HIV-Associated Neurocognitive Disorder (HAND) and the Prospect of Brain-Penetrating Protease Inhibitors for Antiretroviral Treatment

Authors:

Arun K. Ghosh,[*,†]

Anindya Sarkar[†]

Hiroaki Mitsuya [≠,#,§]

[†]Department of Chemistry and Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907, USA;

[≠]Departments of Infectious Diseases and Hematology, Kumamoto University Graduate School of Biomedical Sciences, Kumamoto 860-8556, Japan;

[#]Center for Clinical Sciences, National Center for Global Health and Medicine, Tokyo 162-8655, Japan;

[§]Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

E-mails:
Arun K. Ghosh (* Corresponding author): akghosh@purdue.edu,

Anindya Sarkar: sarkar4@purdue.edu

Hiroaki Mitsuya: mitsuyah@helix.nih.gov

KEYWORDS: CNS penetration, blood-brain barrier penetration, HAND, cART, protease inhibitors, darunavir, bis-THF, antiretroviral agent, neurocognitive disorders, HIV-associated dementia

ABSTRACT:

The advent of combined antiretroviral therapy (cART) has dramatically improved HIV management and patient care of HIV-infected individuals. The cART regimen resulted in a significant reduction of HIV/AIDS-related mortality and greatly improved life expectancies of those patients with access to cART. However, among many HIV-related complications, neurocognitive dysfunction, known as HIV-associated neurocognitive disorder (HAND), has now been a major issue. While the cART regimen has been effective in reduction of HAND in many patients, the prevalence of HAND is increasing as HIV/AIDS patients live longer. HIV infection and its subsequent manifestation of HAND is complex. It is evident that the brain can serve as a sanctuary for HIV replication and HAND can remain in patients even with cART treatment due to poor blood-brain barrier permeability of the majority of current antiretroviral agents. Conceivably, cART needs to have improved central nervous system (CNS) penetration properties for effective treatment and possible prevention of HAND. Therefore, design and development of new antiretroviral agents that can penetrate into the CNS effectively, could block HIV replication and significantly reduce the viral load in the CNS. This may prevent HAND and related symptoms. HIV protease inhibitors (PIs) are a critical component of cART. Over the years, we have designed and synthesized a range of highly potent and novel PIs including the FDA approved drug, darunavir, which has been used as a first-line treatment. In an effort to improve CNS penetration, we have been involved in the design and development of potent PIs with improved *in vitro* brain penetration properties. Herein we provide a brief review that covers insights and discussion of HAND and our work on PI development to ameliorate HIV-associated neurocognitive disorders.

1. INTRODUCTION

The emergence of Human Immunodeficiency Virus (HIV) infection in the early 1980's and the resulting onset of acquired immunodeficiency syndrome (AIDS) swept across the continents. The HIV/AIDS epidemic resulted in an estimated 35 million deaths and orphaned millions of children. Current reports from the joint United Nations program on HIV and AIDS (UNAIDS) estimate that globally there were 36.7 million HIV-infected patients and there were about 1.1 million HIV/AIDS-related deaths at the end of 2015 [1]. The introduction of combined antiretroviral therapy (cART) in the late 1990's led to a dramatic improvement in the management of HIV/AIDS patients and their prognosis. Access to current antiretroviral treatment transformed HIV/AIDS from a life-ending disease to a manageable chronic ailment with near normal life expectancy [2, 3]. The primary target of HIV is the patient's immune system, however the central nervous system (CNS) is also affected at varying degrees. HIV causes a spectrum of neurocognitive dysfunction, also known as HIV-associated neurocognitive disorders (HAND) [4,5]. In particular, HAND has become a major concern for 40-50% of current patients with HIV infection and AIDS. HAND is characterized by impaired short term memory along with mental problems ranging from mild difficulty with concentration, impaired decision-making, and lack of coordination, to progressive dementia. There has been significant reduction of HAND for some patients receiving cART, however HAND has remained prevalent in many HIV-infected patients [6]. Several cohort studies indicated that HAND may persist despite virological suppression and immune recovery with cART regimen [7,8]. With the increase in longevity of HIV/AIDS patients, there is an increasing incidence of HAND and distal polyneuropathy [9]. Furthermore, certain antiretroviral agents also cause adverse effects on the CNS and the peripheral nervous system, and induce the immune restoration inflammatory syndrome (IRIS) [10,11]. In the following sections, we plan to outline cART limitations, current treatment options, and HAND pathogenesis.

Current cART regimens are far from ideal. HIV/AIDS patients need to take cART for the rest of their lives. Some of the major challenges of existing therapeutics are: (a) toxic drug effects, (b) poor patient adherence to complex drug regimens, (c) failure to reestablish otherwise normal immunological functions at an advanced stage of the disease, (d) an increased risk of cancers with prolonged survival, (e) immune reconstitution inflammatory syndrome (IRIS), and (f) CNS complications mainly due to extended survival of patients. Inadequate antiretroviral drug penetration into the CNS may be responsible for cART's failure to reestablish immunological state [12]. CNS complications accompanying HIV include HAND syndrome, vacuolar myelopathy, and certain peripheral neuropathies [10].

In 1981, AIDS was described clinically for the first time and neurocognitive dysfunction due to HIV infection was initially identified [13]. The term AIDS dementia complex was proposed in 1986 to describe the acquired and persistent cognitive decline demonstrated by

patients with HIV infection and AIDS [8, 14, 15]. In the present day, neurological complications occur in more than 40% of patients with HIV infection. At autopsy, about 80% of cases demonstrate evidence of neuropathological abnormalities [13, 16]. The most comprehensive and largest study investigating cognitive impairment in HIV is the CNS HIV AntiRetroviral Therapy Effects Research (CHARTER) cross-sectional cohort study that revealed cognitive impairment in 843 out of 1555 patients infected with HIV-1 [17]. Table 1 outlines a unanimous definition of HAND and was previously reported [18-20]. HAND includes the following classifications: asymptomatic neuro-cognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD).

Table 1. Stages of HIV-associated neurocognitive disorder (HAND) per the Frascati criteria [18-20].

	Average occurence in patients receiving cART	Neurocognitive status*	Functional status**
Asymptomatic neurocognitive impairment (ANI)	28%	Cognitive impairment observed in ≥2 domains (functioning ≥1 SD below normal)	No observations
Mild neurocognitive disorder (MND)	17%	Cognitive impairment observed in ≥2 cognitive domains (functioning ≥1 SD below normal)	Minor interference; deficiency reported by patient (reduced mental perception, incompetence at work; Others support the observations
HIV-associated dementia (HAD)	5%	Cognitive impairment in ≥2 domains (functioning ≥ 2 SD below normal)	Noticeable impairment in everyday living

* Test for neurocognitive status comprises evaluation of at least 5 of the following: attention–information processing, language, abstraction and executive functions, complex perceptual motor skills, memory, simple motor skills, sensory perceptual skills. (SD = standard deviation).

** Functional status is generally evaluated by the patient but may also be verified by others. Other causes of dementia and/or probable influences of substance use must be excluded.

