HIV Persistence and Viral Reservoirs

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To my family and friends

ABSTRACT

Although antiretroviral therapy (ART) can effectively inhibit replication of human immunodeficiency virus type 1 (HIV-1), the virus is able to persist in cellular and anatomical viral reservoirs. Latently infected resting memory $CD4^+$ T-cells are an important cellular reservoir, and the central nervous system (CNS) an important anatomical reservoir for HIV-1 infection. The overall aim of this thesis was to gain greater understanding of HIV-1 persistence, in regards to latent infection as well as the central nervous system.

The initial viral decay rate after initiation of ART has been proposed as a measure of relative regimen potency. We compared initial viral decay in subjects treated with three ART regimens, and found that efavirenz-based therapy gave a faster initial viral decay than protease inhibitor (PI) treated subjects. In turn, lopinavir/ritonavir-based therapy gave a faster initial viral decay than atazanavir/ritonavir-based therapy. This may reflect different inherent antiretroviral potency between the treatment regimens.

Latently infected $CD4^+$ T-cells constitute a major barrier for the eradication of HIV-1 infection. We investigated if a high dose of intravenous immunoglobulin (IVIG) given in addition to effective ART could reduce the size of the pool of latently infected resting cells, and found a reduction in the pool size in five of seven individuals where the latent reservoir was quantifiable. Our findings suggest that the reservoir became accessible through IVIG treatment, and indicate that novel modes of intervention can have an effect on the latent reservoir.

Increased levels of intrathecal immune activation are often found in cerebrospinal fluid (CSF) of treated patients despite effective systemic suppression of HIV-1. We investigated intrathecal immune activation, measured as neopterin and IgG-index, in patients with several years of successful therapy, and found that although ART has a substantial effect on lowering viral replication and immune activation in the CSF, a majority of patients still have ongoing intrathecal immune activation despite effective suppression of the virus for extended periods of time.

Occasional cases of CSF viral escape have been reported. We investigated the occurrence of CSF viral escape in neuroasymptomatic patients effectively treated with commonly used ART regimens. We found that 7 (10%) of 69 patients had evidence of CSF viral escape, which is more common than previously recognized and may have important implications for future treatment strategies and the use of new drug combinations.

Keywords: HIV-1; antiretroviral therapy; latency; cerebrospinal fluid; central nervous system; efavirenz; lopinavir; atazanavir; neopterin; viral decay.

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SAMMANFATTNING PÅ SVENSKA

Humant immunbristvirus (HIV) infekterar och skadar viktiga celler i kroppens immunförsvar. När immunförsvaret blivit så nedsatt att kroppen inte längre kan försvara sig mot infektioner leder det till immunbristsyndromet AIDS. HIV är spritt över hela världen och många miljoner människor smittas av viruset och dör av dess skadeverkningar varje år. Idag finns ett flertal effektiva läkemedel som bromsar virusets förmåga att föröka sig, men trots detta kan infektionen inte botas, utan återkommer snabbt om man slutar med medicineringen. Viruset har förmågan att gömma sig i så kallade reservoarer, där behandlingen inte har någon effekt. En typ av reservoar utgörs av ”sovande” immunförsvarsceller. Där kan virus finnas vilande (”latent”) inne i värdcellens arvsmassa i en inaktiv form som inte påverkas av behandling. En annan typ av reservoarer är anatomiska vävnader som har egenskaper som gör att behandlingen där skiljer sig åt från resten kroppen, och en viktig sådan vävnad är det centrala nervsystemet (CNS). I den här avhandlingen har jag studerat olika aspekter av kvarvarande, eller ”persisterande”, infektion med HIV.

En förutsättning för att kunna bromsa virusets förmåga att föröka sig och skada immunförsvaret är att vi har tillgång till effektiva bromsmediciner. Ett sätt att jämföra hur effektiv en behandling är, är att mäta hur snabbt virusnivåerna i blodet sjunker efter att man påbörjar medicinering. Vi har jämfört hur snabbt viruset sjunker efter start av medicinering mellan tre olika kombinationsbehandlingar mot HIV baserade på endera av läkemedlen efavirenz, lopinavir eller atazanavir. Vi fann att patienter som behandlades med kombinationer av läkemedel innehållande efavirenz sjönk snabbare i virustal än vad patienterna gjorde som behandlades med någon av de andra kombinationerna. Detta kan innebära att kombinationer innehållande efavirenz är mer potenta i att bromsa viruset än de båda andra kombinationerna, men för att se om detta stämmer måste man se hur bra behandlingarna fungerar på längre sikt.

Sovande immunförsvarsceller, så kallade ”minnesceller”, är viktiga för att vi snabbt ska kunna försvara oss mot infektioner, och dessa celler kan leva i kroppen under mycket lång tid i väntan på att de ska behövas. Problemet är att minnescellerna kan infekteras med HIV och bära med sig viruset lika länge som de lever, och detta är en av de viktigaste anledningarna till att infektionen finns kvar i kroppen trots effektiv behandling. Vi har studerat om intravenöst immunoglobulin, förkortat IVIG (så kallat ”gammaglobulin”) givet i höga doser kan minska andelen av minnescellerna i kroppen som bär på viruset. Vi fann att andelen infekterade minnesceller minskade hos en

majoritet av de patienter vi undersökte efter att de fått behandling med IVIG i tillägg till vanlig behandling med bromsmediciner. Detta tyder på att det går att påverka reservoaren av infekterade minnesceller med nya typer av behandling, även om det återstår mycket forskning innan vi vet om det är något som innebär någon fördel för patienter på lång sikt.

HIV infekterar även hjärnan och kan där orsaka nervskador, och hos patienter med långt gången infektion en typ av demensliknande sjukdom som kan vara svårt handikappande för patienterna. Lyckligtvis är detta ovanligt om man har tillgång till behandling, eftersom bromsmedicinering är effektiv även i CNS. Däremot är det troligt att viruset kan finnas kvar i hjärnan trots behandling, precis som det kan göra i övriga kroppen. Det är inte säkert att läkemedel fungerar fullt ut i CNS, eftersom hjärnan omges av en skyddande barriär, den så kallade blod-hjärn-barriären, som hindrar många läkemedel från att tränga in i CNS. Dessutom infekterar viruset celltyper i hjärnan som kan leva under mycket lång tid och därmed skulle kunna bära på viruset länge.

Vi undersökte tecken på inflammation (dvs. ett retningstillstånd som orsakas av en infektion med ett smittämne) i ryggvätskan hos patienter som fått effektiv HIV-behandling under flera års tid. Trots att de inte haft något mätbart virus i kroppen under lång tid kunde vi se att de flesta ändå hade tecken på inflammation i hjärnan om vi jämförde med friska personer. Detta kan tyda på att virus kan fortsätta att föröka sig i hjärnan trots att man får effektiv bromsmedicinering, men för att ta reda på om det verkligen är så måste man göra ytterligare forskning på området.

Vi har också undersökt hur vanligt det är att man kan hitta virus i ryggvätskan på patienter som får så effektiv behandling att vi inte kan mäta något virus i blodet. Vi fann att tio procent av de patienter vi undersökte faktisk hade påvisbart virus i ryggvätskan, vilket är en betydligt större andel än vad man vetat om tidigare. En möjlig förklaring till att det är så är att vissa av de nyare läkemedlen inte kan ta sig in i hjärnan tillräckligt effektivt. För att kunna ta reda på om det verkligen är så måste vi göra fler undersökningar, där man tittar på hur det förhåller sig hos ett större antal patienter.

HIV som finns kvar i kroppen trots effektiv behandling förhindrar att infektionen kan botas. För att komma närmare en slutlig bot av infektionen måste vi lära oss mer om vilka läkemedel som är mest effektiva och hur man kan komma åt virus som inte påverkas av behandlingen, till exempel i vilande minnesceller och i hjärnan. Min förhoppning är att de arbeten som ingår i denna avhandling kan bidra på något sätt till all den ökade kunskap som behövs för att bättre behandla HIV.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Arvid Edén, Lars-Magnus Andersson, Örjan Andersson, Leo Flamholc, Filip Josephson, Staffan Nilsson, Vidar Ormaasen, Veronica Svedhem, Christer Säll, Anders Sönnerborg, Petra Tunbäck, Magnus Gisslén. **Differential Effects of Efavirenz, Lopinavir/r and Atazanavir/r on the Initial Viral Decay Rate in Treatment Naïve HIV-1 Infected Patients**
AIDS Research and Human Retroviruses, in press

II. Annica Lindkvist*, Arvid Edén*, Melissa M Norström, Veronica D Gonzalez, Staffan Nilsson, Bo Svennerholm, Annika C Karlsson, Johan K Sandberg, Anders Sönnerborg and Magnus Gisslén. **Reduction of the HIV-1 reservoir in resting CD4+ T-lymphocytes by high dosage intravenous immunoglobulin treatment: a proof-of-concept study**
*AIDS Research and Therapy 2009, 6:*15; *equal contributors

III. Arvid Edén, Richard W. Price, Serena Spudich, Dietmar Fuchs, Lars Hagberg, and Magnus Gisslén. **Immune Activation of the Central Nervous System Is Still Present after >4 Years of Effective Highly Active Antiretroviral Therapy**
Journal of Infectious Diseases 2007; 196:1779–83

IV. Arvid Edén, Dietmar Fuchs, Lars Hagberg, Staffan Nilsson, Serena Spudich, Bo Svennerholm, Richard W Price, Magnus Gisslén. **HIV-1 viral escape in cerebrospinal fluid of subjects on suppressive antiretroviral treatment**
Submitted

CONTENT

ABBREVIATIONS

ADC	AIDS dementia complex
ANI	Asymptomatic neurocognitive impairment
ART	Antiretroviral therapy
BBB	Blood-brain-barrier
BCB	Blood-CSF-barrier
CCR5	Cysteine-cysteine chemokine receptor
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CPE	CNS penetration effectiveness
CSF	Cerebrospinal fluid
CXCR4	Cysteine-x-cysteine chemokine receptor
DNA	Deoxyribonucleic acid
FI	Fusion inhibitor
GALT	Gut-associated lymphoid tissue
GU tract	Genitourinary tract
HAART	Highly active antiretroviral therapy
HAD	HIV associated dementia
HAND	HIV-associated neurocognitive disorders
HIV	Human immunodeficiency virus
HPC	Hematopoietic progenitor cell
IL-2	Interleukin 2
IL-7	Interleukin 7
IVIG	Intravenous immunoglobulin
LTR	Long terminal repeat
MND	HIV-1 associated mild neurocognitive disorder
NNRTI	Non-nucleoside reverse transcriptase inhibitor

NRTI	Nucleoside reverse transcriptase inhibitor
PCR	Polymerase chain reaction
PI	Protease inhibitor
RNA	Ribonucleic acid
SIV	Simian immunodeficiency virus
STI	Structured/strategic treatment interruption
T_{reg}	Regulatory T-cell
WBC	White blood cell

1 INTRODUCTION

1.1 The HIV epidemic

In 1981, the first cases of what was later termed acquired immunodeficiency syndrome (AIDS) was described in previously healthy young Californian men suffering from *Pneumocystis carinii* (*jiroveci*) pneumonia (PCP)[1]. Only a few years later the causative agent, the human immunodeficiency virus type-1 (HIV-1) was identified [2]. Since its discovery, increased understanding of the scope of the global epidemic has led to the recognition that the HIV-epidemic constitutes one of the most dramatic challenges to human health and development worldwide. The HIV epidemic now affects more than 30 million people globally, with an estimated 2.7 million newly infected people, and 2 million AIDS-related deaths occurring in 2007 [3]. Since the beginning of the epidemic, an estimated 25 million people have died of HIV-related causes. Sub-Saharan Africa remains the region most heavily affected, containing 67 % of the worlds HIV-infected individuals (Figure 1), and in these countries, the HIV epidemic has had dramatic consequences for society, affecting the age distribution of national populations, slowing economic growth and increasing poverty [3].

