of effective birth control), from the time of enrollment until 4 months after the last exposure to pembrolizumab
- Participants who are physically able to father a child must agree to use 2 effective methods of contraception (birth control) from the time you enroll in the study until 4 months after your last exposure to pembrolizumab

    - Effective methods of contraception for this study include:
        1. hormonal contraception (birth control pills, birth control patches, injected hormones, hormonal implants or vaginal ring),
        2. Intrauterine device,
        3. Barrier methods (condom or diaphragm) combined with spermicide, and
        4. Surgical sterilization (hysterectomy, tubal ligation, or vasectomy).
    - If you have had a hysterectomy, tubal ligation, or vasectomy (or have a partner with a hysterectomy, tubal ligation or vasectomy), you do not have to use 2 methods of birth control.

3.3 Exclusion criteria

- Clinically significant medical disorders that might expose the patient to undue risk of harm confound study outcomes or prevent the patient from completing the study as identified on screening studies and by patient history. Examples of such conditions include known cardiac disease such as congestive heart failure, chronic obstructive pulmonary disease, uncontrolled hypertension, kidney disease, liver disease, endocrine disease, pulmonary disease, heart disease, progressive CNS disease such as Parkinson's disease, dementia, prior tuberculosis infection or ongoing CNS opportunistic infection.
- Patient has received immunomodulatory/immunosuppressive therapy (including IV steroids but excluding local injections) in the preceding 6 months.
- Patient with known autoimmunity that would include but is not limited to disorders such as hypo/hyperthyroidism, myasthenia gravis, diabetes mellitus type 1, hemolytic anemia, and immune mediated hepatitis (but excluding patients with hypothyroidism already on thyroid replacement therapy).
- Prior history of cancer (excluding non-invasive squamous and basal cell carcinoma)
- Any opportunistic infection in the prior 2 years (excluding thrush) including latent TB or a positive TB Quantiferon Gold test
- Patient has received other investigational drugs within 3 months before enrollment
- Positive serological or PCR evidence of active or prior infection with HTLV-1/II, Hepatitis B or C. Patients with hepatitis B core (+), surface antibody (+), surface

antigen (–) and hepatitis B DNA (-) are eligible to participate in the study (provided they are on tenofovir, lamivudine or TAF). Participants with prior hepatitis C who are hepatitis C antibody (+) but hepatitis C RNA (-) with normal liver enzymes and no evidence of cirrhosis on clinical liver ultrasound are eligible to participate in the study.

- ☐ Metal in the body which would make having an MRI scan unsafe, such as pacemakers, stimulators, pumps, aneurysm clips, metallic prostheses, artificial heart valves, cochlear implants or shrapnel fragments, or history of welding or metal worker
- ☐ Claustrophobia
- ☐ Inability to lie comfortably on the back for up to two hours.
- ☐ Abnormal anti-thyroid panel (anti-TPO and anti-TG) test at screening visit.
- ☐ Abnormal screening/baseline blood tests exceeding any of the limits defined below or as deemed exclusionary by the investigators on review:
  - AST and ALT values >1.1x ULN
  - Fasting triglyceride > 300 mg/dL.
  - Total bilirubin >1.1 x ULN (unless participant is taking atazanavir or has Gilbert syndrome)
  - Creatinine Clearance or eGFR <60 ml/minute (adjusted for race)
  - Hemoglobin < 10 g/dL
  - Absolute neutrophil count < 1000/µL
  - Platelet count <130,000/mm3 (if platelet clumping is present on hematology slide review, platelet count <100,000/mm3 is considered exclusionary to study)
  - Hemoglobin A1c ≥ 6%
  - Thyroid-stimulating hormone (TSH) and adrenocorticotropic hormone (ACTH) within normal limits. If TSH is not within normal limits then the participant may be eligible if thyroxine (T4) is within normal limits. Participants are not excluded if they are on a stable dose of replacement thyroid medication; dose may be adjusted as needed.

- ☐ An employee or staff of the NIH

4. Study Design and Methods

4.1 Study overview

The study is a single center, single arm, and open label, baseline versus treatment trial of pembrolizumab in HIV patients. Twelve patients with viremically suppressed HIV-1 infection will receive a single dose IV pembrolizumab 200mg infusion; the treatment phase will be followed by a post-treatment follow-up for an additional 52 weeks. Study-related evaluations may be done in an outpatient setting unless inpatient setting is indicated at the NIH Clinical Center as outlined in Table 4. Telehealth may be used for conducting routine visits to the NIH Clinical Center.

In the event that circumstances beyond our control preclude the travel of one or more of the study participants to the NIH Clinical Center for a visit, or otherwise would put their health at greater risk (i.e. epidemic or pandemic), we will endeavor to establish capabilities for remote visits (through an NIH approved platform). Specifically, for safety visits, the study team will arrange for

local laboratory studies to be done, and conduct a telehealth visit with the participant. Unscheduled visits, due to safety, may also be conducted remotely through telehealth and/or offsite laboratory and radiologic studies and/or in conjunction with a local physician with the guidance of the study PI. The laboratory studies may be performed through LabCorp, Quest, or with the local physician, and results will be sent to the study team for safety monitoring and oversight. Radiology studies will be arranged through a local, non-study physician, or directly by the study team, at a site close to the participants home area if travel to the NIH is not possible. In the event that subjects are unable to come to the Clinical Center because of safety issues or travel restrictions, required in person procedures, physical exams, neurologic exams, research blood draws, lumbar punctures, or MRIs, will be conducted at the next planned study visit time point or as soon as is safe and feasible.

Patients have up to 9 visits at the NIH Clinical Center, with visits on Weeks 12 and 18 as optional travel visits. On weeks 12, and 18 patients may have an interval history over the phone and present to an NIH-contracted lab (either Quest and/or LabCorp) for safety blood draws. Offsite study visits are allowed if subject is able to access Quest and/or LabCorp for blood draws within the +/- 1-week study visit timepoint and is not able to come to the NIH Clinical Center. Offsite study visits are allowed at screening and all study timepoints after week 3 in the event of an epidemic or pandemic that limits participant travel to the NIH Clinical Center. In the event of an epidemic or pandemic affecting the week 24 MRI and lumbar puncture timepoints, these studies can be moved to the week 52 visit. If the week 52 is missed due to a pandemic or epidemic, a make-up final study visit comprising all the expected week 52 procedures (including potential LP and MRI from a missed week 24 visit) will be allowed as soon as the participant is able to safely travel to the NIH Clinical Center. During a pandemic or epidemic affecting patient travel, serial missed research collections will not be considered major study deviations and will be reported to the IRB at the time of continuing review.