Although recent studies show an increase in ANI and MND [13], there has been a decline in HAD, implying that compliance to cART may be helpful for limiting neurological disorders [17]. The CNS is one of the main targets of HIV-1 infection. The CNS is usually affected during primary infection and can be nonthreatening in the first few years, without producing any symptoms. Later, it can lead to HIV-1 encephalitis (HIVE), or AIDS Dementia Complex (ADC), displaying cognitive, behavioral and motor failure. At this stage, many opportunistic infections can also affect the CNS. [21]

1.1. Pathogenesis:

At present, the pathogenesis of HAND is not fully understood. It is believed that within a few days of HIV-1 infection, the virus crosses the blood-brain barrier (BBB). The "Trojan horse" theory is the generally accepted explanation [22]. According to this theory, the virus crosses the BBB and enters the CNS through exchange of lymphocytes and activated monocytes, which later separate into macrophages. [19, 23] The virus can later continue to stay in CD4+ T cells, macrophages, and astrocytes. HIV-1 infected cells in the CNS release neurotoxic products, resulting in the death of neurons. This is facilitated by the toxic effects of HIV-1 viral proteins (gp120, gp 41, Nef, Vif, Vpr, Vpu, and Tat) [15,19, 24] and the inflammatory neurotoxic products released from infected cells in the CNS such as nitric oxide, arachidonic acid, and pro-inflammatory cytokines [23]. HIV-1 brain infection generally affects the basal ganglia, deep white matter, and the hippocampus [10, 19]. Pathologically, it is characterized by foci of infected macrophages and microglial, multinucleated cells [22,25,26], extensive white matter pallor, and an increase in microglial and astrocytic cells [21]. HAND has typically been related to low CD4+ T lymphocyte counts (< 200 cells/ microliter) even when there are no CNS opportunistic infections [27].

1.2. Diagnosis:

The diagnosis of HAND is challenging and cannot be made accurately if the patient is affected neurologically due to substance abuse, increased age, drug toxicity and concurring medical conditions like cerebrovascular diseases or sleep disorders. Cognitive symptoms for HAND include amnesia, decline in rational abilities, short-term memory loss and lack of concentration. Behavioral symptoms manifest as depression, lethargy, anxiety, or decreased impulsiveness. Motor symptoms include shivering, slow movements, convulsions, inability to perform fine-finger operations, clumsiness, poor coordination and overactive deep tendon reflexes [19, 28]. Neuroimaging techniques (CT, MRI, SPECT, PET, MRS) have been investigated as tools for both diagnosis and pathogenetic studies of HAND. An elevation in the axonal neurofilament light chain (NFL) protein and the neuronal protein tau are often observed in the cerebrospinal fluid of HAND patients [29].

1.3. Treatment:

Combined antiretroviral therapy (cART) has important effects on HIV infection in the CNS [30, 31]. cART can reduce HIV-1 replication in the blood and brain, leading to a reduction in the number

of activated monocytes. Therefore, less activated monocytes reach the brain, resulting in a decrease in production of neurotoxins and neuroinflammation [8, 32]. However, since clinical trials targeting CNS HIV-1 infection have been difficult to execute, current treatment options derive frequently from observations and conclusions from normal therapy [21]. At present, there are no specific treatments for HAND or related ailments.

1.4. Impact of cART:

Current cART has a number of major limitations. As a chronic treatment, HIV/AIDS patients need to receive cART for the rest of their lives. Compliance with a complex regimen is required and nonadherence to therapy can lead to failure. Toxic drug effects leading to side effects and toxicity are problematic. Viral resistance to drug therapy has been a persistent challenge with patients who initially respond well to treatment experiencing drug failure over time. With extended survival of patients, the more severe spectrum of HAND with HIV-1 encephalitis (HIVE) and especially HIV-associated dementia have become more prominent.

In the first few years of the outbreak of AIDS, the mortality rate from this disease was high. In some developing countries with poor access to resources, the situation continues to be similar [33-35]. However, in developed countries, the extensive use of cART has suppressed this problem to some extent [21, 36]. In fact, after the initiation of protease inhibitors and triple drug therapy, CNS diseases along with systemic AIDS were seen to decrease ten times [37]. According to a study by the Neurobehavioral Research Center on HIV, cognitive recovery was also observed in some patients shortly after beginning cART [38]. At present, the overall understanding that antiretroviral therapy improves neurological dysfunction seems convincing, although the development of HAND continues to occur despite treatment and is escalating as HIV/AIDS patients live longer.

HAND remains widespread in the cART epoch due to latent reservoirs of HIV-1 infection located in the brain and low penetration of drugs into the CNS. Since CNS can shelter the virus in spite of viral containment in the blood, these hidden reservoirs have been a major obstacle to the suppression of HIV [19, 39]. Many cART drugs do not effectively cross the BBB and have no access to these reservoirs in the CNS [40]. Therefore, in chronic CNS HIV-1 infection, the viral population can progress independently [41] being compartmentalized in the cerebrospinal fluid (CSF), and may not be reflected by plasma concentration [42, 43]. Better CNS penetration is thus expected to achieve better neurocognitive improvement due to greater suppression of CNS viral replication [32]. The CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study classified ART drugs into 3 classes based on CNS penetration as outlined in Table 2 reported previously [44].

Table 2. CNS penetration-effectiveness score (CPE score) [44].

Type of Inhibitor	CPE Score (CNS penetration-effectiveness)		
	0 (poor)	0.5 (intermediate)	1 (good)
Nucleoside Reverse Transcriptase Inhibitors (NRTI)	Adefovir Zalcitabine Didanosine Tenofovir	Emtricitabine Lamivudine Stavudine	Abacavir Zidovudine
Non- Nucleoside Reverse Transcriptase Inhibitors (NNRTI)		Efavirenz	Delavirdine Nevirapine
Protease Inhibitors (PI)	Nelfinavir Ritonavir Saquinavir Saquinavir-r Tipranavir-r	Amprenavir Atazanavir Atazanavir-r Fosamprenavir Indinavir	Amprenavir-r Darunavir Fosamprenavir-r Lopinavir-r Indinavir-r
Integrase Inhibitors		Elvitegravir Raltegravir	
Entry Inhibitors	Enfuvirtide T-I249		Vicriviroc Maraviroc

cARTs are known to cause oxidative stress in neurons and endogenous antioxidant activation, leading to damage of neurons. CNS toxicities, especially nucleosides, [28, 39, 45] are attributed to drug effects on mitochondrial DNA polymerase [21, 46]. It has also been observed that the restored immunity as a result of cART might sometimes lead to IRIS, resulting in PML (Progressive multifocal leukoencephalopathy) caused by the JC virus [47, 48], or an increased susceptibility to diseases like Parkinson's and Alzheimer's [19]. Efavirenz, an NNRTI, is known to affect the CNS. Thus far, protease inhibitors have not been shown to affect CNS function. Protease inhibitors (PIs) are an important component of cART. A number of FDA-approved PIs have been shown to cross the BBB to some extent. It may be possible to design and develop PIs with further improvement of BBB penetration properties.

2. DISCUSSION

Over the years, we have designed and developed a range of conceptually intriguing and highly potent nonpeptide HIV-1 protease inhibitors (PIs) with drug-like properties [49, 50]. Our protein X-ray structure-based molecular design led to the development of a variety of cyclic-ether-derived ligands and templates where the ether oxygens are positioned to effectively mimic the carbonyl oxygens of peptide bonds. These molecular templates were specifically designed with defined stereoconfiguration and structural complementarity to effectively fill in the hydrophobic pockets in the active site. Another of our major design objectives has been to design PIs that can combat HIV-1 variants resistant to the currently approved anti-HIV agents. Towards this goal, our inhibitor design strategies focused on maximizing inhibitor interactions with the HIV-1 protease active-site, particularly promoting extensive hydrogen bonding interactions with the protein backbone atoms [51-54]. Our research culminated in numerous potent PIs and one of them is the FDA approved drug, Darunavir (DRV, Fig. 1**)** which contains an X-ray structure-based designed ligand, 3(*R*),3a(*S*),6a(*R*)-bistetrahydrofuranylurethane (*bis*-THF) [55-57]. DRV has been used worldwide as a first-line therapeutic for HIV-1-infected individuals. Due to the presence of a unique moiety, *bis*-THF, DRV can exhibit dual action of blocking HIV-1 protease's enzymatic activity [57, 58] and dimerization [59], thus leading to its promising antiretroviral activity and delayed emergence of DRV-resistant strains. However, the penetration of DRV into the brain is only mediocre (the ratio of DRV concentration in the brain to that in blood is 0.6%) [60], possibly due to inadequate penetration of DRV through the BBB. Since the CNS is a major sanctuary of HIV-1 infection, improving drug concentration in the CNS may be an important strategy to address HAND. [16, 19] Due to reduced drug entry into the CNS, the potent drug concentration is usually sub-therapeutic. This leads to selection of resistant mutants and assists the development of viral resistance [61].