Although the prevalence of infection has declined since the year 2000, infection rates remains high, and the total number of people living with HIV has increased due to higher infection rates than the number of HIV-related deaths. While the epidemic in sub-Saharan Africa appears to have stabilized, it continues to grow alarmingly in other regions of the world, such as Eastern Europe and Asia. Heterosexual transmission is the most important mode of transmission worldwide, and remains the driving force behind the epidemic in southern Africa, while intravenous drug use is a major contributor to the epidemics in Eastern Europe and Asia. Since the overlap between intravenous drug use and commercial sex work in these regions is considerable, there is a significant risk for the development of an extensive sexually transmitted epidemic in these regions [3]. In Sweden, HIV prevalence remains low, although a slight increase has occurred in recent years [4]. However, condom use in Sweden is low and has decreased in recent years [5], as illustrated by

the massive increase in incidence of Chlamydia infection in the last decade, which is a cause for concern regarding the risk for HIV transmission [6].

1.2 The origin of HIV

Two distinct viruses cause AIDS in humans, HIV-1 and HIV-2 [7]. Of the two, HIV-1 is the virus primarily responsible for the global HIV epidemic, while HIV-2 is more geographically restricted [8]. HIV-1 can further be divided into three groups; M (main), N (non-M, non-O) and O (outlier). The M group is the cause of the global epidemic, and can be further divided into subtypes (A, B, C, D, F, G, H, J and K), circulating recombinant forms (CRF) and unique recombinant forms (URF) [8-11]. Compelling phylogenetic evidence demonstrates that HIV-1 and HIV-2 originate from simian immunodeficiency virus (SIV) in African non-human primates. The natural reservoir of HIV-1 is the chimpanzee subspecies *Pan troglodytes troglodytes* which harbors the closely related SIV_{cpz} virus [12-13] that has, with the possible exception for group O [14], been transmitted to humans as HIV-1. These primates are found in southern Cameroon, and this region is considered as the epicenter of the HIV-1 epidemic (Figure 2) [13, 15]. HIV-2 closely resembles the SIV_{sm} found in West African sooty mangabey (*Cercocebus torquatus atys*) monkeys [16-17]. Both animals come into close contact with humans both as sources of meat and as pets, and direct exposure to animal blood through butchering or consumption of contaminated animals is a likely route of transmission to humans [18]. Although both HIV-1 and HIV-2 can cause immunodeficiency, HIV-2 has a lower transmission rate and is less virulent compared to HIV-1 and does not cause AIDS in all infected individuals [16-17]. HIV-2 is not discussed further in this thesis.

HIV-1 likely entered the human population in the beginning of the twentieth century [18-20]. The earliest known case of HIV-1 was retrospectively identified in a plasma sample obtained in 1959 in Leopoldville, now Kinshasa, in the Democratic Republic of Congo [21]. However, even if virus was present in humans as early as the year 1900, the epidemic did not pick up speed until later in the century. Several possible factors may have contributed to the acceleration of the HIV-1 epidemic; increased travel, urbanization, enslavement, prostitution and societal disruption in the beginning of the century have been proposed to have facilitated the spread of the epidemic. In addition, the increased use of injections using unsterile medical equipment

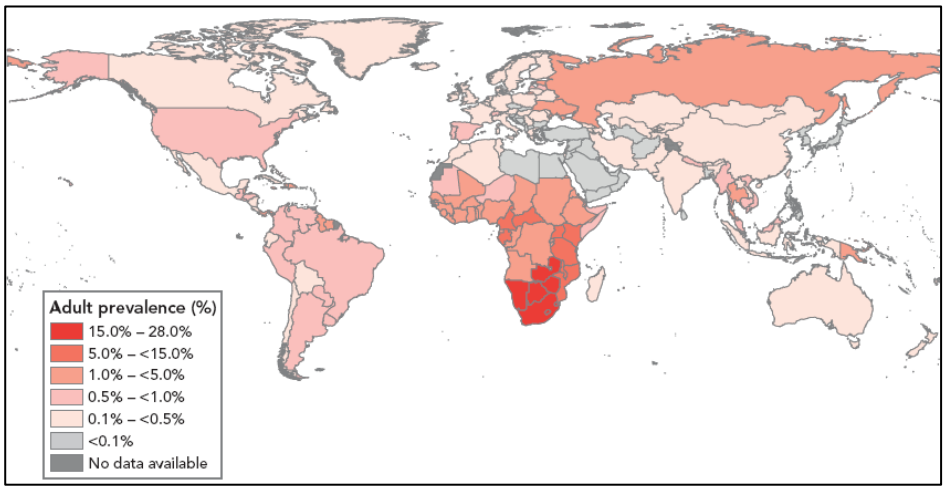

Figure 1. A global view of HIV infection. Highest prevalence rates are found in sub-Saharan Africa, where up to a quarter of the population is infected with HIV. High rates of transmission are now seen in Asia and Eastern Europe. (Source: UNAIDS 2008 Report on the global AIDS epidemic)

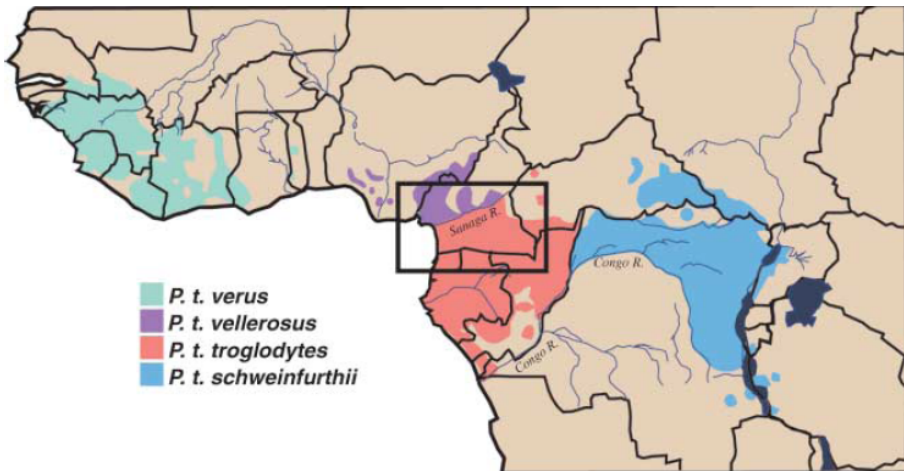

Figure 2. Natural ranges of the four chimpanzee species in western Africa. The natural reservoir of HIV-1 is the subspecies P. t. troglodytes resident in southern Cameroon. (From [13]. Reprinted with permission from AAAS.)

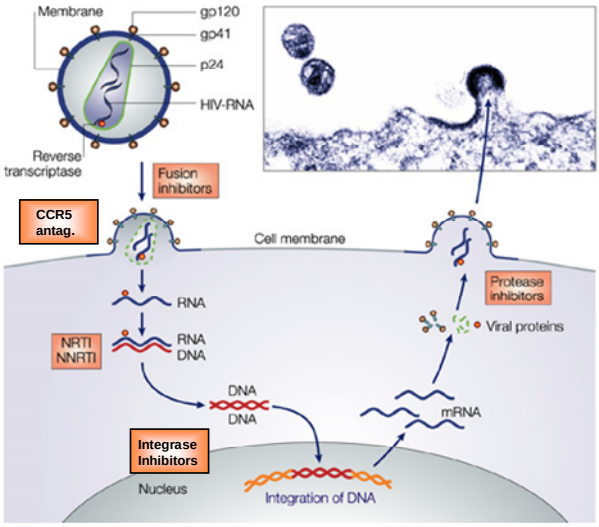

Figure 3. The life-cycle of HIV. HIV-1 enters the target cell by fusion. Subsequent steps in the viral life-cycle involve reverse transcription of viral RNA, integration of proviral DNA into the host cell genome and assembly of viral proteins into new virions budding from the cell surface. Adapted from [22] (Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Microbiol, copyright 2003, reference [22].)

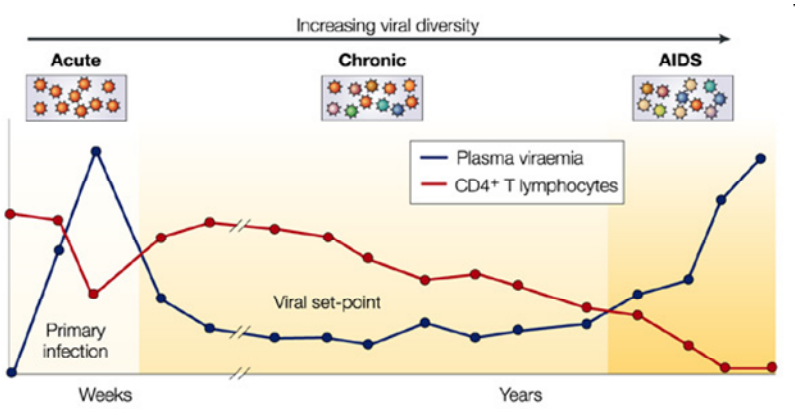

Figure 4. The natural course of untreated HIV-1 infection. After an initial peak, viral load stabilizes at a set-point (blue line). With disease progression, $CD4^+$ T-cell count gradually declines over a period of years (red line). (Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Microbiol, copyright 2003, reference [22].)

during medical treatment or vaccination campaigns may have promoted viral adaptation to the human host by serial passage in humans [18, 23-24].

1.3 The lifecycle of HIV

HIV-1 is a retrovirus belonging to the genus *Lentivirus*, and as a retrovirus carries an RNA genome that is transcribed into DNA by the use of viral reverse transcriptase after the virion enters the target cell. The viral genome contains 9 genes encoding 16 viral proteins; three major genes (*gag, pol, env*) encoding structural proteins and three viral enzymes: protease, integrase and reverse transcriptase (RT); two regulatory (*rev, tat*) and four accessory (*vif, vpu, nef, vpr*) genes [25]. The viral surface protein gp120 of HIV-1 binds to the cluster of differentiation (CD) 4 receptor on the host cell, inducing a conformational change that enables binding to a β–chemokine coreceptor, either CCR5 or CXCR4 [25-28]. The CD4 receptor is expressed on the surface of T lymphocytes, monocytes, macrophages, microglia and dendritic cells [29]. During the earlier part of the infection, viral strains (called R5 or M-tropic strains) use the CCR5 coreceptor, primarily expressed on activated memory $CD4^+$ T-cells and macrophages. At later stages of the disease, about 50 % of infected individuals experience a shift in viral tropism to a predominately CXCR4-tropic (X4 or T-tropic strains) or mixed R5/X4 (dualtropic strains) viral population. The shift to the use of CXCR4, expressed mainly on naïve T-cells, is usually accompanied by a rapid decline in $CD4^+$ T-lymphocytes numbers and clinical progression to AIDS [26, 28-33]. After binding to the cell surface, fusion of the viral and cell membranes allows the virus to enter the cell (Figure 3). By reverse transcription, the RNA genome is transcribed into a DNA intermediate (unintegrated provirus) that is subsequently transported to the nucleus and integrated into the host cell genome by viral integrase [25]. The process of reverse transcription is very error-prone, likely due to the lack of proof-reading capacity of RT. As a consequence, the virus is highly mutagenic, allowing it to evade neutralizing antibodies and to develop resistance to antiretroviral agents [34-36]. Following integration, production of viral proteins and assembly of new virions takes place at the cell surface [25].

1.4 Natural course of HIV-1 infection

After infection with HIV-1, the virus rapidly multiplies in the infected host, and reaches high levels in plasma within weeks of transmission (Figure 4) [37]. Parallel to the rapid rise in viremia, the CD4 cell count falls [38]. During this primary infection phase, a majority of infected patients develops clinical symptoms, called acute retroviral syndrome, typically characterized by fever, fatigue, sore throat, myalgia, headache, lymphadenopathy and rash [39-40]. At this stage, infected individuals have a high risk of disease transmission due to the high levels of plasma viremia. After an additional period of a few weeks, viral load begins to decrease as HIV-1 specific immune responses develop [41]. During the subsequent chronic phase of infection, plasma viremia stabilizes at a viral set-point, which varies significantly between individuals. The level of the viral set-point has been shown to be predictive of the long-term prognosis, where higher levels of viremia is associated with a more rapid loss of $CD4^+$ T-cells and progression to AIDS [42-44]. During the chronic phase of disease, patients have few clinical symptoms; however, virus replication proceeds at high rates in blood and lymphoid tissues as $CD4^+$ T-cells are continuously destroyed and replenished [45-47]. Over a period of years, the CD4 cell count is gradually depleted, and with progressive immunosuppression, the infected individual becomes susceptible to opportunistic infections and malignancies leading to the diagnosis of AIDS (Figure 4). The time from primary infection to development of AIDS is highly variable, but in average is around 10 years [48]. The diagnosis of AIDS is defined by the occurrence of clinical AIDS-defining conditions. . In the American classification system designed by the Centers for Disease Control and Prevention (CDC), a $CD4^+$ T-cell count $<200 \times 10^6$/l is also defined as AIDS.