Visits to the off-site Quest and/or LabCorp are coordinated by the study team, and the patient presents to the site for phlebotomy and urine collection only for safety laboratory work listed in Table 6. For off-site week 12 and 18 visits, the study team arranges a phone call with the patient to assess the interval history within the +/- 1-week interval. Study visits are accepted within a +/- 1-week window and brain FDG-PET/CT is accepted within a +/- 2-week window of the time points noted in Table 4. Each visit is expected to last no longer than 8 hours.

Table 4: Study Timeline

	Screening (Week -52 to 0)	Week -12 to 0	Week 0	Week 3	Week 6	Week 12	Week 18	Week 24	Week 52
Medical history	√	√							
Interval history			√	√	√	√	√	√	√

Physical exam		√	√	√	√[#]	##	##	√[#]	√[#]
Neurologic exam		√	√	√	√[#]	##	##	√[#]	√[#]
Pregnancy testing*	√	√	√	√	√	√	√	√	√
Research Blood Draw		√	√	√	√[#]	##	##	√[#]	√[#]
Safety Blood Draw	√	√		√	√	√	√	√	√
Lumbar Puncture		√		√				##	#
Leukapheresis		##		##				##	
MRI		√		√				√[#]	#
FDG-PET/CT		√		√					
Pembrolizumab 200mg therapy			√						

***For women of child-bearing potential only**

[#]In the event of an epidemic or pandemic, the timing of these study procedures are optional and can be performed when the subject is safely able to travel to the Clinical Center, at least once before participant is able to go off study. For the MRI and lumbar puncture, if they are missed at the week 24 timepoint due to an epidemic/pandemic, they can be moved to the week 52 visit. If this is unable to be completed at week 52, a make-up final study visit will be allowed as soon as the participant is able to safely travel to the NIH Clinical Center.

[##]Optional

4.2 Recruitment

Patients are recruited from the Section of Infections of the Nervous System protocol 13-N-0149 and through advertisement to the general public, and through the recruitment plan stipulated in protocol 13-N-0149.

Additionally, recruitment of HIV+ subjects is done from existing clinic OP-8 NIAID protocols, and referrals from the ID clinics at Washington Hospital Center, Family Medical & Counseling Services, CHARTER/NNTC sites, P30 sites, ACTG sites, and other physicians (Appendix E). Flyers may be posted on bulletin boards at grocery stores, community centers, bookstores, NIH Clinical Center, libraries or placed in advocacy group offices, in doctor's office waiting rooms, libraries, retail establishments with approval of the venue or in accord with their policy. Flyers may be made available at outreach exhibits, speaking engagements, support group meetings, professional meetings, association/trade meetings with approval of the venue or in accord with their policy. Flyers may be given directly to those requesting study information. Flyers may be posted electronically on websites such as such as NIH or NIMH websites, advocacy support group websites and local community online or print publications, such as Capitol Hill Community News. Flyers may be sent electronically to those requesting study information. IRB-approved advertisements may be placed in local print publications of newspapers, magazines and support or health care organizations, including Washington Post, Express, Washingtonian, Bethesda

Magazine, Washington Examiner, Military papers, Washington Jewish Week, NAMI Advocate, MHA, CHADD, and/or the NIH Record/CC News.

IRB-approved advertisements may be placed in national print publications of newspapers and magazines, including New York Times, Newsweek, Time, Oprah. IRB-approved advertisements are posted on the sides of Metro and Ride On buses, within the Metro Rail system, in bus shelters, on billboards, on scrolling digital boards in the Washington DC-Virginia-Maryland area.

Based on current and historical referral patterns, we anticipate this will permit the study to screen an average of 1-2 subjects per month.

4.3 Screening

Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images.

Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed above may be performed. We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the waiver as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent this study:

- Assessments performed another NIH protocol within the timeframes may also be used to determine eligibility once a participant has signed the consent.

Consent under 17-N-0145 is obtained before any study procedures, including screening procedures, are done. Screening can occur at any time during the life-cycle of the protocol. Screening participants did not start the baseline phase until after the SMC's 90-day post-study drug SMC safety review whereby further enrollment was allowed by the SMC in October 2019. In July 2021, the data and safety monitoring entity was changed from an SMC to a data and safety monitoring board (DSMB). Screening can occur within 52 weeks of the baseline visit and include the following procedures and laboratory evaluations:

a. History (Appendix F is used to obtain history)
b. Clinical blood draw
    - Table 5: Screening laboratory evaluations

Laboratory Testing

CBC/diff/plts
Hem A1c
PT/PTT
Pregnancy test (urine or serum)
Fasting Lipid panel
Anti-thyroid panel (anti-TPO and anti-TG)
Acute Care Panel/Hepatic Panel/Mineral Panel
amylase/lipase
TSH
CRP
HIV RNA Viral Load
TBNK
Adrenocorticotropic Hormone
ESR
Anti-nuclear antibody
Anti-ENA
TB Quantiferon gold
Hepatitis scr (HBsAG, Anti HBs,Anti HBc, Anti HCV, Anti HAV, HCV RNA Quant)
HTLV - ½

4.4 Baseline

The following evaluations take place within a 12-week period of time and include:

a. History and physical examination
b. Standardized neurological examination
c. Clinical blood draw and laboratory testing
   - Table 6: Baseline laboratory evaluations

Laboratory Testing
CBC/diff/plts
Hem A1c
PT/PTT
Pregnancy test (urine or serum)
Fasting Lipid panel
Acute Care Panel/Hepatic Panel/Mineral Panel
amylase/lipase
TSH
CRP
HIV RNA Viral Load
TBNK
Adrenocorticotropic Hormone
ESR
Anti-nuclear antibody

Anti-ENA

d. Research blood draw
e. MRI brain with and without contrast
f. Brain FDG-PET/CT
g. Lumbar Puncture