In our effort to improve BBB penetration, while at the same time maintaining excellent antiviral activity and drug properties, we recently reported a number of HIV PIs, GRL-04810, GRL-05010, GRL-0739, and GRL-10413 that show excellent activity against laboratory HIV-strains and multidrug-resistant clinical isolates, with minimal cytotoxicity, and good CNS penetration in vitro [62-65]. Structures of these four GRL-PIs are shown in Fig. 1.

The introduction of fluorine in biomolecules is a popular approach to improve BBB penetration by increasing lipophilicity, along with enhancing metabolic stability, membrane permeability and protein-ligand interactions [66]. Mindful to preserve the key backbone hydrogen-bonding interactions through the *bis*-THF ring oxygen atoms, it was presumed that fluorine substitution might result in non-covalent interactions in the HIV-protease active site. [20, 67] The addition of fluorine atoms is known to increase lipophilicity and possibly enhance CSF penetration [68, 69]. This was the basis for the design of GRL-04810 and GRL-05010, which contain a

bis-THF moiety in which two fluorines are attached on the same carbon [62, 63]. We also designed, synthesized and investigated the nonpeptidic HIV-1 protease inhibitor GRL-0739 [64], which contains the hydrophobic cyclohexyl-bis-THF, and GRL-10413, which contains a 3-chloro-4-methoxy-phenylmethyl P1 moiety [65].

We investigated these PIs against a panel of multidrug-resistant HIV-1 variants. GRL-04810, GRL-05010, GRL-0739 and GRL-10413 suppressed various HIV-1 strains, including multidrug-resistant clinical HIV-1 isolates with reasonably low EC_{50}'s and favorable cytotoxicity profiles as shown in Table 3 [62-65]. They were also highly active against various protease inhibitor-selected laboratory HIV-1 variants (including DRV-resistant variants). In fact, GRL-04810 and GRL-05010 remain active against the variants selected with GRL-04810 and GRL-05010. GRL-0739 was also active against HIV-2_{ROD} (EC_{50} 0.0058 μM). Also, the binding effects of human serum proteins on the antiretroviral activity of GRL-0739 were insignificant compared to other FDA-approved PIs. The selectivity index of GRL-10413 at 102,000 is considerably better than that of other HIV-1 PIs examined in this study (Table 3). GRL-10413 does not allow HIV-1 to acquire resistance to GRL-10413 or other conventional PIs (no amino acid substitutions were observed over 50 passages of selection with GRL-10413).

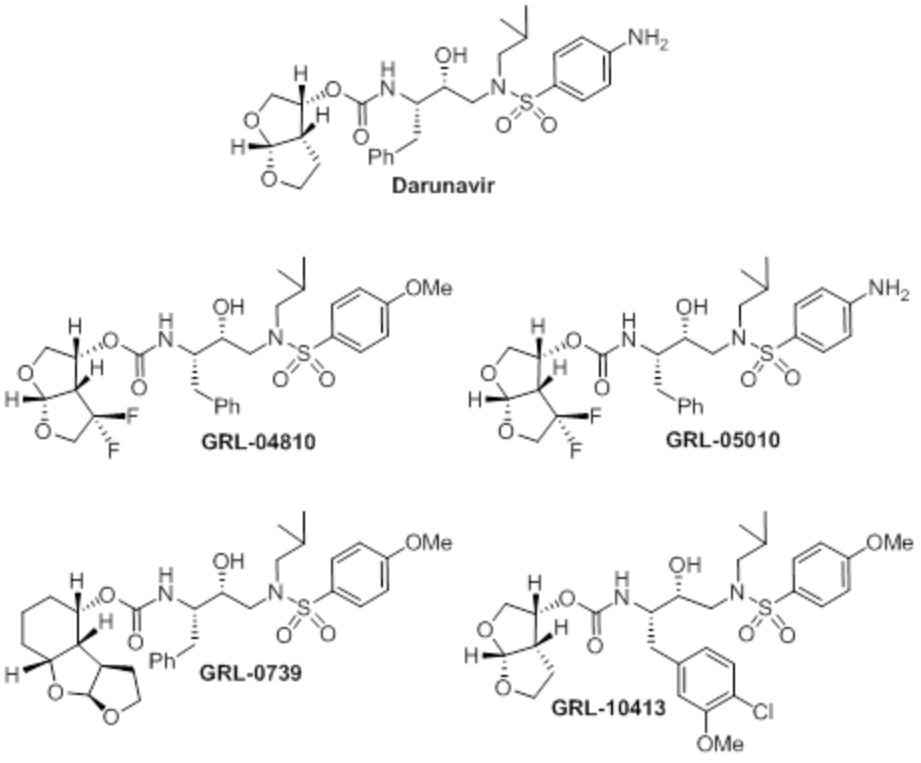

Fig 1. Structures of Protease Inhibitors

TABLE 3. Antiviral activity of PIs against HIV-1_{LAI} and cytotoxicity against MT-2 cells.

Compound	EC_{50} (μM) against HIV-1_{LAI}	CC_{50} (μM)	Selectivity index (CC_{50}/EC_{50})
GRL-04810	0.0008 ± 0.0002	17.5 ± 0.9	21,879
GRL-05010	0.003 ± 0.001	37.0 ± 0.4	12,333
GRL-0739	0.0019 ± 0.0007	21.0 ± 1.6	11,053
GRL-10413	0.00035 ± 0.00003	35.7 ± 1.4	102,000
SQV	0.028 ± 0.003	19.1 ± 1.0	682
APV	0.034 ± 0.009	48.2 ± 9.9	1,418
ATV	0.0048 ± 0.0003	32.4 ± 1.0	6,750
LPV	0.036 ± 0.003	26.7 ± 4.2	742
DRV	0.0056 ± 0.0008	100.6 ± 8.8	17,964

All the GRL PIs reported earlier were based on APV structure. Hence, they were not as active against APV-resistant variants. However, GRL-10413 could stop HIV-$1_{APV}{}^{R}{}_{5\ \mu M}$ replication very efficiently, implying that GRL-10413 has a distinctive antiviral attribute compared to PIs reported earlier. Therefore, both the anti-HIV activity and minimal cytotoxicity of all four protease inhibitors are desirable, although the efficacy and emergence of adverse effects should ultimately be determined by clinical trials.

We then evaluated *in vitro* BBB penetration properties of GRL-04810, GRL-05010, GRL-0739 and GRL-10413. We hoped that this could also shed some light on the drug transport mechanism across the BBB [70]. The BBB model for drug transport assays (BBB Kit; PharmaCo-Cell, Ltd., Nagasaki, Japan) employed here was composed of pericytes, astrocytes from rats and endothelial cells from monkeys, stored at -80°C. The physiological consistency of this model was augmented by the fact that it employed these three kinds of cells, yielded reproducible results, had complex tight junctions and transcytotoxic activity. This model was thus considered ideal for investigating migration of drug molecules across the BBB [71]. A solution of Dulbecco's modified Eagle's medium (DMEM)/F-12 medium, heparin, basic fibroblast growth factor (bFGF), insulin, transferrin, sodium selenite, hydrocortisone, and gentamicin was added to both the blood and brain side of the model. The membranes were collagen-coated. The system was allowed to remain at 37°C for 4 days, and the experiments were conducted over the following two days, as the transendothelial electrical resistance (TEER) becomes maximum in that period. Membranes with a TEER value of >150 Ω/cm^2 were chosen for the experiment. The compounds were added to the blood side of the wells. After 30 minutes, a spectrophotometer with an absorbance at 230 nm was used to measure the quantity of drug that crossed the BBB. Caffeine and sucrose, the most and least lipophilic substances used, served as positive and negative controls, respectively. As seen from Table 4, all four tested PIs yielded much greater concentrations compared to most of the FDA approved PIs.