1.5 Antiretroviral treatment of HIV-1

The first antiretroviral drug to become available for the treatment of HIV-1 infection, the nucleoside reverse transcriptase inhibitor (NRTI) zidovudine, was introduced as early as 1987, only a few years after the virus was identified. However, monotherapy with zidovudine or other NRTIs developed subsequently had only transient effects at best due to the rapid emergence of drug resistance, and did not prevent disease progression [49]. The turning point came in 1995 and 1996, when the first protease inhibitors

(PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) were registered for use. By combining drugs with different mechanisms of action, a potent inhibition of viral replication was achieved, and such drug combinations were aptly named highly active antiretroviral therapy (HAART). The potency of HAART led to hopes that the virus could be eradicated by treatment, and that therapy should be initiated as early as possible ("Hit early and hard" [50]). HAART was subsequently shown to have dramatic effects on disease progression in clinical trials [51-52]. The hope for cure of the infection by HAART treatment was tempered by the discovery of a reservoir of latently infected cell capable of sustaining HIV-1 infection even during potent therapy [53-55]. It was also recognized that antiretroviral drugs had important side effects, leading to a shift in treatment strategy to waiting as long as possible before treatment initiation. However, when available, HAART has had a dramatic impact on reducing AIDS-related disease and death [56-57].

Table 1. Antiretroviral drugs currently used in Sweden.

Class	Group	Generic name	Abbreviation	Trade name
Reverse transcriptase inhibitors				
	Nucleoside analogues (NRTI)			
		abakavir	ABC	Ziagen
		didanosin	ddI	Videx
		emitricitabin	FTC	Emtriva
		lamivudin	3TC	Epivir
		stavudin	d4T	Zerit
		tenofovir	TDF	Viread
		zidovudin	AZT, ZDV	Retrovir
	Non-nucleoside analogues (NNRTI)			
		efavirenz	EFV	Stocrin
		nevirapin	NVP	Viramune
		etravirin	ETR	Intelence
Protease inhibitors (PI)				
		atazanavir	ATV	Reyataz
		darunavir	DRV	Prezista
		fosamprenavir	fAPV	Telzir
		indinavir	IDV	Crixivan
		lopinavir	LPV	Kaletra
		nelfinavir	NFV	Viracept
		saquinavir	SQV	Invirase
		ritonavir*	RTV	Norvir
		tipranavir	TPV	Aptivus
Integrase inhibitors (II)				
		raltegravir	RAL	Isentress
Entry inhibitors				
	Fusion inhibitors (FI)			
		enfuvirtid	T-20	Fuzeon
	CCR5 antagonists			
		maraviroc	MVC	Celsentri

* only used for boosting other PIs

The principle for antiretroviral therapy (ART) of HIV-1 infection is to combine three active drugs from at least two different drug classes (Figure 3) to achieve potent inhibition of viral replication. Over 20 different drugs from 4 different classes are now available for treatment of HIV-1 in Sweden (Table 1) [58]. Current Swedish guidelines recommend the use of two NTRIs in combination with either a ritonavir-boosted PI (PI/r) or a NNRTI as first-line therapy for previously treatment naïve patients [58].

The virological goal of ART is to maintain plasma HIV-1 RNA below the detection level of clinical assays (<50 copies/ml), which is often achievable in adherent patients without multiple drug resistance. Thereby immune function is maintained and disease progression prevented. However, adherence to therapy is crucial for the success of therapy. The high error-rate of viral reverse transcriptase leads to rapid emergence of drug resistance mutations if suboptimal drug concentrations fail to effectively inhibit viral replication [34].

2 PERSISTENCE, LATENCY AND VIRAL RESERVOIRS

It has become evident that suppression of viral replication by the use of antiretroviral therapy is not in itself sufficient for clearing the infection. Virus persists despite treatment, and if antiretroviral therapy is halted, a rapid rebound in viremia is usually seen [59-60]. Residual, low-level viremia can be detected in most treated subjects if sensitive assays are used [61]. Viral persistence may potentially arise from reactivation of long-lived cells infected before therapy was initiated. Alternatively, persistent viremia may result from incomplete suppression of viral replication by antiretroviral therapy, related either to insufficient efficacy of antiretroviral drugs or to insufficient penetration of drugs into distinct anatomical compartments [62-63]. A viral reservoir may thus be defined as a compartment where replication-competent virus can persist for a longer time than is the case in the main pool of actively replicating virus, whether it arises from activation of long-lived cell populations or from anatomical compartments [64]. In this section, I will review important aspects of viral persistence and cellular as well as anatomical reservoirs.

2.1 Dynamics of viral decay

When antiretroviral therapy is initiated, plasma viral load decreases as antiretroviral drugs suppress viral replication and prevent infection of new target cells. Free plasma virus has a short half-life of up to 6 hours [65], and consequently viral load in plasma is strongly correlated to the lifetime of productively infected cells releasing HIV into the blood. The rate of viral decay after initiation of therapy is therefore dependent on the half-life of the cells producing HIV [62]. Several phases of viral decay during antiretroviral therapy can be identified (Figure 5).

After a short lag of 1-2 days, a rapid, exponential decrease in plasma viral load is seen during the first days of therapy [46, 66]. The absolute majority of plasma virus in untreated HIV infection is produced by activated and productively infected $CD4^+$ T-cells, cells that have a short half-life of 1-2 days [46, 63, 65-68], and the rapid initial drop in plasma HIV-1 RNA after

treatment initiation is attributed largely to the subsequent block of infection of this cell population [22, 69]. The initial rapid first phase is followed by a more gradual second phase of viral decay over the following months. During the second decay phase, patients on successful therapy suppress plasma viral load to below the limit of detection of standard assays used in clinical practice (50 HIV-1 RNA copies/ml) [51-52]. The slower decay rate of the second phase has been attributed to other populations of virus-producing cells, with longer life spans than activated CD4+ T-cells, and an estimated half-life of 14-21 days [70]. The source of the phase 2 viremia is not entirely clear, although phase 2 decay has been attributed to virus produced by macrophages, partially activated $CD4^+$ T-cells, or release of trapped viral particles from follicular dendritic cells [64, 70-74].

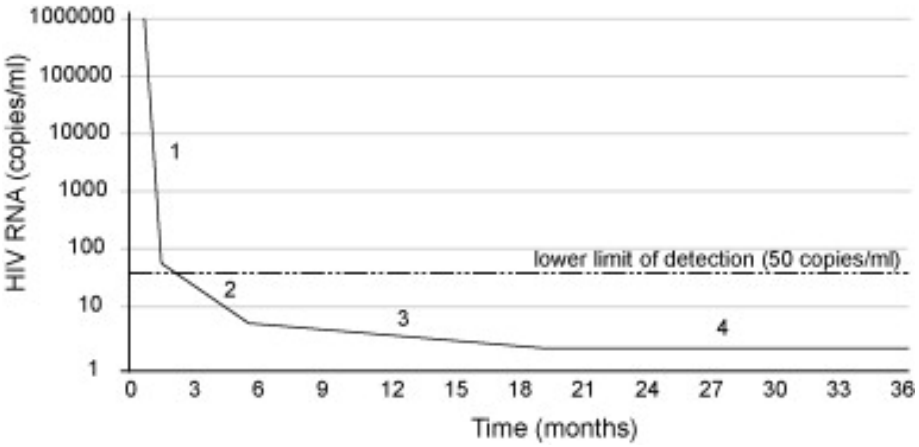

Figure 5. Phases of viral decay after initiation of ART. During phase 1, a rapid drop in plasma viral load is seen. During the more gradual phase 2, viral load decreases below the detection limit of clinical assays. During phases 3 and 4, viral load is stable, or decays at a very slow rate. Dotted line shows the limit of detection (50 copies/ml) of clinical PCR assays. (Reprinted from Antiviral Research, reference [62], Copyright (2010), with permission from Elsevier.)

Based on the rate of the decay of phase 2 viremia, it was initially estimated that the HIV-1 infection could be eliminated in 2-3 years with completely inhibitory treatment [70]. However, as mentioned above, it soon became apparent that additional sources of virus were not eliminated in such a short time span [55, 64, 74-77]. One important barrier to eradication is latently

infected, resting memory $CD4^+$ T-cells, which will be discussed in further detail below. Using more sensitive PCR assays, capable of detecting and quantifying low-level viremia [78], it was shown that a majority of subjects on suppressive antiretroviral therapy with plasma HIV-1 RNA below the limit of detection of clinical assays (<50 copies/ml) still had low-grade, but detectable viremia ≥1 copy/ml [61]. This residual viremia was found to be related to pre-treatment levels of HIV-1 RNA in treated subjects, but not to the specific antiretroviral regimens used. Additional longitudinal studies showed that a third and fourth phase of viral decay under antiretroviral therapy could be identified [62, 79].

As is the case with the initial viral decay, the decay of residual viremia appears to be biphasic. During suppressive therapy, a third phase of decay with a slow rate of decline of viral load corresponds to a cell population with a half-life of approximately 9 months. Latently infected $CD4^+$ T-cells, which have a reported half-life of 6-44 months [74, 80], is a possible source of the plasma virus in the third phase of decay, and likely also contribute to the fourth phase of viremia, during which no observable decline in HIV-1 RNA can be detected. The lack of observable decay during the fourth phase of HIV-1 RNA viremia may suggest that a small number of infected cells with a high degree of stability are present in individuals with chronic HIV-1 infection [79], and it has been hypothesized that infection of a cell that has proliferative capacity, such as a stem cell of monocyte-macrophage lineage, may contribute to the stability of residual phase four viremia [81-82].

2.2 Viral latency

HIV has the ability to establish a latent infection, where viral provirus is present in the host cell, without resulting in active transcription or production of new virions. Latent infection can occur either at the pre-integration or post-integration level of the viral life cycle [83]. Pre-integration latency occurs when HIV enters non-dividing resting lymphocytes, where reverse transcription can take place, but subsequent integration of viral DNA into the host cell genome and production of new virus particles is halted [84-86]. The unintegrated viral DNA is labile and decays rapidly, with an estimated half life of 1-5 days [84, 87-88], thus making it unlikely that pre-integration viral DNA contributes to long-term viral persistence in any significant way.

Post-integration latency is thought to be established when active $CD4^+$ T-cells are infected with HIV-1 before reverting to a resting state as memory

cells. The result is a stably integrated form of the virus, where proviral DNA can persist as integrated DNA in the host cell genome [89]. In a resting state, memory cells have a low metabolic rate and transcriptional activity, and the integrated provirus can remain transcriptionally silent as long as the host cell remains in a resting state. Upon activation of the host cell, viral production can resume; however, in the resting state latently infected cells do not produce virus and are thus not affected by antiretroviral drugs [62, 85, 90]. Although not significant in untreated individuals, the ability of HIV to establish a latent infection has important implications for treatment of the infection, as it provides a mechanism for the virus to persist when active replication is suppressed by antiretroviral drugs. Latently infected memory CD4 cells are present in patients despite effective ART [53-55], constituting a major barrier for eradication of the infection.