4.5 Study Procedures

Each study visit and procedure occur as indicated in Table 4 and Appendix A. These time points are approximated. To accommodate for patient scheduling needs or clinical necessity, study visits are accepted within a +/- 1-week window of the time points noted. Clinical history, physical and neurological examination are expected to last no more than 2 hours and are for clinical care and research. The week 3 neurological and physical examination are required and the week 52 timepoint (or its equivalent ending) should be completed. Lumbar puncture is expected to last no more than 1 hour and are for clinical care and research. The lumbar puncture is required at baseline and at the week 3 timepoint. The third lumbar puncture is optional and could be deferred to the week 52 timepoint as the long-term antibody/immune response at the 24 and 52 week mark are likely to be similar. Leukapheresis is optional and may be performed at baseline, week 3 and week 24. Interim examinations do not impact outcome measures and are highly unlikely to impact subject safety. If interim examinations cannot be completed, this will not adversely affect the study or safety of the participants. Brain FDG-PET/CT are accepted within a +/- 2-week window of the time points noted due to scheduling restraints and prior brain FDG-PET/CT within 12 months is accepted as the baseline scan. FDG-PET/CT lasts no more than 3 hours. FDG-PET/CT scan at baseline and week 3 is for clinical care and research. MRI studies of the brain are performed at baseline, week 3 and 24 for both clinical care and research. Brain MRI is expected to last no more than 2 hours. For women of childbearing potential, urine or serum pregnancy testing is performed at screening, baseline, on the day of pembrolizumab administration, and at each follow up visit. Blood draws occur approximately every three to six weeks for the first 36 weeks of the trial (64 weeks), with a 1-year safety visit, not to exceed Clinical Center guidelines. Safety labs can be drawn according to the trial schedule with an NIH contracted commercial laboratory if preferred by the patient at visits week 12 and 18. Blood draw is expected to last no more than 1 hour. We draw no more than 30 teaspoons of blood at any one time. We draw no more than 5.86 cups or 277 teaspoons of blood during the entire study. As specified in Table 4, blood draws are for both clinical care and research at the time points noted. All laboratory results are reviewed by NIH clinicians in a timely manner upon receipt. Visits at week 12 and 18 include a phone visit for interval history from an NIH clinician if the patient chooses not to come to the NIH clinical center. Screening may be done remotely. Baseline and visits at week 0, 3, 6, 24, and 52 must be at the NIH clinical center. Screening visits occurred prior to the 90-day SMC review, but participants did not begin baseline timepoint until after a 90-day post-drug safety review that allowed further study drug dosing of participants.

Two forms of contraception are required from study enrollment until 4 months' post-study drug when pregnancy is possible. In light of the potential risk of pembrolizumab to a developing fetus, participants are counseled at each study visit concerning the importance of consistently using two forms of effective birth control.

Participation is staggered with interim review of safety and futility. An initial cohort of 4 study patients who have completed week 0 – study drug visit, and an SMC review of the sentinel 90-day post-drug safety data was required prior to additional subject enrollment. Given that the SMC review was favorable, the trial continued to enroll the remaining study subjects. Futility was evaluated at the sentinel safety data review prior to continuing study enrollment.

4.6 Neurologic Evaluations

History and physical examination is performed at baseline and visits specified in Appendix A along with neurological examination. This procedure lasts approximately 1-2 hours.

4.7 Laboratory evaluations

4.7.a Safety blood draw:

Blood is obtained at each visit specified in Appendix A for patient safety.

Table 7: Safety laboratory testing

Laboratory Testing
CBC/diff/plts
Hem A1c
PT/PTT
Pregnancy test (urine or serum)
Fasting Lipid panel
Acute Care Panel/Hepatic Panel/Mineral Panel
amylase/lipase
TSH
CRP
HIV RNA Viral Load
Adrenocorticotropic Hormone
ESR
Anti-nuclear antibody
Anti-ENA
CD4/CD8 counts*

- Or lymphocyte TBNK, interchangeable laboratory evaluations.

4.7.b Additional research blood draw:

Research blood is collected at the baseline visit and subsequent study visits listed in Appendix A. This is used for immunophenotypic analysis and to characterize the cell mediated and humoral immune responses, as well as virology. Any remaining serum or plasma is preserved at less than -60 degrees C for future immunological testing.

Additionally, up to 120 ml of whole blood in syringe with preservative-free heparin (or an equivalent amount in standard research tubes) may be drawn at each research blood time point for immunologic and virologic testing. When 120mL of whole blood is drawn, it occurs on a day separate from other research laboratory testing to comply with clinical center blood draw limits and guidelines if the amount of research blood in additional to

clinical lab blood draw is expected to exceed Clinical Center guidelines, or the amount of whole blood in the syringe to be drawn on the same day is adjusted to abide by clinical center blood draw guidelines. No more than 550mL is drawn in any 8-week period. Lymphocytes are isolated from this sample and are used to quantify viral RNA expression and/or T-cell responses to HIV antigens, as well as other virologic and immune testing. This includes but is not limited to immune testing and may include measurement of circulating bulk T, B-lymphocyte and NK cell populations and peripheral blood as well as enumeration of HIV-specific CD8+ T cells by HIV tetramers and/or cytokine secretion and the capacity of CD8+ T-cells to proliferate in response to HIV antigens. Any remaining lymphocytes are cryopreserved and stored in liquid nitrogen for additional ancillary immunological and virologic testing.

Table 8: Research Laboratory Testing

Blood Lymphocyte Phenotype TBNK
Flow Cytometry (CSF and blood)
Blood HIV-specific T-cell responses
Luciferase Immunoprecipitation Assay (LIPS) – mandatory at CSF paired timepoints, optional at non-CSF paired study visits
Single copy HIV RNA analysis - mandatory at CSF paired timepoints, optional at non-CSF paired study visits

4.7.c *<u>Lumbar puncture:</u>*

A lumbar puncture is done before drug administration at the baseline visit and at week 3 study visit as listed in Appendix A. There is an optional lumbar puncture at the 24-week study visit. Up to 25 mL of CSF is collected and may be evaluated for HIV-1 RNA, cell count and differential, total protein, glucose, IgG indices, flow cytometry and the presence of HIV-1 antibodies, standard CSF pathogens, CSF immune profile, and/or cytokine profile. Additional testing can be performed as necessary for clinical or research confirmation. Lymphocytes may be isolated from the CSF and used for virologic and immune testing as described in additional research blood draw. This procedure lasts from 30 minutes to 2 hours. The lumbar puncture is usually done on an inpatient or day hospital floor of the Clinical Center. The procedure may be performed under fluoroscopic guidance by a credentialed neuroradiologist in Diagnostic Radiology, if there is a patient medical or scheduling need to do so. This involves a small amount of ionizing radiation (0.023 rem per year) per procedure. This is substantially less than the limits imposed by the guidelines of the NIH Radiation Safety Committee (5.0 rem per year for adults). All persons of child-bearing potential will have a pregnancy test performed within 24 hours prior to any LP being performed under fluoroscopy. Sedation with low-dose benzodiazepines may be used for subjects with anxiety during the LP.

4.7.d Leukapheresis:

The patient may undergo leukapheresis (generally, 2 liters of whole blood will be processed to target a yield of 1-2 x10e9 mononuclear cells) in the Department of Transfusion Medicine (DTM) Dowling Apheresis Clinic according to DTM standard operating procedures. The procedure requires dual venous access and takes approximately 3-4 hours to complete. The procedures are carried out in such a way that the extracorporeal volume is no more than 15% of the blood volume in adults. Lymphocytes are isolated from this sample and used to characterize HIV biology including assessment of live virus reservoir when possible, as well as other virologic and immune testing. This includes but is not limited to immune testing and may include measurement of circulating bulk T, B-lymphocyte and NK cell populations and peripheral blood as well as enumeration of HIV-specific CD8+ T cell killing and/or cytokine secretion and the capacity of CD8+ T-cells to proliferate in response to HIV antigens. Any remaining lymphocytes will be cryopreserved and stored in liquid nitrogen for additional ancillary immunological and virologic testing.