Table 4. Evaluation of the apparent blood brain barrier permeability coefficient using an in vitro model

Compound	Class	Initial luminal tracer concentration (μM)	Final abluminal tracer concentration (μM)	P_{app} (10^{-6} cm/s)
RAL	Integrase inhibitor	100	0.68 ± 0.23	10.2 ± 3.5
FTC	NRTI	100	1.11 ± 0.44	16.6 ± 6.7
MVC	CCR5 inhibitor	100	1.06 ± 0.41	16.6 ± 6.3
AZT	PI	100	1.50 ± 0.12	22.7 ± 1.9
IDV	PI	100	2.42 ± 0.12	36.7 ± 1.7
SQV	PI	100	0.33 ± 0.03	4.9 ± 0.4
APV	PI	100	0.70 ± 0.14	10.6 ± 2.1
LPV	PI	100	0.94 ± 0.05	14.2 ± 0.7
ATV	PI	100	1.02 ± 0.10	15.4 ± 1.4
DRV	PI	100	0.65 ± 0.23	9.9 ± 4.2
GRL-04810	PI	100	3.16 ± 0.48	47.8 ± 8.8
GRL-05010	PI	100	4.08 ± 0.65	61.8 ± 12.1
GRL-0739	PI	100	1.80 ± 0.66	27.3 ± 10.1
GRL-10413	PI	100	1.40 ± 0.21	21.1 ± 3.1
Caffeine	Positive control	100	6.60	76.4 ± 2.4
Sucrose	Negative control	100	0.03 ± 0.005	0.33 ± 0.13

The apparent permeability coefficient (P_{app}) is a quantitative evaluation of the efficacy with which a compound can penetrate the BBB model in vitro [70]. $P_{app} = (\Delta[C]_{abluminal}/\Delta t) \times (VA/A \times [C]_{luminal})$, ($\Delta[C]_{abluminal}$: drug concentration in the brain side; Δt: experimental time; VA: brain side volume; A: surface area of the membrane; $[C]_{luminal}$: drug concentration in the blood side. The P_{app} values of GRL-04810 (47.8 x 10^{-6} cm/s) and GRL-05010 (61.8 x 10^{-6} cm/s) were significantly greater than the P_{app} values of other antiviral drugs tested. Compounds with P_{app} values greater than 20 x 10^{-6} cm/s generally have efficient penetration across the BBB; those with values of 10 x 10^{-6} to 20 x 10^{-6} cm/s have an average penetration, whereas those with values lower than 10 x 10^{-6} cm/s penetrate the BBB poorly [71].

To assess the efficacy of the drugs that successfully crossed the brain interface in the BBB assay, the susceptibility of HIV-1_{LAI} to the recovered drugs from the

abluminal side was determined in an MTT assay employing MT-2 cells [62, 64, 65]. The antiviral activity of the recovered GRL-04810, GRL-05010 and GRL-10413 was superior to that of DRV and the rest of the tested PIs. All of them significantly suppressed the replication of HIV-1_{LAI} and HIV- $1_{ERS104pre}$ more than other antiretroviral agents including DRV. This implies unambiguously that after crossing the BBB, the drugs were molecularly unchanged and retained their strong HIV-1 protease inhibiting capability. These results indicate that these drugs can possibly suppress the replication of HIV-1 in CNS and thus control chronic inflammation, which is a crucial factor in the inception and development of HAND.

The addition of fluorine atoms is generally predicted to bestow greater lipophilicity on certain compounds. It is well-known that the lipophilicity of a drug is important for its oral bioavailabilty, absorption and BBB permeability [68, 69]. The shake flask method [62] is a rational way for determining the partition (log P) and distribution coefficients (log D) of drugs. The log P evaluates the lipophilicity of a molecule [72], while the log D does the same for the ionized form of the compound, in a Tris-buffered saline solution (pH 7.4). In the logP study, GRL-04810 showed a concentration of 72 μM in the octanol (lipidic) interface, which was significantly higher than GRL-05010 (14 μM) and DRV (12 μM). The log D for GRL-05010 (-1.01) and DRV (-1.03) were reasonable [73, 74], while GRL-04810 was the most lipophilic (logD -0.29).

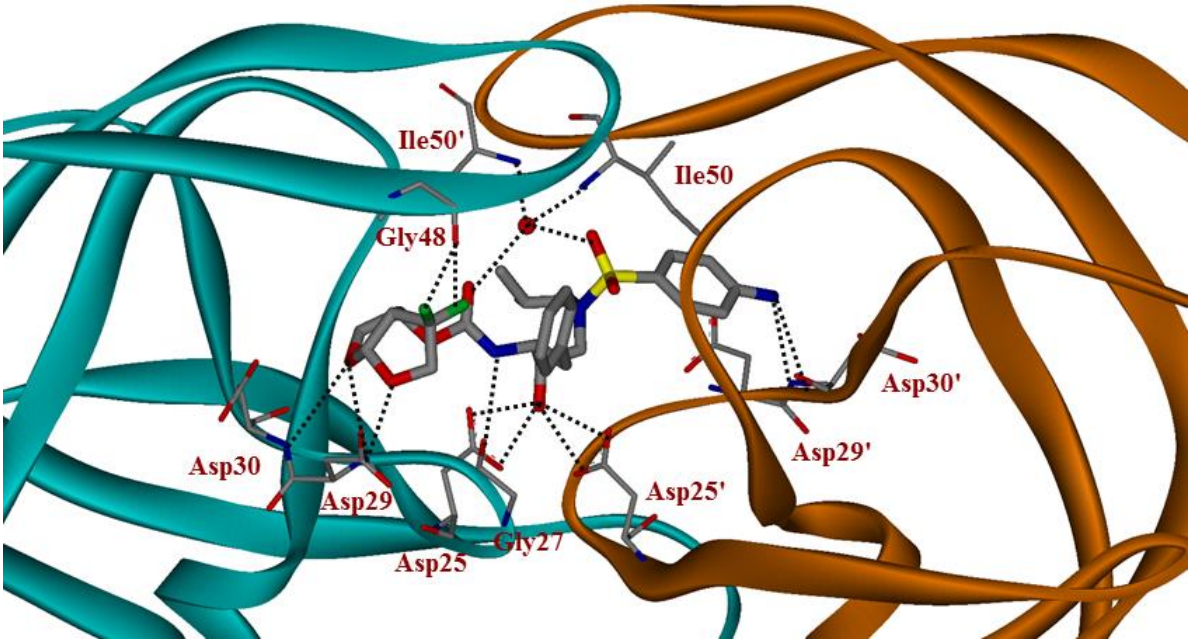

Figure 2. GRL-05010-bound X-ray structure of HIV-1 protease. The inhibitor carbon atoms are shown in grey, water molecules are red spheres, and the hydrogen bonds are indicated by dotted lines. Nonbonded interactions of *gem*-difluorides with the carbonyl of Gly48 in the protease flap region are shown.