The pool of latently infected memory CD4 cells is established already during primary HIV-infection [91], and although the size of the latent reservoir is estimated to be quite small [54, 92], it is highly stable. Initial estimates calculated a half life of latently infected cells to roughly 6 months, which indicated that continuous effective ART with suppression of viral replication would be able to eliminate the latently infected CD4 cells over a time of seven to ten years [74]. However, additional studies have shown that latently infected memory CD4 cells decay very slowly even in subject treated for several years with antiretroviral therapy, with a half life that may be as long as 44 months or more. This indicates that it would take over 60 years of effective therapy to deplete the latent reservoir, making eradication of infection under current treatment regimens all but impossible [77, 80]. In addition, it has recently been shown that HIV-1 can infect bone marrow derived hematopoietic progenitor cells (HPC) establishing both active and latent infection. These cells may be long lived and could carry latent HIV-1 for extended periods of time [93]. In another recent study by Chomont and colleagues, it was shown that integrated HIV-1 DNA can be found in different subsets of memory $CD4^+$ T-cells in individuals on ART, mainly in central memory (T_{CM}) and transitional memory (T_{TM}) T-cells. In patients responding well to treatment or starting therapy early in the course of infection, thus maintaining higher CD4 cell counts, T_{CM} cells appeared to be the main long term reservoir. The low degree of proliferation in these cells allows them to survive for long periods of time, providing a possible long-lasting reservoir for HIV-1. In subjects with low CD4 cell counts, HIV-1 DNA was preferentially found in T_{TM} cells that persist by low-level homeostatic proliferation, also making them a very stable viral reservoir [94].

2.3 Persistent viremia

As has been discussed above, persistent low-level viremia is a common feature in patients treated with highly active antiretroviral therapy. An important question to address is whether residual viremia is the result of ongoing cycles of replication even under suppressive ART, or rather signifies a release of virus from stable reservoirs, infected before the initiation of therapy (Figure 6). When the presence of residual viremia was initially identified, it was assumed that viral replication was responsible for the plasma virus detected despite effective treatment [76]. However, this view has been challenged based on additional studies on the nature of residual viremia [82].

Viral evolution during ongoing therapy would suggest the presence of ongoing replication; however, studies on viral evolution have given somewhat conflicting results. Some studies have found signs of viral evolution [74, 95]. A recent study of a therapeutic vaccine found sequence evolution that was correlated to episodes of quantifiable residual viremia in a small subset of subjects, although residual viremia related to virus from the latent reservoir was found in others [96]. Several reports have shown no viral evolution in treated patients [81, 97-100], indicating that ART completely stops viral replication at least in some subjects [82]. In a study of patients interrupting therapy during structured treatment interruptions (STI), rebounding virus populations resembled pretreatment virus and did not show evidence of genetic evolution over time [101]. The lack of new resistance mutations detected in patients on ART with suppressed plasma viremia (<50 copies/ml) further argues against ongoing viral replication, and points to the release of virus from stable cellular reservoirs as an important source for residual viremia [97-98, 100, 102]. Virus isolated from resting memory $CD4^+$ T-cells has been shown to be closely related to residual plasma virus populations found in subjects with ongoing ART, thus pointing to the latent reservoir as the source of residual viremia in these patients [81-82, 97, 100].

It has been suggested that ongoing replication, if present, would permit replenishment of the latent reservoir [103-106]. However, by the study of predominant plasma clones (PPC) present in a subset of individuals under ongoing ART, Sedaghat and colleagues failed to demonstrate any temporal evolution of sequences in the latent reservoir, indicating that replenishment of the reservoir due to ongoing viral replication does not occur [81-82, 107]. Although results from viral evolution studies may have varying conclusions,

it is important to note that for some individuals, no evidence of ongoing replication can be seen, thus indicating that ART has the potential to fully inhibit viral replication at least in some cases. Differences in residual viremia for varying treatment regimens have been reported in a cross-sectional study lacking pretreatment characteristics [108]; however, the correlation between the level of residual viremia and pretreatment viral load, but lack of correlation to antiretroviral drug regimen demonstrated in longitudinal studies further implicates events occurring before the initiation of therapy as crucial to the residual viremia seen in patients on ART [61, 79].

If persistent viremia results from ongoing cycles of HIV-1 replication despite antiretroviral treatment, it can be assumed that intensifying treatment by adding additional active drugs to the treatment regimens used would have an effect on the level of residual viremia. However, in recent reports, this has not been the case. In patients with suppressive ART (HIV-1 RNA <50 copies/ml), adding an additional active drug from a drug class not previously used by the study subjects had no effect on the level of residual virema [109]. Addition of a fusion inhibitor or integrase inhibitor to standard ART regimens did not affect the decay rate of the latent reservoir [110], the frequency of infection of resting memory $CD4^+$ T-cells, or low-level residual viremia [111]. After intensification with abacavir or efavirenz to protease-inhibitor based regimens, a decrease in the number of episodes of transiently detectable viremia (viral blips), has been reported [105]. However, viral blips may be a consequence of low-level variations in plasma HIV-1 RNA close to the level of detection, representing release from stable reservoirs and not ongoing viral replication [102]. Thus, intensification of ART has not convincingly been proven effective in reducing residual viremia in subjects with ART. Moreover, when simplifying ART to boosted protease inhibitor monotherapy, increased levels of residual viremia preceded virologic failure in subjects for whom monotherapy was not effective in controlling viremia, and viral replication was later evident [112].

Interestingly, in a recent study, a transient increase in episomal 2-LTR circles was seen in a subset of subjects after intensification of suppressive ART with raltegravir [113]. Raltegravir inhibits integration of linear HIV-1 cDNA into the host cell genome; instead viral DNA is converted to episomal cDNAs [114]. The increase in episomal cDNA after adding raltegravir to previous treatment regimens may represent ongoing viral replication in a subset of the patients studied [113]; however, another recent study found no discernable effect on residual viremia after raltegravir intensification, and thus no indication of ongoing replication, in patients with highly suppressive therapy [115].

2.4 Sanctuary sites

Studies of residual viremia and trials of intensification of ART do not fully exclude the possibility that HIV-1 may persist in sanctuary sites where ongoing replication may be possible either because of limited penetration of antiretroviral drugs or special biological properties of these compartments. Anatomical locations such as the central nervous system (CNS) and genitourinary (GU) tract, as well as tissues such as the gut-associated lymphoid tissue (GALT) are regarded as distinct compartments of HIV-1 infection [62-63].

A majority of HIV-1 replication during untreated infection takes place in lymphoid organs, such as lymph nodes and the GALT [116]. GALT $CD4^+$ T-cells are depleted during untreated infection, and immune reconstitution after initiation of ART is impaired [117]. The high frequency of infected cells as well as possible cross-infection between the blood and GALT compartment may indicate persistent replication, and the possibility that the GALT may act as a reservoir for HIV-1 infection [118].

The GU tract is also considered as a potential reservoir for HIV-1 infection. HIV-1 has been detected in several cell types in seminal fluid [119-120]. Differences in viral load and viral sequences between seminal fluid and blood in untreated individuals [121], as well as reduced penetration of antiretroviral drugs into seminal tissue [122-123], suggest that the GU tract may act as a separate compartment of infection. Antiretroviral therapy reduces viral load in seminal fluid, although detectable virus is still found in some individuals on suppressive therapy, indicating that the GU tract may be a potential reservoir for viral persistence in HIV-1 infection [76, 124-126].

Another important compartment of HIV-1 infection is the central nervous system, to which I will turn in the following section.

3 HIV-1 AND THE CENTRAL NERVOUS SYSTEM

Human immunodeficiency virus type-1 is a neurotropic virus, and infection of the CNS begins during the primary systemic infection [39, 127-128]. HIV-1 remains detectable in cerebrospinal fluid (CSF) of most infected individuals at all stages of the disease [129-130]. Occasionally, patients experience neurological symptoms during primary infection, mainly in the form of aseptic meningitis [131-132]. However, the majority of CNS complications to chronic HIV-1 infection occur as immune function deteriorates with progressive disease, including CNS opportunistic infections and malignancies, and HIV associated dementia (HAD), also described as the AIDS dementia complex (ADC) [131, 133-134]. Opportunistic diseases commonly seen in advanced HIV-1 disease include cerebral toxoplasmosis, progressive multifocal leucoencephalopathy (PML), cryptococcal meningitis, CNS lymphoma and cytomegaloviral (CMV) encephalitis [135]. HAD, seen in about 20% of untreated individuals with advanced disease is directly caused by the HIV-1 infection itself.

HAD is a clinical syndrome including cognitive, motor, and behavioral dysfunction [136-137]. The diagnosis of HAD is based on a clinical and neuropsychological evaluation of symptoms and the exclusion of other ongoing CNS diseases or preexisting comorbidities that can explain neuropsychological impairment [138]. With the advent of ART, the incidence of HAD has been greatly reduced [139], and is now almost exclusively seen in untreated patients, or patients failing ART because of drug resistance or nonadherence [140]. Moreover, patients with HAD frequently experience improvement in neurocognitive impairment after initiation of treatment, although to a varying degree; residual symptoms or signs can remain despite therapy [140-144].

In addition to HAD, which represents a severe complication to the disease, more subtle forms of neurological manifestations are also related to chronic HIV-1 infection. Treatment has reduced the incidence of HAD, but it is recognized that neurocognitive impairment remains prevalent in HIV-1 infected patients [145-146]. Collectively termed HIV-associated neurocognitive disorders (HAND), such impairments are, in addition to HAD, classified as asymptomatic neurocognitive impairment (ANI) or HIV-1

associated mild neurocognitive disorder (MND) [138]. However, the diagnosis of less significant neurocognitive impairment in chronic HIV-1 disease is problematic, as other disorders affecting the general population, or affecting those with risk factors for acquiring HIV-1 infection, may influence diagnostic results making identification of HIV-1 related disease difficult [140]. Reduced performance in neuropsychological testing in HIV-1 infected individuals may be biased by other co-morbidities such as complications of substance abuse, age-related degenerative disease, or mental illness affecting adherence to medication. In addition, diagnostic neuropsychiatric testing does not necessarily differentiate active disease from residual symptoms related to previous neurological injury. For this reason, the complimentary use of biomarkers to detect ongoing neuronal injury or inflammatory activity has been suggested as a pathobiological tool in the evaluation of CNS disease in HIV-1 infected individuals [147-148].

3.1 Biomarkers of CNS infection

Because of its proximity to, and shared barriers with the brain, CSF represents an accessible compartment for evaluating CNS responses to HIV-1 infection and antiretroviral treatment of the infection [149]. HIV-1 infection generates a chronic inflammatory reaction in the CNS measurable in CSF by analysis of immunological markers and the presence of white blood cells (WBC) [130, 149-150]. Several immunological markers have been evaluated in relation to HIV-1 infection of the CNS [148]. Here, I will briefly overview the biomarkers relevant to this thesis.

HIV-1 RNA is detectable in CSF in a majority of untreated individuals during all stages of the disease [129-130], although CSF viral load can vary considerably between individual patients [151], and is usually lower than in plasma [130]. High levels of CSF HIV-1 RNA are seen during primary infection and in patients with HAD, as well as during concomitant opportunistic infections [129, 152-154]. In patients with successful systemic suppression of HIV-1 RNA during ART, a parallel suppression of CSF HIV-1 RNA is usually seen as well [149, 155].

Elevated WBC count, pleocytosis (defined as >4 $x10^6$ cells/l), is a frequent finding in the CSF of untreated patients, more common in the early stages of infection, and is correlated to CSF viral load [149, 156-157]. Of WBC, 85-95% are lymphocytes, mainly T-cells, and the rest monocytes [140]. As immune function deteriorates with progressive disease, CSF pleocytosis

becomes less common, and CSF WBC is generally markedly lower when blood CD4 cell count reaches <50 $x10^6$ cells/l [140]. CSF WBC is usually also normalized after initiation of ART [149].

HIV-1 infection in the CNS also induces intrathecal antibody production, measurable as elevated immunoglobulin G (IgG) index or by detection of specific oligoclonal bands in CSF. Intrathecal antibody production measured as elevated IgG-index increases during disease progression [156, 158-159].