4.8 Neuroimaging evaluations

4.8.a Magnetic resonance imaging (MRI):

MRI of the brain is completed at the baseline visit (for applicable participants), week 3 and week 24 Study Visits. MRI is performed with FDA-approved contrast (typically gadobutrol) of the cerebrum MRI of the brain lasts 1-2 hours. A macrocyclic gadolinium chelate is used in this study, and NIH Clinical Center Radiology and Imaging Sciences guidelines for gadolinium administration are followed.

Under certain circumstances, participants in this study may receive a linear chelate. Examples of situations in which this may occur include the following: (1) prior sensitivity to all macrocyclic agents; (2) participation in a longitudinal study in which a linear chelate was previously given, there is a clear advantage to maintaining the same gadolinium protocol, and T1 signal change in the basal ganglia or dentate nuclei was not observed; (3) after consultation with, and approval by, a radiologist credentialed at the NIH Clinical Center.

An FDA approved gadolinium guide is available on the FDA website: https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-warns-gadolinium-based-contrast-agents-gbcas-are-retained-body. Participants are given a copy of the gadolinium guide.

Sedation with a low-dose benzodiazepine may be used for subjects with anxiety during the MRI.

4.8.b Positron Emission Tomography/ Computed Tomography (PET-CT):

Brain ^{18}FDG-PET/CT is completed at the baseline visit and repeated at the 3-week study visit to assess for CNS and HIV-reservoir site inflammation. PET/CT is performed with FDA-approved glucose analog 2-deoxy-2(^{12}F) fluoro-D-glucose (FDG). For patients who have

had a brain FDG-PET/CT in the prior 12 months, this scan can serve as the baseline neuroimaging study. FDG-PET/CT of the brain lasts no more than 3 hours.

4.9 Drug dosing

Pembrolizumab is dosed at the recommended 200mg, per FDA guidelines for approved conditions. Prior data on our HIV-positive patients with progressive multifocal leukoencephalopathy (PML) who received pembrolizumab for the treatment of JC virus reactivation show that PD-1 saturation on T-cells is present for up to 4-6 weeks post 2mg/kg infusion (data not published).

4.10 Drug administration

Pembrolizumab 200mg is administered as a one-time intravenous infusion over 30 minutes during the treatment phase of the study. In our experience the study drug is very well tolerated with 650mg of oral acetaminophen and 25mg oral diphenhydramine 30 minutes prior to infusion. Unless there is a contraindication to one or either of these medications, all study subjects receive pre-medication 30 minutes prior to the pembrolizumab infusion due to the 0.1% risk of infusion reactions seen in clinical trials.[31] Post-infusion, patients are observed for safety purposes in the clinical center and admitted overnight if indicated for drug-related infusion side effects to be discharged the following day.

4.11 End of participation

Following the completion of study procedures or withdrawal from the study, patients will receive ongoing care by their primary neurologist or infectious disease physician. Patients who self-referred to NINDS (for example, by finding the study listing on the NIH Clinical Center website) will be given a list of outside neurologists and/or infectious disease specialist after the initial evaluation if they do not have outside HIV management. Should any patient decide to discontinue participation, information relevant for the management of the patient's condition will be discussed with the patient and the primary physician, and any treatment recommendations will be made. All patients will be discharged to their primary care physician for long-term management. Patients will be given study results that are pertinent to their ongoing clinical care.

4.12 Disease community engagement

We plan to continue to engage the community and to disseminate trial results by engaging and presenting data to the ACTG community, to the NIMH Center Series, to the NIAID VRC HIV Cure Meetings, to the NIH Intramural Neuro-HIV retreat, at CROI and at the AAN.

5. Management of Data and Samples

5.1 Storage

All collected samples, including serum; blood, CSF, and peripheral blood mononuclear cells (PBMC) derived from patients, are coded and stored in secured freezers on the NIH campus or at the NCI at Frederick Central Repository. NIH samples are transferred and retained at the end of the study under a repository protocol. Loss or destruction of samples will be reported to the IRB.

Study data is collected and stored in a 21 CFR Part 11 compliant database.

5.2 Data and sample sharing plan

Data and samples may also 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. Repositories receiving data and/or samples from this protocol may be open-access or restricted access. The intent is for all samples to be analyzed in laboratories on the NIH campus or associated contract laboratories. Cell lines may be shared with other laboratories in a manner similar to that described previously.

Samples and data are 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 is not be provided to collaborators, but remains at NIH. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Imaging data without any personal or identifying information attached may be shared with other investigators or public data repositories in the spirit of the Final NIH Statement on Sharing Research Data (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.htmL). This provides the research community with open access to datasets contributed by labs around the world. Information is either unlinked or coded, with demographics limited to age (accurate to the year), sex (male, female), group membership (e.g., disease/treatment state) and handedness. For coded data, the investigators retain the key to the code, which is maintained on secured computers at NIH. Data is transferred using secure file transfer protocols.

For subjects enrolled in 13-N-0149, data and samples may be shared between investigators of 13-N-0149 and the present study. The data and samples may be shared with patients names and identifying information. Sharing these data and samples helps minimize patients need to repeat procedures if data or samples are already collected, as they are co-enrolled in both studies.

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

6. Additional Considerations

6.1 Research with investigational drugs or devices

This study involves the use of Pembrolizumab, an FDA-approved medication for the treatment of non-resectable or metastatic melanoma, as well as refractory non-small cell lung cancer. This study was granted IND 133900 on May 25th, 2017.

6.2 Gene therapy

This study does not involve gene therapy

6.3 MRI

The following MRI scanners (NIH CC Radiology and Imaging Sciences Department) are used in this study:

3.0 Tesla (Siemens)

- Device model: Prisma
- Description of each component: FDA-approved head coil, pulse sequences, software and monitoring equipment
- This device is classified as an FDA approved device (#K173592). This device is being used as marketed.

3.0 Tesla (Philips)

- Device model: Achieva
- Description of each component: FDA-approved head coil, pulse sequences, software and monitoring equipment
- This device is classified as an FDA approved device (#K190461). This device is being used as marketed.

6.4 PET/CT

The PET/CT is classified as an FDA approved device. This device is being used as marketed, and as per FDA approved indications.

7. Risks and Discomforts

The most common adverse reactions to pembrolizumab (reported in ≥ 20% of cancer patients) include fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia and diarrhea.[32] These are further elaborated on in Tables 3 and 4.[31, 32]

As pembrolizumab is a humanized monoclonal IgG4 kappa immunoglobulin there is a potential risk of allergic reactions, including anaphylaxis. If infusion toxicities occur, the infusion will be stopped. The patient will be examined by a study investigator at the time of event.