We conducted crystallographic analysis to determine how the PIs interact with HIV-1 protease. The interactions of DRV (PDB entry 4HLA) with HIV-1 protease seen in a crystal structure share key interactions with both GRL-04810 and GRL-05010 (PDB ID: 4U8W). As shown in Fig. 2, the inhibitor GRL-05010 is bound in the active site cavity via a network of strong hydrogen bonding interactions with backbone atoms, as well as with the catalytic aspartates of HIV-1 protease. The surface view of GRL-05010-bound HIV-1 protease is shown in Fig. 3. Both oxygen atoms present in the bis-THF group of GRL-04810 and GRL-05010 have hydrogen bonding interactions with the backbone nitrogens of Asp29 and Asp30 in the S2 site of the protease. As seen in the crystal structure [63], GRL-05010 maintains a polar interaction of the carbamate NH group with the backbone carbonyl oxygen of Gly27. The carbamate carbonyl oxygen and the sulfonamide oxygen are hydrogen bonded with the amides of Ile50 and Ile50' in the flaps of HIV-1 protease through a bridging water molecule. The methoxy oxygen of GRL-04810 forms a hydrogen bond with the NH of Asp30'. On the other hand, the NH nitrogen of GRL-05010 forms strong polar interactions with the Asp30' NH and with the backbone carboxyl of Asp30'. In particular, intense interactions can be observed between the carbonyl oxygen atom of Gly48 in the flap region of the protease and both fluorine atoms on the bis-THF ligand. Not only does this enhance the binding affinity, but it also provides stability to the flexible flap region. Also, the fluorine atoms can fill the vacant cavity near the flap

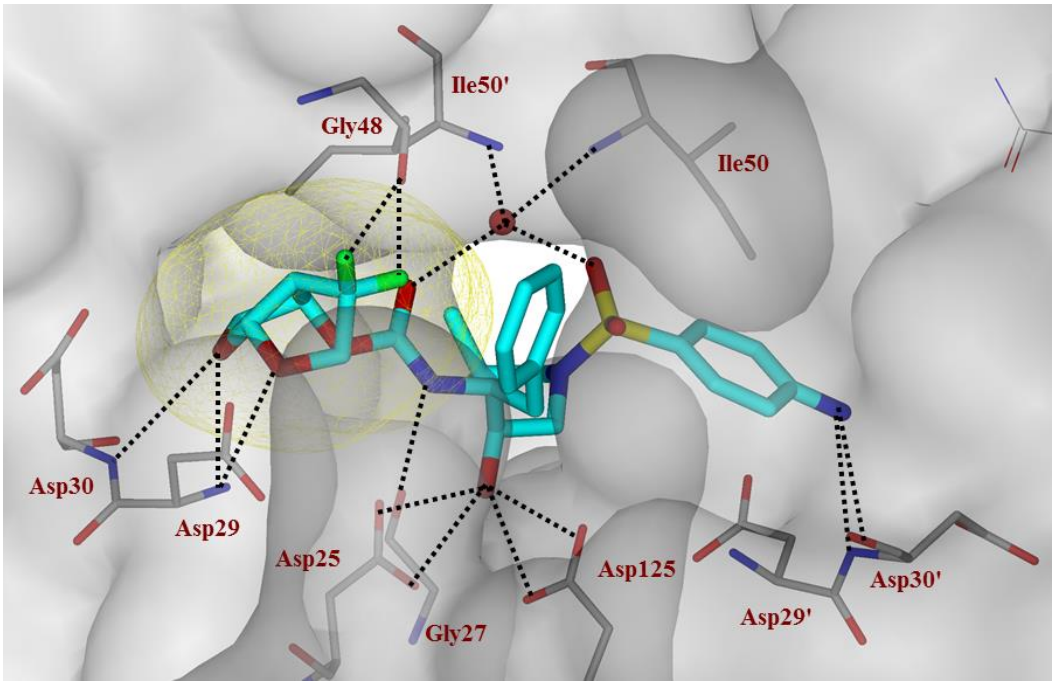

Figure 3. GRL-05010-bound X-ray structure of HIV-1 protease with protein surface is shown in gray. Inhibitor carbon atoms are shown in torquois and fluroro-bis-THF surface is shown inside the yellow wire mesh. All hydrogen bonds are indicated by dotted lines.

of the protease. The P1 and P2' aromatic rings fill the S1 and S2' sites, respectively, whereas the P1' isobutyl side chain efficiently fills the hydrophobic pocket at the S1' site. Thus, the excellent potency and affinity of GRL-04810 and GRL-05010 toward HIV-1 protease can be justified by this broad array of interactions.

GRL-0739 shared only a few common interactions with the HIV-protease as darunavir. A new H-bonding interaction of the backbone atoms of Asp29 and Gly27 with a tricyclic moiety oxygen of GRL-0739 through a water molecule was observed. In spite of showing an activity profile better than darunavir, GRL-0739 did not seem to have as many hydrogen bonding interactions as darunavir [75]. While studying the hydrophobic interactions of GRL-0739, it seemed that the tricyclic ring and methoxybenzene moieties of GRL-0739 had much stronger van der Waals interactions and larger surface area than darunavir. Therefore, the fewer number of H-bonding interactions are compensated by the better hydrophobic contacts of GRL-0739, leading to better anti-HIV-1 activity than darunavir [64].

GRL-10413 showed similar interactions as DRV (both hydrogen bonding and VdW contacts). A strong electron density for the chlorine atom in two different configurations was observed. In the major configuration, the positively charged guanidinium group of Arg8 was found to form a bidentate halogen bond with the chlorine. In the minor configuration, the chlorine atom is pointed toward the amide plane between Ile50 and Gly49. In fact, glycine is one of the most common amino acids that is known to interact with halogens. [76]. The influence of halogen bonding on interactions of molecules with proteins is well-studied [77]. Halogens (especially fluorine and chlorine) not only facilitate membrane permeability and metabolic stability, but also enhance binding affinity and specificity [78]. This excellent binding profile of GRL-10413 supports its enhanced antiviral activity [65].

3. CONCLUSION

HIV-Associated Neurocognitive Disorder is a debilitating to devastating complication of HIV infection. Success with combined antiretroviral treatment continues to improve life expectancy, but with increasing age, manifestations of neurocognitive disorder for HIV/AIDS patients are increasing. Since HIV-1 protease inhibitors are an important component of current antiretroviral treatment regimens, targeting the CNS sanctuary of HIV with new protease inhibitors capable of crossing the blood-brain barrier and interrupting HIV in the brain may be key to treating or even preventing HIV-associated dementia. Darunavir is an FDA-approved protease inhibitor widely used in treatment-experienced patients, with significantly greater virological response and immunological benefits compared to the standard care. Our structure-based modification resulted in very potent brain-penetrating protease inhibitors, GRL-04810 and GRL-05010. We have also designed other inhibitors such as GRL-0739 and GRL-10413 which show BBB efficacy. The present data suggest that GRL-04810, GRL-05010, GRL-0739 and GRL-10413 have several advantages: (a) they form extensive

interactions with the residues in the active site of HIV-1 protease, leading to excellent antiviral activity against a broad spectrum of drug-resistant HIV-1 isolates and variants; (b) the hydrophobic interactions, logP and logD values indicate a very good lipophilicity profile and (c) they can efficiently penetrate the BBB and retain their activity. Therefore, they possess desirable features as a drug suitable for treating patients infected with wild-type and/or multidrug-resistant HIV-1 variants, including HAND. The current data warrant further consideration of these novel PIs for the treatment of HIV/AIDS. It is necessary to evaluate their other drug properties including their pharmacokinetics, pharmacodynamics, and oral bioavailability in the clinical setting.