Neopterin is produced primarily by cells of monocyte/macrophage lineage after stimulation by interferon-γ (IFN- γ) [160], and appears to be involved in the antimicrobial function of activated cells [161]. Elevated levels of neopterin reflect immune activation through macrophage activation or in the CNS activation of microglia, and in blood neopterin levels have been found to correlate to disease progression in HIV-1 infection [160]. In untreated individuals, CSF neopterin is commonly elevated, and increases with progressive immunodeficiency and declining CD4 cell count. The highest levels are seen in subjects with HAD [162-164]. With ART, CSF neopterin is markedly reduced, although not to levels seen in uninfected controls. A low-level increase in CSF neopterin is frequently found even in subjects successfully treated with antiretroviral drugs [165].

3.2 Neuropathogenesis

HIV-1 enters the CNS primarily by means of monocytes infected before trafficking across the blood-brain-barrier (BBB), and settling in the CNS as perivascular macrophages [166-168]. The main targets of HIV-1 infection in the CNS are cells of bone-marrow lineage, macrophages and micoglial cells that express CD4 as well as CCR5; these are the cells that are productively infected in the brain (Figure 7) [132, 166, 169-170]. The pathological correlate to HAD is HIV-1 encephalitis, characterized by accumulation of infected macrophages, microglial cells, and multinucleated giant cells formed by fusion of multiple macrophages or microglia, mediated through expression of the viral protein gp 120. Multinucleated giant cells are a characteristic neuropathological finding in HIV encephalitis [132, 170].

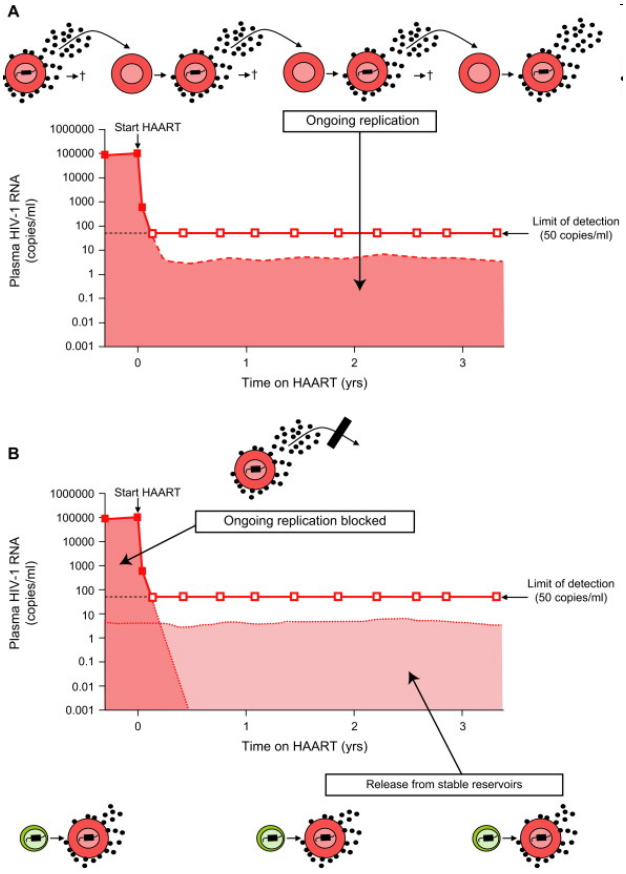

*Figure 6. Origin of low-level residual viremia (RV) in patients using ART. In scenario **A**, RV represents ongoing viral replication. In scenario **B**, ART stops all replication and RV represents release of virus from stable reservoirs. (Reprinted from J Allergy Clin Immunol, reference [82], Copyright (2008), with permission from Elsevier)*

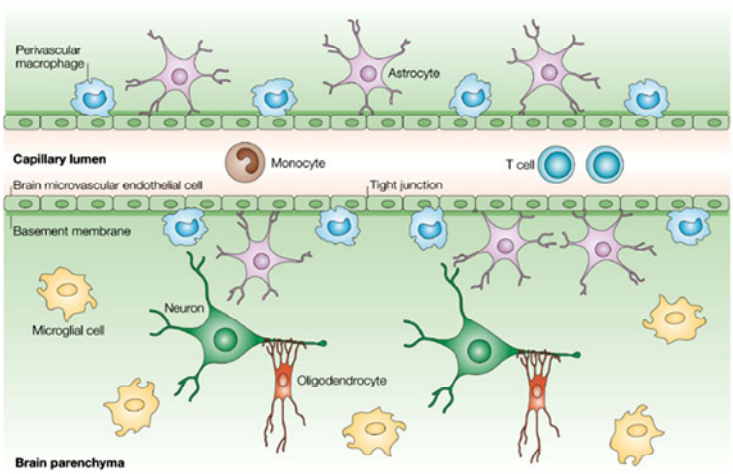

Figure 7. Different cell types in the brain. The primary target cells for HIV-1 infection in the CNS are macrophages and microglia. Macrophages are localized in the perivascular space surrounding the brain capillaries, and are replenished by circulating monocytes. Microglia are found in the brain parenchyma. (Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Immunol, reference [170], copyright 2004)

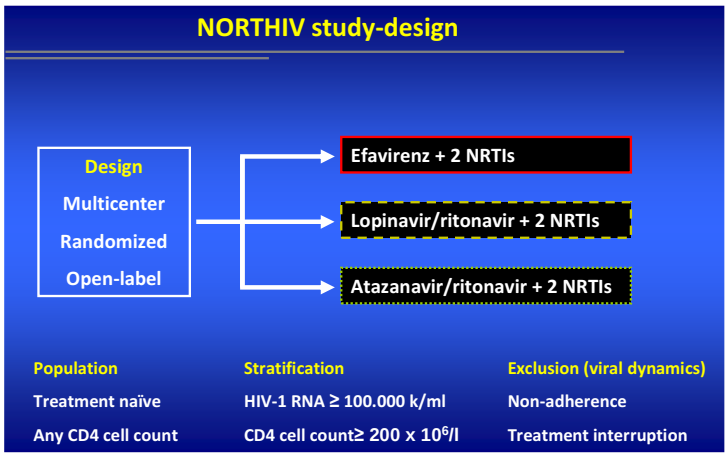

Figure 8. NORTHIV study design. Patients were randomized to one of three study arms. Randomization was stratified according to baseline $CD4^+$ T-cell count and plasma HIV-1 RNA. For the viral dynamics sub-study, patients with known non-adherence or treatment interruption were excluded from the analysis.

Although viral products may have direct toxic effects against neurons or astrocytes, the primary mechanism of neuronal damage in HIV-1 infection is likely to be a consequence of the inflammatory process initiated by virus-infected cells [131, 171], where macrophages act as both the major targets for HIV-1 replication and as the source of important toxins [172]. Secreted cellular products such as cytokines, quinolinic and arachidonic acids and nitric oxide can have neurotoxic effects, and chemokines and pro-inflammatory cytokines promote further cell activation and recruitment of additional macrophages and T-cells, thereby amplifying HIV-1 induced neurotoxicity [132, 170, 173]. The improvement seen in patients after initiation of ART suggests that neurological dysfunction is an active, reversible toxic process initiated by the infection with HIV-1 [140].

3.3 CNS as a reservoir for HIV-1

The CNS is an important potential reservoir for persistent HIV-1 infection. Several features that characterize the CNS influence the infectious process as well as treatment of HIV-1 in the CNS and suggest that it may act as a separate compartment, or sanctuary site, in HIV-1 infection. Importantly, as previously mentioned, cellular targets for HIV-1 infection and viral production differ partly from the systemic infection. The brain is a non-lymphatic organ; the main target cells for HIV-1 enter the CNS primarily through trafficking across the BBB from the systemic circulation before settling in the CNS as perivascular macrophages, with the notable exception of brain-resident microglial cells [132, 166, 170] (Figure 7). Additionally, in the CSF migrating $CD4^+$ T-cells contribute to local viral production as well as the transport of viral strains from the systemic compartment into the CNS [174]. The half life of these cell types differ significantly. As previously discussed, productively infected $CD4^+$ T-cells have a very short half life, while tissue macrophages turn over more slowly. Parenchymal microglia are much more quiescent cells and have a considerably longer lifespan [175].

Compelling evidence from several studies demonstrate that HIV-1 infection in the CNS is compartmentalized from the systemic infection, although to varying degrees at different stages of the infection. Because direct sampling of brain tissue is not possible except in rare circumstances, most studies rely on post-mortem analyses or, more commonly, of CSF. Analyses of HIV-1 in brain tissue from autopsies or biopsies have shown that brain-derived variants

are genetically distinct from HIV-1 isolated in peripheral blood [176-178]. In CSF, viral populations can originate from both the CNS and blood [179-180], and genetic compartmentalization between viral populations in CSF and blood has been demonstrated in several studies [181-183]. In untreated subjects, viral populations in CSF and blood diverge with progressive disease, being closely related in early infection but showing greater compartmentalization over time [184]. After initiation of therapy, compartmentalized variants decay rapidly in neurologically asymptomatic subjects, in parallel with the viral plasma decay rate, suggesting that short-lived cells ($CD4^+$ T-cells) are the main source of CSF virus in these patients. However, in neurologically impaired subjects, the decay rate of compartmentalized virus is reduced, indicating other cellular sources of CSF viral populations in patients with HAD/HIVE [185]. Functional compartmentalization regarding drug resistance profiles and cell tropism have also been demonstrated in the CSF, further indicating that the CNS can act as a separate compartment in HIV-1 infection [186-189].

Anatomically, the CNS is separated from the systemic circulation by the BBB; the CSF compartment is also separated from the periphery by the blood-CSF-barrier (BCB) of the epithelium of the choroid plexus [190]. The main function of these barriers is to maintain a stable environment for the brain; however, the BBB and BCB restrict the penetration of antiretroviral drugs into the CNS compartment [191]. Drug penetration into CSF varies in and among drug classes, although it is important to note that evaluation of drug penetration and potential antiretroviral efficacy in the CNS is largely based on pharmacokinetic data, rather than clinical trials on antiviral efficacy [192-198]. Even less is known regarding the CNS efficacy of drug combinations used for treatment of HIV-1 infection [140, 194, 199]. In addition to lower drug concentrations in the CSF, some antiretroviral drugs may be less effective in chronically infected macrophages, the primary target cell for treatment in the CNS [200].

Despite the potential problems with lower availability of antiretroviral drugs in CSF, patients generally respond well to ART. In subjects on effective therapy, HIV-1 RNA is usually suppressed in CSF as well as in plasma [155, 188, 201-202]; furthermore, as previously mentioned, ART has proved to be effective in preventing neurological complications to chronic HIV-1 infection [139]. Even in patients failing therapy systemically, ART is often more effective in CSF than in blood [188]. Likely, effective treatment of the systemic infection has an important influence on CSF viral load as well. Reduced numbers of productively infected cells in the periphery also reduces

the number of infected cells transitioning into the CSF. Furthermore, reduced levels of systemic immune activation likely contributes to treatment effects in the CSF, as activated CD4+ T-cells are more permissive to infection, and subsequent viral production [174]. However, the slow viral decay rate and compartmentalized viral population seen in patients with advanced infection and more profound immunodeficiency suggest that an important component of CSF virus is derived from more long-lived cells, likely in the CNS itself [174, 185]. Thus, penetration of antiretroviral drugs into the CNS remains an important issue for the treatment of HIV-1 in the brain, as suboptimal drug levels may allow virus to replicate in the CNS despite effective suppression in the blood.

4 AIMS

The overall aim of this thesis was to gain greater understanding of the clinical aspects of HIV-1 persistence, in regards to latent infection as well as the anatomic reservoir that is the central nervous system. The specific aims were:

I. to investigate differences in viral decay rate among three recommended first-line ART combinations in treatment naïve patients as a potential reflection of drug potency

II. to investigate the effect on the pool of latently infected resting $CD4^+$ T-cells of adjuvant treatment with a high dose of intravenous immunoglobulin (IVIG) in addition to suppressive antiretroviral therapy

III. to investigate the effect of long-term suppressive antiretroviral therapy on intrathecal immune activation in cerebrospinal fluid

IV. to investigate the occurrence of detectable HIV-1 RNA in the cerebrospinal fluid of patients with suppressive systemic therapy ("viral escape"), and its relation to intrathecal immune activation and antiretroviral drug regimens

5 PATIENTS AND METHODS

For more detailed information on the methods used in this thesis, I refer to the methods section of the specific papers. However, I will use this section to overview the patient populations upon which the work in this thesis is based.