All patients are informed as to the 15-20% risk of the development of autoimmunity that was seen in patients with either melanoma or non-small cell lung cancer on repeat dosing as listed in table 1.[31] There is also a theoretical risk of immune reconstitution inflammatory syndrome, although no reports exist in the literature to date.

These immune-mediated adverse reactions that may require corticosteroid treatment and/or other supportive therapies. They include the following autoimmune conditions.

- Pneumonitis
- Colitis
- Hepatitis
- Hypophysitis
- Nephritis
- Hyperthyroidism and Hypothyroidism

All patients are advised to report new or worsening cough, chest pain, shortness of breath, diarrhea, severe abdominal pain, jaundice, nausea, vomiting, easy bruising or bleeding, persistent or unusual headache, extreme weakness, dizziness, fainting, vision changes, bleeding in the urine, urinary pain, hair loss, heat or cold intolerance, changes in sweating patterns, severe fatigue, or

changes in weight loss or weight gain as these are frequent symptoms of the above listed autoimmune conditions.

All male participants are advised that pembrolizumab may cause fetal harm. Sexually active participants are instructed to use highly effective contraception, with participants agreeing to use two methods of control including one barrier method, during and for 4 months after the last dose of pembrolizumab, if there is even a remote possibility of fathering a pregnancy.

7.1 Risks of neurological evaluation

There may be minimal discomfort associated with the clinical examination.

7.2 Risks of blood drawing

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

7.3 Risk of intravenous line

There may be some discomfort and bruising at the site of needle entry. Some people may feel light-headed or faint. There is a small risk of bleeding, infection or local inflammation of the skin and/or vein with possible pain and/or swelling.

7.4 Risks of MRI

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner or tattoos, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye, of which they may be unaware. All subjects are screened for these conditions prior to the study, and if they have any, they will not receive an MRI scan.

7.5 Risks of intravenous gadolinium contrast

Symptoms from the contrast infusion are usually mild and may include feeling hot, burning, or coldness in the arm during the injection, a metallic taste, headache, allergic reactions and nausea. In an extremely small number of individuals, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. Unless specifically allowed by the protocol, participants will not receive gadolinium-based contrast agents for research purposes if they have previously had an allergic reaction to them. Individuals with a history of anaphylaxis to other agents or chronic asthma requiring treatment will not receive gadolinium under this protocol unless they have previously received gadolinium and tolerated it well. Participants are asked about such allergic reactions and history of asthma before a contrast agent is administered.

People with kidney disease are at risk for a serious reaction to gadolinium contrast called “nephrogenic systemic fibrosis,” which has resulted in a very small number of deaths. If subjects are 60 years old or greater or have diabetes, kidney disease or liver disease, blood work to assess kidney function will be performed within 4 weeks before any MRI scan with gadolinium contrast. Participants may not receive gadolinium for a research MRI scan if kidney function is not normal. There is no evidence for the potential of gadolinium-related toxicity in people with normal kidney

function. This protocol follows NIH Clinical Center guidelines for kidney-function screening related to gadolinium administration.

Most of the gadolinium contrast is eliminated in the urine. However, recent studies have found very small amounts of residual gadolinium in the body, including the brain, by imaging and at autopsy. Macrocyclic gadolinium-containing contrast agents are substantially less likely to leave gadolinium behind than linear agents. The use of macrocyclic vs. linear agents in this study is delineated in the procedures section above. There is presently no evidence that the retained gadolinium is associated with any adverse effects.

7.6 Risks of lumbar puncture

Adverse effects associated with lumbar punctures include brief pain or tingling paresthesia radiating down the lower extremity due to the needle brushing against a nerve. Should this occur, the needle can be repositioned. Mild lower back pain at the site of needle insertion following the procedure can occur; this can be managed with over the counter non-steroidal anti-inflammatory agents if needed. In approximately one third of patients, a post-dural puncture headache may develop and persist for a few days; in one in 50 to 200 lumbar punctures the post-dural puncture headache can last longer than 7 days. Generally, this headache is not severe and resolves spontaneously within days - 2 weeks. Should the headache persist or be severe, a blood patch can be performed. An extremely rare complication of lumbar puncture includes temporary double vision related to abducens nerve palsy, and infection. Strict aseptic technique is followed. Collecting additional 10-15 mL of CSF per procedure represents negligible risk [35]. In humans the rate of CSF synthesis is approximately 21.5 mL/hour [36], or approximately 500mL/24hours, which represents roughly 4 times the total volume of CSF in an adult patient. Therefore, the volume of 20-25mL of CSF that would be collected for both diagnostic and research purposes will be replenished in its entirety within approximately 1-1.5 hours after collection.

7.7 Risks of sedation

Subjects may be sedated for the MRI or lumbar puncture. Benzodiazepines cause sedation. Other possible side effects include confusion, dizziness and agitation. Monitoring is required in clinic after benzodiazepine administration and staff ensure that patient has a designated ride home, either through staff-arranged taxi or with a driver designated by the patient.

7.8 Risks of PET/CT scanning

There are no known physical hazards to subjects from being inside the PET/CT scanner, which detects injected radioactivity within the body. Additionally, the scanning procedures are conducted in the presence of trained staff, should subjects experience any discomfort and require medical attention. Subjects can communicate with the staff while in the scanner and can withdraw from the study at any time, if they wish to do so. Occasionally subjects become anxious during the scanning procedure, in which case, they can request the operator to stop the scanning and to be removed from the scanning bed.

7.9 Risks of Fluorine-18-fluorodeoxyglucose

Fluorine-18-labeled fluorodeoxyglucose (FDG) is a radioactive glucose analog used for metabolic imaging. After intravenous injection, FDG is taken in organs proportionally in regard to glucose metabolism. The brain has the highest uptake of FDG. Imaging is recommended to begin after an uptake period of 1 hour. The principal route of excretion of FDG is through the urinary system. The dose of FDG administered is approximately 10 mCi per scan.

7.10 Risks of Radiation Exposure

PET/CT scans require exposure to radiation. All tests requiring subject exposure to ionizing radiation are considered potentially hazardous. A detailed breakdown of the radiation exposure risks expected for the subjects enrolled in this protocol are included in the NIH 88-23(a) Application for Authorization to Use Radiation in Research Involving Human Subjects. The radiation received conforms to the NIH Radiation Safety Committee guidelines for subjects participating in research studies, defined as a 5-rem effective dose in 12 consecutive months. The total radiation exposure from two PET/CT from this protocol in one year is 1.39 rem. Lumbar puncture may be done in the radiology department under fluoroscopic guidance for patient medical or scheduling needs. The total radiation exposure during the two procedures is 0.05 rem and for three procedures is 0.075 which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

The total radiation exposure that a participant may receive through participation in this study is 1.47 rem, which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

7.11 Risks of Premedication

Premedication with standard dose diphenhydramine (Benadryl) and acetaminophen (Tylenol) is provided to study participants prior to receiving study drug. The risks of receiving diphenhydramine include drowsiness, dry mouth, urinary retention and constipation. The risks of receiving acetaminophen include allergic reaction, skin rash and liver injury.