4. Abbreviations:

HAND: HIV-Associated Neurocognitive Disorder

HIV/AIDS: Human immunodeficiency virus infection and acquired immune deficiency syndrome

cART: combined antiretroviral therapy

UNAIDS: The Joint United Nations Programme on HIV and AIDS

IRIS: Immune reconstitution inflammatory syndrome

CNS: Central Nervous System

CSF: Cerebrospinal fluid

CHARTER: CNS HIV AntiRetroviral Therapy Effects Research

ANI: Asymptomatic neurocognitive impairment

MND: Mild neurocognitive disorder

HAD: HIV-associated dementia

SD: standard deviation

ADC: AIDS Dementia Complex

HIVE: HIV-1 encephalitis

CT: Computerized (or computed) tomography

SPECT: Single-photon emission computed tomography

PET: Positron emission tomography

MRI: Magnetic Resonance Imaging

NFL: Neurofilament light chain

HAART: Highly active antiretroviral therapy

CPE: CNS penetration-effectiveness score

NRTI: Nucleoside Reverse Transcriptase Inhibitors

NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors

CCR5: Chemokine receptor type 5

PI: Protease Inhibitors

DNA: Deoxyribonucleic acid

PML: Progressive multifocal leukoencephalopathy

JC virus: John Cunningham virus

EC_{50}: Half maximal effective concentration

CC_{50}: 50% cytotoxic concentration

FDA: Food and Drug Administration

BBB: Blood-Brain barrier

TEER: Transendothelial electrical resistance

P_{app}: Apparent permeability coefficient

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

PDB: Protein Data Bank

DMEM: Dulbecco's modified Eagle's medium

bFGF: basic fibroblast growth factor

RAL: Raltegravir

FTC: Emtricitabine

MVC: Maraviroc

AZT: azidothymidine

IDV: Indinavir

SQV: Saquinavir

APV: Amprenavir

LPV: Lopinavir

ATV: Atazanavir

DRV: Darunavir

VdW: Van der Waals

ZDV: Zidovudine

Acknowledgements: The research was supported by grants from the National Institutes of Health (GM53386). This work presented here was also supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health and in part by a Grant-in-aid for Scientific Research (Priority Areas) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Monbu Kagakusho), a Grant for Promotion of AIDS Research from the Ministry of Health, Welfare, and Labor of Japan (Kosei Rohdosho: H15-AIDS-001), and the Grant to the Cooperative Research Project on Clinical and Epidemiological Studies of Emerging and Reemerging Infectious Diseases (Kumamoto University) of Monbu-Kagakusho. We thank Ms. Heather Osswald (Purdue University) for helpful discussions.

5. REFERENCES

[1] Global AIDS Update UNAIDS report - http://www.who.int/niv/pub/arr/global-aids-update-2016-pub/en

[2] Fauci AS, Marston HD. Ending the HIV-AIDS pandemic—follow the science. *N Engl J Med.* 2015;373(23):2197-2199.

[3] Tan IL, McArthur JC. HIV-Associated Neurological Disorders *CNS Drugs.* 2012;26(2):123-134.

[4] Ghafouri M, Amini S, Khalili K, Sawaya BE. HIV-1 associated dementia: Symptoms and causes. *Retrovirology*. 2006;3:28.

[5] McArthur JC, McClernon DR, Cronin MF, Nance-Sproson TF, Saah AJ, St Clair M, Lanier ER. Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain. *Ann Neurol.* 1997;42(5):689-698.

[6] Maschke M, Kastrup O, Esser S, Ross B, Hengge U, Hufnagel A. Incidence and prevalence of neurological disorders associated with HIV since the introduction of highly active antiretroviral therapy (HAART). *J Neurol Neurosurg Psychiatry*. 2000;69(3):376-380.

[7] Sacktor N, Nakasujja N, Skolasky R, Robertson K, Wong M, Musisi S, Ronald A, Katabira E. Antiretroviral therapy improves cognitive impairment in HIV+ individuals in sub-saharan Africa. *Neurology.* 2006;67(2):311-314.

[8] Spudich S. HIV and neurocognitive dysfunction. *Curr HIV/AIDS Rep*. 2013(3);10:235-243.

[9] Alkali NH, Bwala SA, Nyandaiti YW, Danesi MA, *Ann Afr Med* 2013;12(1):1-10.

[10] Navia BA, Cho ES, Petito CK, Price RW. The AIDS dementia complex: II. Neuropathology. *Ann Neurol.* 1986;19(6):525–535.

[11] Letendre SI, McCutchan JA, Childers ME, Woods SP, Lazzaretto D, Heaton RK, Grant I, Ellis RJ, HNRC Group. Enhancing antiretroviral therapy for human immunodeficiency virus cognitive disorders. *Ann Neurol.* 2004;56(3):416-423.

[12] Lohse N, Hansen AB, Gerstoft J, Obel N. Improved survival in HIV-infected persons: consequences and perspectives. *J Antimicrob Chemother*. 2007;60(3):461–463.

[13] Zhou L, Saksena NK. HIV associated neurocognitive disorders. *Infect Dis Rep*. 2013;5(suppl 1): e8. www.ncbi.nlm.nih.gov/pmc/articles/ PMC3892625/pdf/idr-2013-s1-e8.pdf.

[14] Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, Glass JD. Localization of HIV-1 in human brain using polymerase chain reaction/in situ hybridization and immunocytochemistry. *Ann Neurol.* 1996;39(6):705–711.

[15] Müller M, Wandel S, Colebunders R, Attia S, Furrer H, Egger M. Immune reconstitution

inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and metaanalysis. *Lancet Infect Dis.* 2010;10(4):251-261.

[16] Rani P, Thomas FP. Central Nervous System Complications in HIV. *Medscape*. Dec 23, 2015. Retrieved from http://emedicine.medscape.com/article/1167008-overview.

[17] Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, Marcotte TD, Atkinson JH, Rivera-Mindt M, Vigil OR, Taylor MJ, Collier AC, Marra CM, Gelman BB, McArthur JC, Morgello S, Simpson DM, McCutchan JA, Abramson I, Gamst A, Fennema-Notestine C, Jernigan TL, Wong J, Grant I; CHARTER Group. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology.* 2010;75(23):2087-2096.

[18] Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, Gisslen M, Grant I, Heaton RK, Joseph J, Marder K, Marra CM, McArthur JC, Nunn M, Price RW, Pulliam L, Robertson KR, Sacktor N, Valcour V, Wojna VE. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology.* 2007;69(18):1789-1799.

[19] Simoes E, Justino JD. HIV-associated neurocognitive disorders: A review for NPs. *The Nurse Practitioner.* 2015;40(7):1–7.

[20] Clifford DB, Ances BM. HIV-associated neurocognitive disorder. *Lancet Infect Dis.* 2013;13(11):976–986.

[21] Price RW, Spudich S. Antiretroviral therapy and central nervous system HIV-1 infection. *J Infect Dis.* 2008;197(Suppl 3):S294-S306.

[22] Vazeux R, Brousse N, Jarry A, Henin D, Marche C, Vedrenne C, Mikol J, Wolff M, Michon C, Rozenbaum W, et al. AIDS subacute encephalitis. Identification of HIV-infected cells. *Am J Pathol.* 1987;126(3):403-410.

[23] Rawson T, Muir D, Mackie NE, Garvey LJ, Everitt A, Winston A. Factors associated with cerebrospinal fluid HIV RNA in HIV infected subjects undergoing lumbar puncture examination in a clinical setting. *J Infect.* 2012;65(3):239–245.

[24] Cysique LA, Vaida F, Letendre S, Gibson S, Cherner M, Woods SP, McCutchan JA, Heaton RK, Ellis RJ. Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. *Neurology.* 2009;73(5):342-348.

[25] Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science.* 1988;239(4840):586–592.

[26] Brew B, Rosenblum M, Cronin K, Price R. AIDS dementia complex and HIV-1 brain infection: clinical-virological correlations. *Annals of Neurology.* 1995;38(4):563–570.