5.1 The NORTHIV study

The analysis of initial viral decay rates in paper I is based on the NORTHIV study cohort. NORTHIV ("a study on ART **N**aïve patients **O**n different **R**egimens to Treat **HIV**") is a randomized, open label, multicenter clinical trial comparing the efficacy and safety of three different antiretroviral drug regimens in treatment naïve patients in Sweden and Norway (Figure 8). The study protocol was approved by the Research Ethics Committee of the University of Gothenburg, the Regional Committees for Medical Research Ethics in Norway, and the Swedish Medical Products Agency. Between 2004 and 2007, a total of 242 patients were recruited into the study; of these subjects, 239 received at least one dose of the study drugs. Randomization was also stratified according to plasma HIV-1 RNA (above or below 100.000 copies/ml), and $CD4^+$ T-cell count (above or below $200x10^6$ cells/l), at the time of inclusion. The three treatment arms were based on the drug regimens recommended at the time of trial design as first-line choices for initial therapy in treatment naïve HIV-1 infected patients, and included: (a) efavirenz 600 mg q.d. + 2 NRTIs q.d., (b) lopinavir 400 mg b.i.d. + ritonavir 100 mg b.i.d. + 2 NRTIs b.i.d., or (c) atazanavir 300 mg q.d. + ritonavir 100 mg q.d. + 2 NRTIs q.d.. The choice of NRTI "backbone" was up to the recruiting center, and was not regulated in the study protocol. Furthermore, change in backbone was allowed during the study period, and did not constitute a protocol violation leading to exclusion or failure in the overall analysis. One of the study arms was designed for twice-daily dosing (lopinavir-containing treatment regimens), while the remaining two arms contained drug combinations taken once daily. Subjects were followed for a protocol-stated 144 weeks. The main results of the NORTHIV trial have not yet been reported.

5.2 Adjuvant IVIG pilot study

In order to study potential effects of IVIG-treatment we included 9 highly motivated subjects followed at the Department of Infectious Diseases at Sahlgrenska University Hospital/Östra. All subjects had a history of effective viral suppression, with continuous ongoing therapy ≥ 2 years and plasma HIV-1 RNA levels <50 copies/ml for ≥ 1.5 years. In this small, proof-of-concept study, no controls were included.

5.3 Studies on cerebrospinal fluid

The Department of Infectious Diseases at the Sahlgrenska University Hospital/Östra began a longitudinal research project on HIV-1 infection in the CNS as early as 1985. Since that time, CSF responses to HIV-1 disease and therapy have been monitored in subjects willing to undergo lumbar punctures for research purposes. Individual patients undergo yearly paired sampling of CSF and blood; additionally lumbar and venous punctures are performed at the start of, as well as three months after initiation or cessation of therapy. This thesis includes 66 patients thus monitored. In addition, a total of 18 subjects monitored in similar protocols at the Department of Neurology, University of California, San Francisco, are included in the studies on cerebrospinal fluid (papers III and IV). At each recruiting site, study protocols have been approved by respective research ethics committees and all included patients have provided informed consent for participation.

6 RESULTS

6.1 Paper I

It has been suggested that the initial viral decay kinetics after initiation of ART may be representative for the potency of an antiretroviral drug regimen [203]. Presumably, a more effective combination of antiretroviral drugs can inhibit new rounds of viral infection in permissive cells, and thereby viral replication, to a greater degree than less potent therapies. This difference in viral replication would then be measurable as variations in the rate at which plasma viral load falls after initiation of ART. In addition to the long-term evaluation of treatment outcome, we were therefore interested in evaluating the initial viral decay kinetics in the setting of a clinical population representative of Scandinavian HIV-1 infected patients, the NORTHIV study cohort.

To evaluate initial viral decay, we analyzed the decline in plasma viral load from baseline to after four weeks of therapy. For the purpose of studying viral decay kinetics, we excluded patients who did not regularly take the study drugs during the time period, either because of treatment interruption or from lack of adherence. Consequently, 227 of the 239 patients in the NORTHIV study were included in the analysis. A sub-group of 157 patients underwent more frequent sampling with an additional one to three weekly study visits. We used the decline in plasma HIV-1 RNA from treatment initiation to first sampling (days 5-9) as an estimation of phase 1 decay, and from days 14 (12-16) to 28 (24-35) for phase 2 decay. In addition, we also measured the increase in $CD4^+$ T-cell count from treatment initiation to day 28.

The greatest initial viral decay was seen in the efavirenz-treated patients. This group had a significantly larger decline in plasma viral load at all time points compared to atazanavir/ritonavir (atazanavir/r)-treated patients, and to lopinavir/ritonavir (lopinavir/r)-treated patients up to day 21. The lopinavir/r-based treatment group in turn had a significantly greater decrease in plasma viral load compared to the atazanavir/r-based group from days 14 through 28. The larger HIV-1 RNA decline in the efavirenz-based treatment arm was also

followed by a greater increase in $CD4^+$ T-cell count after four weeks of treatment; however, in the two PI-based treatment arms, the increase in $CD4^+$ T-cell count was quite similar. Estimations of phase 1 decay rate again showed a significant difference between the efavirenz-treated patients and the patients receiving PI-based treatment regimens. Median viral decay corresponded to phase 1 half-lives in the study groups ranging from 1.17 in the efavirenz- based arm, to 1.77 in the atazanavir/r-based arm. No difference was seen in estimations of phase 2 decay rates (ranging from 8.8 to 13 days). Overall, our estimations of phase 1 and 2 decay rates were found to be comparable to previously reported findings [70, 204-205]. From our results, we concluded that efavirenz combined with two NRTIs may hold the potential for greater antiretroviral potency than either of the two protease inhibitors studied.

6.2 Paper II

As I have described in the second chapter, the pool of latently infected memory $CD4^+$ T-cells constitutes a major obstacle for the eradication of HIV-1 infection. In this proof-of-concept study, we investigated the effect on the latent reservoir of intravenous immunoglobulin (IVIG), given in addition to suppressive ART. The choice of IVIG as an adjuvant therapy was based on observations made on an HIV-1 infected patient with Guillain-Barré Syndrome, who received treatment with IVIG in addition to ongoing antiretroviral therapy [206]. During IVIG treatment, a temporary elevation of plasma viral load was detected, and when ART was later discontinued, the patient remained aviremic for a period of several months. This raised the question of whether IVIG treatment had contributed to the transient increase in plasma viremia by activating latently infected memory cells, and if the unusually long aviremic period after cessation of ART was a result of a decrease in the size of this cell pool.

To test this hypothesis, we treated 9 patients with a high dose of IVIG for five consecutive days. All subjects had been on continuous ART for ≥ 2 years, with plasma HIV-1 RNA levels < 50 copies/ml for at least 1.5 years. The pool of resting $CD4^+$ T-cells was quantified at baseline, and 8-12 weeks after IVIG treatment. Seven of the 9 patients had detectable levels of replication-competent virus in the isolated resting memory $CD4^+$ T-cells, five

of whom experienced a decrease in the latent reservoir after treatment. In these five patients, plasma viral RNA was detectable within two weeks after IVIG administration, and the highest HIV-1 RNA level was correlated to the size of the latent reservoir at baseline. Moreover, all of the five responding patients had detectable low-level viremia at baseline, compared to only one at follow-up. In two subjects, viral sequences from plasma and activated memory $CD4^+$ T-cells were compared and found to be closely related. We also noted an increase in serum interleukin 7 (IL-7) during the first eight days after IVIG intervention in the five subjects who responded to treatment. In addition, a consistent increase in $CD25^+CD127^-$ regulatory T-cells (T_{regs}) was found in all subjects after IVIG treatment. Our results indicate that treatment with IVIG had an effect on the latent reservoir.

6.3 Paper III

Antiretroviral therapy is commonly effective in lowering HIV-1 RNA levels in CSF as well as in blood. However, intrathecal immune activation can be detected in the CSF of many patients even when RNA is suppressed to levels below the limit of detection of clinical assays (<50 copies/ml). To evaluate the effect of suppressive ART on intrathecal immune activation over time, we identified 15 neurologically asymptomatic or stable patients who had been successfully treated with ART (plasma HIV-1 RNA <50) for ≥3.5 years (median 4.6 years). Ten patients from the Gothenburg cohort and five from the San Francisco cohort (see methods section) were included in the analysis.

Despite several years of suppressive therapy, we found that a majority of the patients had signs of ongoing intrathecal immune activation. Abnormal levels of neopterin as well as IgG-index were found in 60% of the subjects. However, both biomarkers decreased significantly when compared to pre-treatment levels, and all subjects had undetectable HIV-1 RNA in CSF as well as in blood. Although ART has a substantial effect on viral replication and immune activation in CSF, a majority of patients still have ongoing intrathecal immune activation despite effective suppression of the virus for extended periods of time.

6.4 Paper IV

As illustrated in paper III, ART is usually effective in lowering HIV-1 RNA levels in CSF in most patients who achieve systemic suppression with treatment. However, occasional subjects have detectable virus in CSF despite being suppressed to undetectable (<50 copies/ml) levels in blood. Such viral escape in CSF may signify ongoing viral replication in the CNS, posing a risk for neurological complications and potentially emergence of drug resistant virus. We investigated the occurrence of viral escape in CSF in a cross-sectional analysis of patients successfully treated with commonly used and recommended drug regimens.

We included a total of 69 patients in the analysis, 56 from the Gothenburg cohort and 13 from San Francisco. All had undetectable HIV-1 RNA in clinical assays (<50 copies/ml) in blood and had been treated with ART ≥6 months, with no change in treatment regimen for ≥3 months. Subjects were neurologically asymptomatic or stable, and used treatment combinations including efavirenz, lopinavir/r or atazanavir/r in combination with 2 NRTIs; tenofovir, abacavir or zidovudine in addition to emtricitabine or lamivudine. Seven (10%) of the 69 patients had evidence of viral escape in CSF, with HIV-1 RNA > 50 copies/ml. This group of patients had significantly higher levels of intrathecal immune activation measured with neopterin. In addition, these subjects also had significantly longer treatment time, more episodes of treatment interruptions and number of plasma viral blips than the subjects without CSF viral escape. The study size did not allow for conclusive comparisons of the relative efficacy of different antiretroviral drugs or drug combinations. We did not detect any difference in CNS penetration effectiveness (CPE) rank [194] in subjects with, and without, CSF viral escape. Our data suggests that viral escape in CSF is more frequent than previously recognized.

7 DISCUSSION

Although antiretroviral therapy has had a major impact on controlling HIV-1 disease, it has been made evident that elimination of the infection by means of the currently available drugs is not possible. The ability of HIV-1 to establish a stable, integrated, latent infection in resting memory $CD4^+$ T-cells constitutes a major obstacle for the eradication of the infection. Moreover, the presence of anatomical reservoirs, such as the CNS, poses additional challenges to the ultimate goal of achieving a cure for the infection. In this thesis, I have addressed several important topics regarding the persistence of HIV-1 infection. How potent are the antiretroviral drugs currently in use? Can the pool of latently infected resting memory $CD4^+$ T-cells be affected by new modes of therapy? Is the CNS a possible reservoir for the infection? Although these questions are by no means conclusively answered by the work presented here, several observations can be made.

Drug potency and latent resting cell infection

The efficacy of antiretroviral therapy is an unsettled issue. Some argue that the drug combinations currently used have the potential to fully block viral replication in infected patients, while other data suggest that viral replication can be ongoing, despite effective suppression of plasma viremia (Figure 6). This issue has been reviewed in chapter 2. Briefly, studies of viral evolution during ART in some cases support ongoing viral replication [95-96]. On the other hand, the lack of sequence evolution in other studies [81, 99], in addition to the lack of correlation between residual viremia and treatment regimen [61, 79], and the failure of intensification trials to influence the magnitude of residual viremia [109, 111], suggest that current ART regimens do have the potential to fully inhibit viral replication.