7.12 Risks of Leukapheresis

The potential risks associated with leukapheresis include lightheadedness, dizziness, possible fainting, tingling around the mouth and in the fingers and toes, nausea, chills, vomiting, mild muscle cramps, loss of <1 pint of blood, or pain, bruising, or discomfort at the needle insertion sites. Most procedures are performed without an incident. Blood components removed during leukapheresis are generally replaced by the body within a few hours or a few days. Temporary or permanent nerve damage may occur at the needle placement sites. There is also a risk of infection. These are very rare. To date, there have been no cases of permanent nerve damage or infection with apheresis at the NIH.

8. Subject Safety Monitoring

The NIH PI and DSMB are responsible for the safety of the participants and for monitoring and reporting any unanticipated problems or adverse events. The study personnel closely monitor patient safety during study procedures. Credentialed clinical staff monitor all contrast-enhanced MRI scans in accordance with NIH NMR Center policies.

The PI is responsible for overall study safety monitoring. The DSMB serves as the IND sponsor's medical monitor and makes the final determinations of relatedness on behalf of the sponsor (NINDS). The DSMB is charged with reviewing all AEs at a bi-yearly meeting. The DSMB will be given reports as soon as possible but within 24 hours of a known grade 3 or higher AE, or an SAE, on an "ad hoc" basis. The DSMB will provide feedback to the study PI and NINDS within 7 days of received "ad hoc" reports, as well as after each bi-yearly meeting. Participants will be removed from the study if they are unable to cooperate with the study procedures, if a medical exclusionary condition develops, or if the principal investigator judge's discontinuation to be in the participant's best medical interest. If a patient experiences neurologic decline or other clinical conditions requiring immunomodulatory and immunosuppressive drugs including steroids during the trial, they will be discontinued from the trial and will receive standard medical care.

8.1 Expected Adverse Events

There is a potential for autoimmunity with cancer dosing protocols using pembrolizumab. The most frequently and consistently experienced adverse reactions (in at least 20% of patients taking the drug for refractory cancers) reported are fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia and diarrhea.

8.2 Monitoring of adverse events

Patients will be instructed to contact the study investigators and/or seek medical care if they have any questions or concerns. The patient will be instructed to contact the investigator immediately should he/she manifest any new sign or symptom or change in their condition during the period extending from the time of dosing to and including the final study visit at Week 52. Adverse events will be documented according to the criteria outlined in Section 25.4.

Additionally, the patient is instructed to contact the investigator immediately if she becomes pregnant or if he believes that he has fathered a child. Testing would be performed to verify that the subject or subject's partner was pregnant. If the pregnancy test were positive, the study subject and their partner would be offered consultation with an OB/GYN at the NIH for counseling. Subjects and their pregnant partners would be instructed to contact the investigator with any abnormalities experienced during their pregnancy, in the event of fetal loss, or if fetal malformations were present. Additionally, information on any live-birth children up to 12-week post-partum would be collected when possible.

Patients are physically examined by the investigators or patient's physicians approximately every 3 to 6 weeks for the study duration until study visit week 24, except at visit week 12 and 18 as noted in Appendix A. Laboratory safety monitoring occurs every 3 to 6 weeks for the until study visit week 24 as listed in Appendix A.

8.3 Toxicity tables/ criteria to be used

Toxicity is assessed according to the U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Available from:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

8.4 Treatment of adverse events

Treatment of any AE is according to current available best treatment. Treatment of infusion reactions and immune-related adverse events will be treated based on standard of care using Merck protocol guidelines as a reference, included in Section 25.3, Appendix C. The applied measures will be recorded in the patient's chart. All AEs occurring from the time of study drug dosing through the study follow-up period of 1+ years are documented, recorded, and reported as outlined in section 25.4.

8.5 Criteria for Infusion-Related Toxicity

If infusion-related toxicities occur, study drug will be discontinued. If infusion related toxicity occurs, subjects will be monitored until toxicity resolves to less than Grade 1.

8.6 Criteria for individual subject withdrawal

A subject will permanently discontinue from the study if the subject desires to discontinue study drug monitoring under this protocol. Patients have the right to withdraw from the study at any time and for any reason. The investigator also has the right to withdraw patients from the study in the event of an intercurrent illness, adverse events, protocol violations, administrative reasons, or if it is in the best interest of the participant. However, all subjects wishing to withdraw from the study after receiving study drug will be invited to stay in the study to be monitored with clinical safety measures only (including clinical blood draws, neurologic and physical examinations, as well as MRI and FDG-PET studies), forgoing all further research testing from that timepoint forward. Patients who wish to withdraw fully from the study will be considered non-completers and will not be followed after the point of study withdrawal. Subjects who withdraw prior to receiving study drug will be considered dropouts and can be replaced.

In the event that the subject or partner of an enrolled subject becomes pregnant within 4 months of the subject receiving study drug, information about the subject, the subject's partner's pregnancy, the outcome of the pregnancy, and the status of the infant at 8 to 12 weeks of age will also be collected. Participants will receive counseling with an OB/GYN consult.

The reasons for discontinuation of the study drug must be recorded in the subject's Clinical Center patient file. Patients withdrawing before receiving trial drug are discontinued from the study without further follow up. Patients withdrawing after receiving trial drug are offered clinical follow up for the study duration of 26 weeks, and, if they choose to remain enrolled will be followed and all adverse events up to 1 year will be reported and documented in the Clinical Center records. If discontinuation or withdrawal was because of an adverse event grade 3 or higher, the patient will be offered to stay in the trial for clinical follow up only until the event is resolved or stabilized for up to 1 year if patient received study drug. Patients who decline clinical follow up when offered will be withdrawn from the trial with no further follow up.

Participants who withdraw voluntarily after initiation of the study but before receiving study drug are considered dropouts. These subjects can be replaced.

Patients who voluntarily withdraw after receiving study drug but before the 3-week study visit will be considered non-completers.

9. Outcome Measures

9.1 Primary outcome measures

Frequency of grade 3 and higher AE that are definitely or probably causal to pembrolizumab.