[27] Nabha L, Duong L, Timpone J. HIV-associated neurocognitive disorders: perspective on management strategies. *Drugs.* 2013;73(9):893-905.

[28] Manji H, Jäger H, Winston A. HIV Dementia and antiretroviral drugs: 30 years of an epidemic. *J Neurol Neurosurg Psychiatry*. 2013;84(10):1126-1137.

[29] Brew BJ, Pemberton L, Blennow K, Wallin A, Hagberg L. CSF amyloid beta42 and tau levels correlate with AIDS dementia complex. *Neurology*. 2005;65(9):1490-1492.

[30] Spudich S, Lollo N, Liegler T, Deeks SG, Price RW. Treatment benefit on cerebrospinal fluid HIV-1 levels in the setting of systemic virological suppression and failure. *J Infect Dis*. 2006;194(12):1686–1696.

[31] Mellgren A, Antinori A, Cinque P, Price RW, Eggers C, Hagberg L, Gisslén M. Cerebrospinal fluid HIV-1 infection usually responds well to antiretroviral treatment. *Antivir Ther*. 2005;10(6):701–707.

[32] Cysique LA, Waters EK, Brew BJ. Central nervous system antiretroviral efficacy in HIV infection: a qualitative and quantitative review and implications for future research. *BMC Neurol*. 2011;11:148-158.

[33] Snider, WD, Simpson, DM, Nielson, S, Gold JW, Metroka CE, Posner JB. Neurological complications of acquired immune deficiency syndrome: analysis of 50 patients. *Ann Neurol*. 1983;14(4):403-418.

[34] Price, RW. Neurological complications of HIV infection. *Lancet*. 1996;348(9025):445-452.

[35] Robertson K, Kopinsky K, Mielke J, Appiah K, Hall C, Price R, Kumwenda J, Kanyama C, Amod F, Marra C, Taylor T, Lalloo U, Jelsma J, Holding P, Boivin M, Birbeck G, Nakasujja N, Sanne I, Parsons TD, Parente A, Tucker K; Assessment of NeuroAIDS in Africa Conference Participants. Assessment of neuroAIDS in Africa. *J Neurovirol*. 2005;11(Suppl 1):7-16.

[36] Maschke M, Kastrup O, Esser S, Ross B, Hengge U, Hufnagel A. Incidence and prevalence of neurological disorders associated with HIV since the introduction of highly active antiretroviral therapy (HAART). *J Neurol Neurosurg Psychiatry*. 2000;69(3):376–380.

[37] d'Arminio AM, Clinque P, Mocroft A, Goebel FD, Antunes F, Katlama C, Justesen US, Vella S, Kirk O, Lundgren J; EuroSIDA Study Group. Changing incidence of central nervous system diseases in the EuroSIDA cohort. *Ann Neurol*. 2004;55(3):320-328.

[38] Spudich S, González-Scarano F. HIV-1-related central nervous system disease: current issues in pathogenesis, diagnosis, and treatment. *Cold Spring Harb Perspect Med*. 2012;2(6):a007120.

[39] Cross H, Combrink M, Joska J. HIV-associated neurocognitive disorders: Antiretroviral regimen, CNS penetration effectiveness and cognitive outcomes. *S African Medical Journal*. 2013;103(10):758-762.

[40] Gisolf EH, Van Praaq R, Jurriaans S, Portegies P, Goudsmit J, Danner SA, Lange JM, Prins JM. Increasing cerebrospinal fluid chemokine concentrations despite undetectable cerebrospinal fluid HIV RNA in HIV-1-infected patients receiving

antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2000;25(5):426–433.

[41] Kaul M, Garden G, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature*. 2001;410(6831):988–994.

[42] Cunningham PH, Smith DG, Satchell C, Cooper DA, Brew B. Evidence for independent development of resistance to HIV-1 reverse transcriptase inhibitors in the cerebrospinal fluid. *AIDS*. 2000;14(13):1949-1954.

[43] Pierson T, McArthur J, Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. *Annu Rev Immunol*. 2000;18:665–708.

[44] de Oliveira FTM, do Olival GS, de Oliveira ACP. Central Nervous System Antiretroviral High Penetration Therapy. *J AIDS Clin Res*. 2015;6(12):529-531. doi:10.4172/2155-6113.1000529.

[45] Akay C, Cooper M, Odeleye A, Jensen BK, White MG, Vassoler F, Gannon PJ, Mankowski J, Dorsey JL, Buch AM, Cross SA, Cook DR, Peña MM, Andersen ES, Christofidou-Solomidou M, Lindl KA, Zink MC, Clements J, Pierce RC, Kolson DL, Jordan-Sciutto KL. Antiretroviral drugs induce oxidative stress and neuronal damage in the CNS. *J Neurovirology*. 2014;20(1):39–53.

[46] Nolan D, Mallal S. Complications associated with NRTI therapy: update on clinical features and possible pathogenic mechanisms. *Antivir Ther*. 2004;9(6):849–863.

[47] Cinque P, Koralnik IJ, Clifford DB. The evolving face of human immunodeficiency virus-related progressive multifocal leukoencephalopathy: defining a consensus terminology. *J Neurovirol*. 2003;9(Suppl 1):88–92.

[48] Kean, JM, Rao S, Wang M, Garcea RL. Atwood, Walter J, ed. Seroepidemiology of Human Polyomaviruses. *PLoS Pathogens.* 2009;5(3):e1000363. doi:10.1371/journal.ppat.1000363

[49] Ghosh AK, Sridhar PR, Kumaragurubaran N, Koh Y, Weber IT, Mitsuya H. Bis Tetrahydrofuran: a Privileged Ligand for Darunavir and a New Generation of HIV Protease Inhibitors That Combat Drug Resistance. *ChemMedChem*. 2006;1(9):939-950.

[50] Ghosh AK, Kincaid JF, Cho W, Walters DE, Krishnan K, Hussain KA, Koo Y, Cho H, Rudall C, Holland L, Buthod J. Potent HIV protease inhibitors incorporating high-affinity P2-ligands and (R)-(hydroxyethylamino)sulfonamide isostere. *Bioorg Med Chem Lett.* 1998;8(6):687-690.

[51] Ghosh AK, Dawson ZL, Mitsuya H. Darunavir, a conceptually new HIV-1 protease inhibitor for the treatment of drug-resistant HIV. *Bioorg Med Chem.* 2007;15(24):7576-7580.

[52] Ghosh AK, Chapsal BD. "Design of the anti-HIV-1 protease inhibitor darunavir," Chapter 13 in: Introduction to Drug Research and development, 2013, Elsevier, Ltd.

[53] Ghosh AK, Anderson DD, Weber IT, Mitsuya H. Enhancing backbond binding – a fruitful concept for combating drug-resistant HIV. *Angew Chem Int Ed Engl.* 2012;51(8):1778-1802.

[54] Ghosh AK, Chapsal BD, Weber IT, Mitsuya H. Design of HIV protease inhibitors targeting protein backbone: an effective strategy for combating drug resistance. *Acc Chem Res.* 2008;41(1):78-86.

[55] Ghosh AK, Krishnan K, Walters DE, Cho W, Cho H, Koo Y, Trevino J, Holland L, Buthod J. Potent HIV protease inhibitors incorporating high-affinity P2-ligands and (R) (hydroxyethylamino)sulphonamide isostere. *Bioorg Med Chem Lett.* 1998;8(6):687–690.

[56] Ghosh AK, Cho W, Walters DE, Krishnan K, Hussain KA, Koo Y, Cho H, Rudall C, Holland L, Buthod J. Structure based design: novel spirocyclic ethers as nonpeptidal P2-ligands for HIV protease inhibitors. *Bioorg Med Chem Lett.* 1998;8(8):979–982.