In paper I, we measured initial viral decay rates in patients starting treatment with three potent antiretroviral drug combinations. It has been hypothesized that more potent inhibition of new rounds of infection would eliminate virus at a higher rate than seen in less efficient therapy, and the initial viral decay rate has been put forward as an early measure of the inherent antiretroviral potency of a given treatment regimen [203]. Initial viral decay has been

found to correlate with long-term clinical outcome in previous studies [205, 207-208]. Importantly however, some of these trials have included suboptimal treatment regimens, such as monotherapy trials or older treatment combinations. Thus, the relation between initial viral decay and long-term treatment success found may be a reflection of the overall inferiority of some of the regimens studied. In contrast, no correlation was found between viral decay and treatment success in a study comparing two NNRTI-based treatment regimens [209]. Although longitudinal results from the NORTHIV trial are still pending, the differences in viral decay noted in the three treatment groups are in accordance with comparative clinical trials on the long-term efficacy of included drugs [210-211], and may thus represent a true variation in antiretroviral potency for the treatment regimens studied.

Interestingly, treatment with the integrase inhibitor raltegravir has been shown to result in a more rapid viral decay when compared to efavirenz [212]. It has been proposed that raltegravir alters the decay kinetics by its mode of action, reducing the pool of cells able to contribute to the phase 2 decay by blocking integration of proviral DNA into the host cell genome. These findings illustrate that the mechanism of action of a drug class may be important for the decay kinetics observed after treatment initiation. The stage in the viral life cycle at which different drugs act may determine the decay rate after initiation of therapy, independent of drug efficacy [213]. *In vitro* analyses of drug inhibition on viral infectivity have shown that the PIs and NNRTIs currently used (including those studied in paper I) have a very high potential to inhibit viral replication [82, 214], which is in accordance with the success of ART-combinations including these drugs in clinical trials [215-216].

It must also be noted that higher efficacy does not automatically mean that a treatment regimen will do better in clinical practice. Any drug combination with enough potency to inhibit viral infectivity to a high enough degree will perform well in treatment of the infection [214]. In a routine clinical setting, the ultimate success of a treatment regimen is influenced by other factors. Importantly, adherence issues must always be considered in the evaluation of therapeutic efficacy. In addition, side effects are important for the long-term durability of ART, either by influencing the patients' drug intake, or by necessitating changes in therapy due to adverse effects.

Despite the potent drug regimens currently available, residual viremia can be detected in most subjects when using high-sensitivity assays [61, 79]. As I have outlined in the second chapter, the pool of latently infected resting memory $CD4^+$ T-cells is an important source of viral persistence and residual

viremia. Activation of resting cells leading to virus expression and lytic destruction of infected cells, while preventing de-novo infection of susceptible targets by maintaining effective antiretroviral therapy, would potentially allow for the elimination of infection. Several strategies have been investigated in order to decrease the size of the latent reservoir. Interleukin 2 (IL-2) given in addition to ART decreased the size of the latent reservoir in one study [217], but rapid viral rebound was seen after cessation of ART [218-220]. IL-2 has also been given in combinations with OKT-3 (an anti-CD3 monoclonal antibody) with or without addition of hydroxyurea and didanosine, to achieve T-cell activation [221-224]. However, either lack of decrease in total HIV-1 DNA, or rapid rebound of plasma virus after treatment interruption, was noted in these trials [221-222]. Much interest has also been invested in valproic acid (VPA) as a possible promoter of HIV-1 gene expression in resting $CD4^+$ T-cells. VPA is an anticonvulsant drug that inhibits histone deacetylase (HDAC), an enzyme involved in chromatin remodeling, and regulation of HIV-1 gene expression [225]. In a pilot study of four patients, a decline in the latent reservoir was seen in three individuals after addition of VPA and enfuvirtide to effective ART [226]. In an extended study, only 4 of 11 VPA-treated patients had a decline in resting cell infection [227]. Recently however, longitudinal data showed that no long-term effect on resting cell infection could be seen after VPA treatment [111]. Moreover, others failed to demonstrate any difference in resting cell infection in HIV-1 infected patients regularly using VPA for neurological disorders compared to HIV-1 infected controls using ART alone [228-230].

In paper II, we demonstrate an effect of IVIG as adjuvant to effective ART on the latent reservoir. The transient increase in plasma virus seen in the patients who experienced a decline in the size of the latent reservoir likely originated from resting $CD4^+$ T-cells, as suggested by the sequence analyses. It is unlikely that IVIG had a direct effect on HIV-1 expression in resting $CD4^+$ T-cells. However, an indirect effect mediated by cytokines such as IL-7, is possible. IL-7 has been shown to induce proviral activation from resting $CD4^+$ T-cells *in vitro* [231], and induced transient plasma blips in a subset of individuals in a randomized, controlled study [232]. In addition, we noticed a consistent increase in T_{regs} after IVIG-treatment, which has also been shown by others [233-234]. T_{regs} are important in modulating chronic inflammatory responses [235] and interestingly, elite controllers (individuals capable of controlling viral load without ART) maintain higher serum levels of T_{regs} than individuals with progressive disease do [236]. It is possible that IVIG has a modulating effect on the immune activation seen in chronic HIV-1 infection. Our results indicate that IVIG-treatment had an effect on the latent reservoir; however, data must be interpreted with caution. This was a small,

uncontrolled study, and results need confirmation in expanded, controlled trials. Furthermore, it is unclear whether the decrease in size of the latent reservoir observed will influence HIV-1 persistence over time. After VPA-interventions, resting cell infection reverted to baseline levels over time [111]. Homeostatic processes may prevent a stable reduction in the latent reservoir, as indicated in recent work by Chomont et al [94]. Moreover, additional highly stable reservoirs of persistent HIV-1 infection may need to be considered if HIV-1 latency is to be successfully eliminated [93].

CNS persistence

As is the case with the systemic infection, it is not clear if HIV-1 can replicate in the CNS during suppressive ART. For obvious reasons, it is not possible to directly sample brain tissue in living patients except under very special circumstances. Therefore, the absolute majority of studies on CNS responses to ART have been done through evaluation of CSF. As previously described, ART regularly reduces CSF HIV-1 RNA to below standard levels of detection [155, 188, 201-202]. Despite effective suppression of CSF virus however, signs of intrathecal immune activation can be detected in treated patients [165], and it has been suggested that persistent immune activation may represent ongoing low-level viral replication within the brain, generating an inflammatory response measurable in CSF [237]. We show in paper III that intrathecal immune activation, measured as neopterin and IgG-index, remains elevated in a majority of patients even after several years of effective viral suppression. CSF neopterin has been shown to be intimately correlated with CSF viral load, where subjects with highly suppressed CSF virus (<2.5 copies/ml) have significantly lower neopterin levels than subjects with CSF HIV-1 RNA below the routine clinical detection limit of 50 copies/ml [237]. These findings are in agreement with the higher neopterin levels noted in patients with viral escape in CSF (paper IV).

Thus, the presence of HIV-1 is a likely trigger of the intrathecal inflammatory response. However, as CSF virus can originate from blood cells trafficking into the CNS as well as from productive infection within the brain itself [179], elevated CSF neopterin alone does not conclusively demonstrate ongoing viral replication in the CNS. Release of virus from activated, latently infected T-cells migrating into the CSF compartment may trigger an immune response in the CNS measurable as elevated levels of neopterin [140]. Conversely, ongoing low-grade viral replication in long-lived cells resident in

the brain may initiate an inflammatory response even in the absence of HIV-1 RNA levels measurable in CSF. It has also been suggested that the inflammatory response may result from autoimmune phenomena, or a self-sustaining state of cellular activation, initially triggered by HIV-1 but persisting even in the absence of virus during effective therapy [140, 170]. This may also be true for the humoral response, measured as elevated IgG-index (paper III). Although HIV-1 infection triggers intrathecal antibody production, HIV-specific antibodies constitute only a minor part and may result from non-specific immunologic or autoimmune reactions as well as active viral replication [238-239]. It must be noted however, that a significant decrease in intrathecal immune activation was seen in treated patients compared to pre-treatment levels, indicating that suppression of virus has important effects on reducing, if not eliminating, immune responses in the CNS triggered by HIV-1 infection (paper III).

Residual, low-level viremia can be detected in CSF as well as in plasma, although it appears to be a less frequent finding than in blood [188, 202, 240]. The origin of residual CSF viremia is less well characterized than in blood. Latently infected resting memory $CD4^+$ T-cells may become activated as they traffic across the BCB, exposing the CSF compartment to low levels of virus which would implicate a common source of residual viremia in both compartments. In contrast, several factors point to CNS-resident cells as potential reservoirs for persistent CSF viremia. Differentiated tissue macrophages are not affected by the cytopathic effect of HIV-1 to the same extent as activated $CD4^+$ T-cells are, which may enable continuous low-level virus production to take place for the entire life span of the infected cells [241-242]. The perivascular macrophage pool is continuously replenished by bone-marrow derived cells, potentially recruiting additional targets for ongoing infection [175]. Microglia may also be replenished by monocytes [175]; in addition, the long life span of microglia makes this cell population a potentially significant source of persistent HIV-1 infection in the CNS.

The BBB limits the passage of most antiretroviral drugs into the CNS, which may lead to suboptimal concentrations in the brain parenchyma, thus reducing antiretroviral potency in the CNS [243-244]. Although little is known about actual drug concentrations in the brain itself, studies of CSF have shown that not all drugs reach adequate concentrations in the CSF compartment [192]. However, experience has shown that CSF viral load generally responds well to effective systemic therapy regardless of treatment regimen [155, 188, 237]. Cases of viral escape in CSF under suppressive systemic ART have been rare [188], and as mentioned above, residual low-level CSF virus appears to be less common than in plasma. In paper IV

however, we demonstrate that CSF viral escape (here defined as CSF HIV-1 RNA >50 copies/ml while plasma HIV-1 RNA is <50 copies/ml) in a cohort of neuroasymptomatic patients treated with contemporary and recommended ART combinations was more common than previously experienced. Ten percent of systemically suppressed patients had CSF HIV-1 RNA above the detection limit of clinical assays, which was correlated to higher levels of intrathecal immune activation measured as CSF neopterin.

Insufficient drug concentrations due to limited penetration may lower ART potency in the CNS, possibly allowing some degree of viral replication to occur. Over time, this may establish a CNS infection that is independent of viral reseeding from the periphery, eventually leading to viral escape in CSF. Interestingly, subjects with detectable CSF virus had been treated with ART for a significantly longer time than subjects with suppressed CSF viral load (paper IV). Additionally, we found that previous treatment interruptions as well as previous plasma viral blips were more common in the group of subjects with CSF viral escape. Treatment interruptions result in a rapid rebound of plasma viremia, and have proved to increase the overall risk of adverse events and disease progression [245]. Viral load also increases rapidly in the CSF, and results in increased levels of intrathecal immune activation, as well as neuronal injury [246]. After interruption of therapy an increase in CSF levels of neurofilament light protein (NFL), a marker of axonal injury, has been observed suggesting that the rapid elevation of viral load during treatment interruption has potential CNS-damaging effects [246]. Exposure of the CNS to HIV-1 RNA may promote the establishment of an autonomous infection within CNS-resident cells. Viral blips have also been shown to be associated with decreased adherence [247]. The higher frequency of plasma viral blips in subjects with CSF viral escape suggests that intermittent reseeding of the CNS, whether due to limited potency or lack of adherence, may promote persistent infection in the brain.

The importance of drug penetration across the BBB for controlling HIV-1 infection in the CNS is not fully elucidated. Based on pharmacokinetic CSF studies, the CPE rank has been proposed as a simple way to estimate the potency of drug combinations in the CNS [194, 248]. However, other factors may influence antiretroviral potency in the CNS, and indeed, we did not see any correlation between CPE rank and CSF viral escape in our subjects. We could not demonstrate a correlation between CSF viral escape and the specific drugs included in ART regimens; however, variations in CNS penetration may still be important. Differences in efficacy between the studied drugs were not large enough to be detected in our study cohort, although we did see a trend towards significance when comparing the NRTIs

tenofovir, abacavir and zidovudine. Notably, none of the subjects treated with zidovudine, a drug with well demonstrated CNS efficacy, had CSF viral escape (paper IV).