9.2 Secondary outcome measures

i. Change in HIV-specific antibody responses in the CSF and serum using LIPS assay.
ii. Change in CSF cytokine profile post-study drug.
iii. Peripheral CD4+ counts.
iv. HIV RNA in plasma and CSF.
v. Change in FDG-PET/CT metabolic uptake in the CNS.
vi. Change in PD-1 expression in the CSF and blood cells.

10. Statistical Considerations

10.1 General design

This is a proof-of-concept, single-arm, phase 1 safety study of pembrolizumab given as a single dose of 200mg. Each participant is followed for a total of 52-64 weeks for evaluation. This study will also collect CSF and plasma data at baseline and week 3 after treatment to assess immunologic responses against HIV after PD-1 blockade.

10.2 Analytical plan

The primary objective of the study is to describe the safety of pembrolizumab in patients with HIV. The analytical plan will be descriptive and will only include data starting at the baseline data point. Proportions of adverse events will be reported along with exact 95% confidence intervals using the Clopper-Pearson approach (the binomial test function in R will be used for the calculations). Any AE will also be reported.

The secondary objective of the study is to assess the effects of pembrolizumab on various immunologic responses. Paired t-tests (one-sided at 5% significance) will be used to test fold increase (on log scale) of the assays before and after treatment. For example, for the antibodies to Tat, we will test the null hypothesis that the mean log-fold-increase will be log (1.2) versus the alternative that there is no increase in Tat over the 3-week post-treatment period. In addition, 95% confidence interval for the log fold change will be calculated to describe the variability of the endpoint. As a sensitivity analysis, we will also perform a one-sided Wilcoxon signed rank test for the null hypothesis that the log-fold-change is symmetric about log (1.2) at 5% significance. The signed rank test does not require normality of the log-fold-change (as the t-test does) and will ensure the test results are robust.

We will also use the Berlin patient's antibody levels as a benchmark for the post-treatment antibodies of the study participants. Specifically, we will calculate the 95% confidence interval for each antibody mean and compare the interval to the Berlin patient's average values for the antibody against each of the following proteins: gp41, gp120, matrix, p24, nucleocapsid, reverse transcription, integrase, protease, and tat.

Other secondary endpoints will be analyzed in a similar manner. For change in PD-1 expression, change in CSF cytokine, and change in FDG-PET/CT uptake, paired t test and signed rank test will be conducted. For all variables, 95% confidence intervals for the log change will be calculated. When data are available for the Berlin patient, we will also compare the confidence interval of these secondary endpoints to that of the Berlin patient.

10.3 Sample size and power considerations

We plan to enroll a total 20 subjects and expect 12 patients will receive study drug to have complete follow up for safety (12 months) and CSF measurements (3 weeks).

The sample size of this study was determined based feasibility, while aiming to enable the study team to collect data to decide on the next steps—in two ways: (1) the primary analysis will provide descriptive statistics of the AE rates, and (2) the secondary analysis will provide estimate of variability of the antibodies. These data are useful for the planning of a proof of concept phase 2 study where safety will continue to be monitored.

In addition, we aim to be able to identify any unexpected severe AEs that may warrant trial stopping. Specifically, with 12 patients, there is about 86% probability to detect any unexpected severe AEs that occurs at a rate of 15% or higher. This probability calculation provides an assurance for detecting SAEs that are unexpected that may occur at a high rate (i.e. 15%). We note that this is not intended to serve as a hypothesis test, but rather a monitoring mechanism.

We will use the data to assess the variability in the antibody response and detect possible signals. For example, for the anti-Tat antibody analysis, we assume that the standard deviation of a log-fold-increase is 0.25, based on Burbelo et al.[13] Under this assumption, the one-sided t-test at 5% level (described above) will have 81% power to conclude that the mean log-fold-increase in less than 1.2 under the alternative of a unity fold change. Under the same assumption, a 5% Wilcoxon signed rank test (one-sided) has 74% power. These calculations indicate that the pilot will be able to provide data on the variability in these antibodies and therefore to understand the treatment effects (clinically important difference divided over variability), if not demonstrate statistical significance.

11. Human Subjects Protection

11.1 Subject selection

All adult patients with HIV-1 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 are 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 exclusion of children

Due to the complex nature of this clinical trial, the greater than minimal risk, and the off-label use of pembrolizumab, children are not allowed to participate in this trial.

11.3 Justification for exclusion of other vulnerable subjects

Due to the complex nature of this clinical trial, and the off-label use of pembrolizumab, those who are not able to provide informed consent are not allowed to participate in this trial because the goals of the study can be achieved without by enrolling only those with capacity to consent, therefore the risks outweigh the benefits for this population.

11.4 Justification for sensitive procedures

There are no sensitive procedures in this trial.

11.5 Safeguards for vulnerable populations and sensitive procedures

Because pembrolizumab is rated as a category D drug in pregnancy with evidence of adverse effects in animal reproductive studies and absence of controlled studies in humans, participants are required to use appropriate methods of contraception for the trial. Women over age 55 who have not had a period for one year are considered menopausal and do not need pregnancy testing or contraception. Women under the age of 55 are excluded unless there is a history of surgical menopause, tubal ligation or other history of surgical and/or medical sterility.

12. Anticipated Benefit

Indirect benefit(s): This study does not offer direct benefit to participants but is likely to yield generalizable knowledge about HIV viral reservoirs and will inform future clinical trials aimed at curative interventions.

13. Consent Documents and Process

13.1 Designation of those obtaining consent

Study investigators designated as able to obtain consent in the study personnel page, obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

13.2 Consent procedures

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place.

In the event that circumstances beyond our control preclude the travel to the NIH Clinical Center for a visit, or otherwise would put their health at greater risk (i.e. epidemic or pandemic), we will endeavor to establish capabilities for remote consent. The initial consent process as well as re-

consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator with the agreement of the participant.

A physical copy of the consent form may be sent to the subject if a telephone consent process is used. The consent interview may then be conducted when the subject can read the consent form during the discussion. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

Consent will be documented with required signatures on the physical copy of the consent (which includes the printout of an electronic document sent to the participant), or on the electronic document. The process for documenting signatures on an electronic document is described below.

When an electronic document is used for the documentation of consent, this study will use the iMedConsent platform which is 21 CFR, Part 11 compliant to obtain the required signatures. During the consent process, participants and investigators will view the same document simultaneously in their respective locations.

The identity of the participant will be determined by a prompt which will require the provision of information from an official identification document, prior to obtaining the signature. Both the investigator and the participant will sign the electronic document using a finger, stylus or mouse.

A copy of the informed consent document signed and dated by the subject are given to the subject. Confirmation of a subject's informed consent is documented in the subject's medical records prior to any testing under this protocol.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant. During the consent process, participants and investigators will view the same approved consent document simultaneously in their respective locations.

Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult

room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

The investigator obtaining consent documents the consent process in the participant's medical record. All instances of use of the CC Short Written Consent Form are reported to the IRB at the time of annual review. Interpreters are present for other protocol procedures as necessary.