[57] Koh Y, Nakata H, Maeda K, Ogata H, Bilcer G, Devasamudram T, Kincaid JF, Boross P, Wang YF, Tie Y, Volarath P, Gaddis L, Harrison RW, Weber IT, Ghosh AK, Mitsuya H. Novel bis-tetrahydrofuranylurethane-containing nonpeptidic protease inhibitor (PI) UIC-94017 (TMC114) with potent activity against multi-PI-resistant human immunodeficiency virus in vitro. *Antimicrob Agents Chemother.* 2003;47(10):3123–3129.

[58] Ghosh AK, Xu CX, Rao KV, Baldridge A, Agniswamy J, Wang YF, Weber IT, Aoki M, Miguel SG, Amano M, Mitsuya H. Probing multidrug-resistance and protein-ligand interactions with oxatricyclic designed ligands in HIV-1 protease inhibitors. *ChemMedChem*. 2010;5(11):1850–1854.

[59] Koh Y, Matsumi S, Das D, Amano M, Davis DA, Leschenko S, Baldridge A, Shioda T, Yarchoan R, Ghosh AK, Mitsuya H. Potent inhibition of HIV-1 replication by novel non-peptidyl small molecule inhibitors of protease dimerization. *J Biol Chem.* 2007;282(39):28709–28720.

[60] Calcagno A, Yilmaz A, Cusato J, Simiele M, Bertucci R, Siccardi M, Marinaro L, D'Avolio A, Di Perri G, Bonora S. Determinants of darunavir cerebrospinal fluid concentrations: impact of once-daily dosing and pharmacogenetics. *AIDS.* 2012;26(12):1529–1533.

[61] Langford D, Marquie-Beck J, de Almeida S, Lazzaretto D, Letendre S, Grant I, McCutchan JA, Masliah E, Ellis RJ. Relationship of antiretroviral treatment to postmortem brain tissue viral load in human immunodeficiency virus-infected patients. *J Neurovirol.* 2006;12(2):100–107.

[62] Salcedo-Gómez PM, Amano M, Yashchuk S, Mizuno A, Das D, Ghosh AK, Mitsuya H. GRL-04810 and GRL-05010; difluoride-containing nonpeptidic HIV-1 protease inhibitors(PIs) that inhibit the replication of multi-PI-resistant HIV-1 in vitro and possess favorable lipophilicity that may allow blood-brain barrier penetration.

Antimicrob Agents Chemother. 2013;57(12): 6110–6121. doi.org/10.1128/AAC.01420-13

[63] Ghosh AK, Yashchuk S, Mizuno A, Chakraborty N, Agniswamy J, Wang YF, Aoki M, Salcedo-Gómez PM, Amano M, Weber IT, Mitsuya H. Design of gem-difluoro-bis-tetrahydrofuran as P2 ligand for HIV-1 protease inhibitors to improve brain penetration: synthesis, X-ray studies, and biological evaluation. *ChemMedChem.* 2015;10(1):107–115. doi.org/10.1002/cmdc.201402358.

[64] Amano M, Tojo Y, Salcedo-Gómez PM, Parham GL, Nyalapatla PR, Das D, Ghosh AK, Mitsuya H. A novel tricyclic ligand-containing nonpeptidic HIV-1 protease inhibitor, GRL-0739, effectively inhibits the replication of multidrug-resistant HIV-1 variants and has a desirable central nervous system penetration property in vitro. *Antimicrob Agents Chemother*. 2015;59(5):2625–2635. doi.org/10.1128/AAC.04757-14.

[65] Amano M, Salcedo-Gómez PM, Zhao R, Yedidi RS, Das D, Bulut H, Delino NS, Sheri VR, Ghosh AK, Mitsuya H. A modified P1 moiety enhances in vitro antiviral activity against various multidrug-resistant HIV-1 variants and in vitro central nervous system penetration properties of a novel nonpeptidic protease inhibitor, GRL-10413. *Antimicrob Agents Chemother*. 2016;60(12):7046–7059.

[66] Purser S, Moore PR, Swallow S, Gouverneur V. Fluorine in medicinal chemistry *Chem Soc Rev.* 2008;37(2):320-330.

[67] Zhou P, Zou J, Tian F, Shang Z. Fluorine Bonding — How Does It Work In Protein−Ligand Interactions? *J Chem Inf Model.* 2009;49(10):2344–2355.

[xx] Valcour V, Sithinamsuwan P, Letendre S, Ances B. Pathogenesis of HIV in the central nervous system. *Curr HIV/AIDS Rep*. 2011;8(1):51–60.

[68] Bright TV, Dalton F, Elder VL, Murphy CD, O'Connor NK, Sandford G. A convenient medical-microbial method for developing fluorinated pharmaceuticals. *Org Biomol Chem.* 2013;11(7):1135–1142.

[69] Liu P, Sharon A, Chu CK. Fluorinated nucleosides: synthesis and biological implication. *J Fluor Chem.* 2008;129(9):743–766.

[70] Cecchelli R, Berezowski V, Lundquist S, Culot M, Renftel M, Dehouck MP, Fenart L. Modelling of the blood brain barrier in drug discovery and development. *Nat Rev Drug Discov.* 2007;6(8):650–661.

[71] Nakagawa S, Deli MA, Nakao S, Honda M, Hayashi K, Nakaoke R, Kataoka Y, Niwa M. Pericytes from brain microvessels strengthenthe barrier integrity in primary cultures of rat brain endothelial cells. *Cell Mol Neurobiol.* 2007;27(6):687–694.

[72] Hughes JD, Blagg J, Price DA, Bailey S, Decrescenzo GA, Devraj RV, Ellsworth E, Fobian YM, Gibbs ME, Gilles RW, Greene N, Huang E, Krieger-Burke T, Loesel J, Wager T, Whiteley L, Zhang Y. Physiochemical drug properties associated with in vivo toxicological outcomes. *Bioorg Med Chem Lett.* 2008;18(17):4872–4875.

[73] Ryckmans T, Edwards MP, Horne VA, Correia AM, Owen DR, Thompson LR, Tran I, Tutt MF, Young T. Rapid assessment of a novel series of selective CB2 agonists using parallel synthesis protocols: a lipophilic efficiency (LipE) analysis. *Bioorg Med Chem Lett.* 2009;19(15):4406–4409.

[74] Waring MJ. Defining optimum lipophilicity and molecular weight ranges for drug candidates—molecular weight dependent lower log *D* limits based on permeability. *Bioorg Med Chem Lett.* 2009;19(10):2844–2851.

[75] Amano M, Koh Y, Das D, Li J, Leschenko S, Wang YF, Boross PI, Weber IT, Ghosh AK, Mitsuya H. A novel bis-tetrahydrofuranylurethane-containing nonpeptidic protease inhibitor (PI), GRL-98065, is potent against multiple-PI-resistant human immunodeficiency virus in vitro. *Antimicrob Agents Chemother*. 2007;51(6): 2143–2155. doi .org/10.1128/AAC.01413-06.

[76] Xu Z, Yang Z, Liu Y, Lu Y, Chen K, Zhu W. Halogen bond: its role beyond drug-target binding affinity for drug discovery and development. *J Chem Inf Model*. 2014;54(1):69–78. doi.org/10.1021/ci400539q

[77] Wilcken R, Zimmermann MO, Lange A, Joerger AC, Boeckler FM. Principles and applications of halogen bonding in medicinal chemistry and chemical biology. *J Med Chem*. 2013;56(4):1363–1388. http://dx.doi.org /10.1021/jm3012068.

[78] Müller K, Faeh C, Diederich F. Fluorine in pharmaceuticals: looking beyond intuition. *Science.* 2007;317(5846):1881–1886. doi.org/10.1126 /science.1131943.