13.3 Consent documents

The consent form contains all required elements. The consent form is attached in iRIS.

14. Data and Safety Monitoring

14.1 Data and safety monitor

Data and safety is monitored by a Data and Safety Monitoring Board (DSMB).

14.2 Data and safety monitoring plan

Parameters that will be monitored for the study as a whole:

a. MRI brain
b. FDG-PET/CT
c. Clinical and laboratory data

The clinical data, including clinical laboratory parameters and physical examination with MRI results are reviewed by the study investigators weekly in a clinical care meeting. If a clinical care meeting is not able to be scheduled due to holiday, conflicting schedules, or inclement weather, the PI will review the clinical data within one week of the patient's visit. Additionally, research data will be reviewed within one week of acquisition in weekly laboratory meeting, or at the clinical care meeting.

The DSMB serves as the sponsor's medical monitor and will make the final determinations of relatedness on behalf of the IND sponsor (NINDS). The DSMB is charged with reviewing all AEs at a bi-yearly meeting. This meeting may take place in person, as a tele-conference, web-meeting or via email. The DSMB will operate under the rules of an approved charter. Reviewers will have access to unblinded and interim study data and will be given a pre-meeting report a week prior to the scheduled interim bi-yearly meetings. After the bi-yearly interim meetings and the 90-day safety meeting, the DSMB chair will provide a report to the study PI and NINDS Office of the Clinical Director within 14 days.

The DSMB will be given reports within 24 hours of a known grade 3 or higher AE, or SAE, on an ad hoc basis. The DSMB will be given reports within 7 days of any known grade 2 AE involving the following irAE diagnoses: Pneumonitis, Colitis, Hepatitis, Hypophysitis, Nephritis, Hyperthyroidism and/or Hypothyroidism. The DSMB will provide feedback to the study PI and the IND sponsor (NINDS) within 7 days of received ad hoc notifications.

14.3 Criteria for stopping the study or suspending enrollment or procedures

The study will be stopped/suspended and DSMB review will be triggered if one or more subjects develop:

- Any SAEs deemed to be possibly, probably, or definitely attributable to study medication by the Investigator and/or the data and safety monitoring board.
- One grade 4 AE or lab abnormality deemed to be possibly or probably related to the study medication by the Investigator and/or the safety monitoring committee.
- Two grade 3 AEs or lab abnormalities that are similar in nature and deemed to be possibly or probably related to the study medication by the Investigator and/or safety monitoring committee.
- An anaphylactic reaction deemed to be at least possibly related to the study medication by the Investigator and/or the safety monitoring committee.
- A pattern of significant symptoms, physical findings, or laboratory abnormalities (adverse events) that, although individually minor, collectively represent a safety concern in the opinion of the investigator and/or the safety monitoring committee and are judged to be at least possibly related to study medication. If this pattern is noted by the investigator during the infusion of study treatment, it should be stopped immediately and the subject should be further evaluated and treated.

The FDA will be notified immediately if the study is suspended for reasons of safety. In the event that the study is stopped or suspended, the DSMB will convene to review the interim study data until the time of stoppage or suspension. The study will resume only in the event that DSMB review determines an adverse event meeting the stopping criteria (significant symptoms, physical findings and/or laboratory abnormalities) to be unlikely due to or unrelated to study drug.

15. Quality Assurance (QA)

15.1 Quality assurance monitor

The PI and the IND sponsor's Contract Research Organization (CRO) perform quality assurance monitoring for this study.

15.2 Quality assurance plan

The PI and co-investigators meet monthly to review quality control on all data. Additionally, clinical data (including clinical labs and physical exam) collected on individual subjects is reviewed by study investigators within one week of each visit. A Contract Research Organization (CRO) provides on-site monitoring of this protocol. An initial visit was conducted by the CRO, following final approval of the protocol by the IRB and FDA. During the site initiation visit, the study team and the monitor determined the frequency of monitoring visits. The sponsor via the CRO, is responsible for providing adequate oversight of the investigation to ensure adequate protection of the rights, welfare, and safety of human subjects and the quality and integrity of the resulting data. The purpose of the QA audit is to assess compliance with applicable regulatory requirements, good clinical practice guidelines, NINDS policy, as well as to provide recommendations for improving the management of clinical research data.

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

Reportable events will be tracked and reported in compliance with Policy 801.

17. Alternatives to Participation

FDA approved medications to treat HIV infection is combination antiretroviral therapy. Patients will continue antiretroviral therapy on this trial. There is no FDA approved drug therapy for evaluation of a viral reservoir in HIV infection. The alternative to participate is therefore to remain on FDA approved therapy with no additional medication. The study drug is FDA approved for the treatment of specific refractory cancers, but not for the evaluation of HIV reservoirs.

18. Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators and their staff. 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.

All research activities are conducted in as private a setting as possible.

19. Confidentiality

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 receives 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 are kept in password-protected computers on the NIH campus. De-identified results from clinical trials will be posted on cctrials.gov

19.2 For stored samples

Data and samples are stored in secured areas. Samples kept at NIH are stored in a coded fashion in a locked room. Clinical data is stored on a secure NINDS server. Identifiable information is not released without the patient's signed release form, except when authorized by law.

20. Conflict of Interest

20.1 Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

20.2 Conflict of interest

There are no conflicts-of-interest to report.

21. Technology Transfer

A technology transfer agreement is in place for this protocol.

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the Technology Transfer office of NINDS has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

Samples and data derived from this protocol may be analyzed in conjunction with outside collaborators, and in these instances materials and data transfer agreements will be obtained, or existing agreements will be followed.

22. Research and Travel Compensation

Participants are compensated for research-related discomfort and inconveniences in accord with NIH guidelines. NIH provides partial travel to and from the Clinical Center in Bethesda, Maryland within the United States per established policy. Compensation rates are comparable to those at NIH and an escort fee is not provided. Compensation is prorated for parts completed if subjects do not complete the study.

Table 9. Subject Reimbursement.

	Inconvenience units	Pay for inconvenience	Time (h)	Pay for time	Total Occurrences	Pay	Pay for Study Completion
FDG-PET/CT	15	150.00	3	40.00	2	190.00	380.00

MRI	10	100.00	2	30.00	3	130.00	390.00
Lumbar puncture	12	120.00	2	30.00	3	150.00	450.00
Antecubital venous catheter	3	30.00			5	30.00	150.00
Leukapheresis	17.5	175.00			3	175.00	525.00
Gadolinium administration	2	20.00			3	20.00	60.00
Blood Draw	3	30.00	1	20.00	9	50.00	450.00
Clinic Visit	4	40.00	1	20.00	9	60.00	540.00
Total							2945.00

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