The clinical significance of ongoing intrathecal immune activation with or without detectable CSF virus is not fully established. ART has been effective in preventing severe forms of HIV-1 related neurological disease [139], suggesting that persistent immune activation may be clinically benign. However, less apparent neurocognitive impairment may be prevalent in HIV-1 infected patients [145-146], and may signify ongoing low-grade neurological damage. Recent reports have demonstrated CSF viral escape linked to concurrent neurological symptoms, illustrating the potential impact of suboptimal viral control in the CNS [249-251]. This may have important implications for future antiretroviral therapy, as new treatment strategies, for example NRTI-sparing regimens, as well as the implementation of new drug combinations with less CNS-penetrating properties, become more common in clinical practice.

Concluding remarks

The potency of antiretroviral drug regimens is not fully elucidated. Interesting new ways of evaluating the relative inhibitory effect of ART have been proposed recently, where *in vitro* measurement of the reduction in viral infectivity, or instantaneous inhibitory potential (IIP), of an antiretroviral drug may influence antiviral activity *in vivo* [82, 214]. However, in the future additional clinical studies are needed to further clarify the efficacy of recommended drug regimens both from a virological and a clinical perspective. It is not fully established if initial viral decay rate reflects the potency of a given drug regimen or is related to pharmacological mechanisms of HIV-1 inhibition, and if the difference in decay seen between different drugs have an impact on residual viral replication *in vivo*. This is especially true in the CNS, where current knowledge of the efficacy of drug combinations is insufficient. As I have discussed here, intrathecal immune activation is a common finding, and viral escape can be detected in individuals on modern drug regimens. If these findings represent an actual lack of therapeutic effect in the CNS, new strategies may need to be developed in the treatment of neurological HIV-1 disease. The most immediate way of investigating this issue is to expand our observations into larger, preferably randomized and controlled, clinical trials.

The origin of residual virus in CSF is not well known. As CSF represents a compartment shared between the peripheral circulation and the CNS, viral strains may originate from any of these sources. Sequence analyses have been performed by many groups in plasma. Very few, if any, such studies have investigated residual CSF virus and this could potentially be a very interesting focus for future research. Although CSF is an accessible compartment, patients are sometimes unwilling to partake in sampling, and the procedure requires some degree of proficiency. However, with sufficient knowledge and organization in the clinic and the lab, such studies may be possible to perform.

Furthermore, the pool of latently infected resting memory $CD4^+$ T-cells constitute an important barrier to the elimination of HIV-1 disease. We have shown that this reservoir is probably accessible by adjuvant interventions; however, lasting effects and clinical benefit is unclear. In the case of IVIG treatment, our findings are interesting but must be interpreted with caution. Again, expanded, controlled trials as well as long-term follow-up are needed to properly evaluate the potential benefit of such strategies.

Although it is not known if viral replication in the CNS can take place during potent ART, we and others have shown that the CNS is a compartment that must be taken into consideration when approaching the subject of HIV-1 persistence. While ART can have a great impact on controlling the infection, intrathecal immune activation as well as viral escape demonstrates that treatment does not fully suppress the CNS responses caused by HIV-1 infection, whether it is due to ongoing viral replication in the brain or release of virus from stable reservoirs in the periphery. If ongoing viral replication occurs in blood during treatment with ART is also an unsettled issue. As in the CNS, the impact of ART on HIV-1 disease cannot be overestimated, even if eradication of the infection has proved to be unachievable thus far. If this ultimate goal is to be realized, many of the issues addressed in the studies included in this thesis remain to be elucidated.

8 CONCLUSIONS

- The NNRTI efavirenz, when used in combination with 2 NRTIs, lowers plasma viral load at a faster rate than ART combinations containing either of the PIs lopinavir/r or atazanavir/r. In turn, the rate of decline is greater with lopinavir/r-based than atazanavir/r-based therapy. This may reflect different inherent antiretroviral potency between the treatment regimens

- Addition of IVIG to effective ART reduced the size of the pool of latently infected resting memory $CD4^+$ T-cells. Although findings need replication in controlled trials, the results indicate that novel modes of intervention can have an effect on the latent reservoir

- Despite several years of effective virologic suppression, a majority of subjects still have elevated levels of intrathecal immune activation. The nature of the immune response is not entirely clear, but may result from ongoing replication of virus in the brain or exposure of the CNS to low levels of virus originating from the systemic circulation.

- As many as ten percent of effectively treated, neurologically asymptomatic individuals have viral escape in CSF. The cause of viral escape is again not clearly defined. Viral escape in CSF may result from insufficient drug penetration, allowing virus to replicate despite systemic suppression. Autonomous CNS infection established through intermittent reseeding of the CNS compartment during treatment interruptions or temporary increases in viremia may be of importance for CSF viral escape.

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REFERENCES

1. Gottlieb MS, Schroff R, Schanker HM, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981;305:1425-31
2. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983;220:868-71
3. UNAIDS. Report on the global AIDS epidemic, 2008
4. Smittskyddsinstitutet. Statistik för hivinfektion 2009. www.smittskyddsinstitutet.se, 2009
5. Wulff M, Lalos A. The condom in relation to prevention of sexually transmitted infections and as a contraceptive method in Sweden. Eur J Contracept Reprod Health Care 2004;9:69-77
6. Smittskyddsinstitutet. Statistik för klamydiainfektion. www.smittskyddsinstitutet.se, 2010
7. Clavel F, Guetard D, Brun-Vezinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. Science 1986;233:343-6
8. McCutchan FE. Global epidemiology of HIV. J Med Virol 2006;78 Suppl 1:S7-S12
9. Abecasis AB, Lemey P, Vidal N, et al. Recombination confounds the early evolutionary history of human immunodeficiency virus type 1: subtype G is a circulating recombinant form. J Virol 2007;81:8543-51
10. Robertson DL, Anderson JP, Bradac JA, et al. HIV-1 nomenclature proposal. Science 2000;288:55-6
11. Sharp PM, Bailes E, Robertson DL, Gao F and Hahn BH. Origins and evolution of AIDS viruses. Biol Bull 1999;196:338-42
12. Gao F, Bailes E, Robertson DL, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature 1999;397:436-41
13. Keele BF, Van Heuverswyn F, Li Y, et al. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. Science 2006;313:523-6
14. Van Heuverswyn F, Li Y, Neel C, et al. Human immunodeficiency viruses: SIV infection in wild gorillas. Nature 2006;444:164
15. Vidal N, Peeters M, Mulanga-Kabeya C, et al. Unprecedented degree of human immunodeficiency virus type 1 (HIV-1) group M genetic diversity in the Democratic Republic of Congo suggests that the HIV-1 pandemic originated in Central Africa. J Virol 2000;74:10498-507
16. de Silva TI, Cotten M and Rowland-Jones SL. HIV-2: the forgotten AIDS virus. Trends Microbiol 2008;16:588-95
17. Reeves JD, Doms RW. Human immunodeficiency virus type 2. J Gen Virol 2002;83:1253-65

18. Hahn BH, Shaw GM, De Cock KM and Sharp PM. AIDS as a zoonosis: scientific and public health implications. Science 2000;287:607-14
19. Korber B, Muldoon M, Theiler J, et al. Timing the ancestor of the HIV-1 pandemic strains. Science 2000;288:1789-96
20. Worobey M, Gemmel M, Teuwen DE, et al. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. Nature 2008;455:661-4
21. Zhu T, Korber BT, Nahmias AJ, Hooper E, Sharp PM and Ho DD. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. Nature 1998;391:594-7
22. Simon V, Ho DD. HIV-1 dynamics in vivo: implications for therapy. Nat Rev Microbiol 2003;1:181-90
23. Chitnis A, Rawls D and Moore J. Origin of HIV type 1 in colonial French Equatorial Africa? AIDS Res Hum Retroviruses 2000;16:5-8
24. Gisselquist D. Emergence of the HIV type 1 epidemic in the twentieth century: comparing hypotheses to evidence. AIDS Res Hum Retroviruses 2003;19:1071-8
25. Levy JA. HIV and the pathogenesis of AIDS. 3rd ed. ed. Washington DC, 2007
26. Berger EA, Murphy PM and Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol 1999;17:657-700
27. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J and Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 1998;393:648-59
28. Levy JA. HIV pathogenesis: 25 years of progress and persistent challenges. Aids 2009;23:147-60
29. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 1998;280:1884-8
30. Bleul CC, Wu L, Hoxie JA, Springer TA and Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc Natl Acad Sci U S A 1997;94:1925-30
31. Connor RI, Sheridan KE, Ceradini D, Choe S and Landau NR. Change in coreceptor use correlates with disease progression in HIV-1--infected individuals. J Exp Med 1997;185:621-8
32. Moore JP, Kitchen SG, Pugach P and Zack JA. The CCR5 and CXCR4 coreceptors--central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS Res Hum Retroviruses 2004;20:111-26
33. Singh A, Collman RG. Heterogeneous spectrum of coreceptor usage among variants within a dualtropic human immunodeficiency virus type 1 primary-isolate quasispecies. J Virol 2000;74:10229-35
34. Coffin JM. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science 1995;267:483-9

35. Preston BD, Poiesz BJ and Loeb LA. Fidelity of HIV-1 reverse transcriptase. Science 1988;242:1168-71
36. Roberts JD, Bebenek K and Kunkel TA. The accuracy of reverse transcriptase from HIV-1. Science 1988;242:1171-3
37. Daar ES, Moudgil T, Meyer RD and Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med 1991;324:961-4
38. Picker LJ. Immunopathogenesis of acute AIDS virus infection. Curr Opin Immunol 2006;18:399-405
39. Schacker T, Collier AC, Hughes J, Shea T and Corey L. Clinical and epidemiologic features of primary HIV infection. Ann Intern Med 1996;125:257-64
40. Tindall B, Cooper DA. Primary HIV infection: host responses and intervention strategies. Aids 1991;5:1-14
41. Koup RA, Safrit JT, Cao Y, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994;68:4650-5
42. Lefrere JJ, Roudot-Thoraval F, Mariotti M, et al. The risk of disease progression is determined during the first year of human immunodeficiency virus type 1 infection. J Infect Dis 1998;177:1541-8
43. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126:946-54
44. Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA and Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167-70
45. Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 1993;362:359-62
46. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM and Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995;373:123-6
47. Piatak M, Jr., Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 1993;259:1749-54
48. Pantaleo G, Graziosi C and Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus infection. N Engl J Med 1993;328:327-35
49. Volberding PA, Lagakos SW, Grimes JM, et al. The duration of zidovudine benefit in persons with asymptomatic HIV infection. Prolonged evaluation of protocol 019 of the AIDS Clinical Trials Group. Jama 1994;272:437-42
50. Ho DD. Time to hit HIV, early and hard. N Engl J Med 1995;333:450-1

51. Gulick RM, Mellors JW, Havlir D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N Engl J Med 1997;337:734-9
52. Hammer SM, Squires KE, Hughes MD, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N Engl J Med 1997;337:725-33
53. Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci U S A 1997;94:13193-7
54. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295-300
55. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997;278:1291-5
56. Murphy EL, Collier AC, Kalish LA, et al. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. Ann Intern Med 2001;135:17-26
57. Palella FJ, Jr., Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338:853-60
58. Josephson F, Albert J, Flamholc L, et al. Treatment of HIV infection: Swedish recommendations 2009. Scand J Infect Dis 2009;41:788-807
59. Harrigan PR, Whaley M and Montaner JS. Rate of HIV-1 RNA rebound upon stopping antiretroviral therapy. Aids 1999;13:F59-62
60. Montaner JS, Harris M, Mo T and Harrigan PR. Rebound of plasma HIV viral load following prolonged suppression with combination therapy. Aids 1998;12:1398-9
61. Maldarelli F, Palmer S, King MS, et al. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. PLoS Pathog 2007;3:e46
62. Dahl V, Josefsson L and Palmer S. HIV reservoirs, latency, and reactivation: prospects for eradication. Antiviral Res 2010;85:286-94
63. Pomerantz RJ. Residual HIV-1 infection during antiretroviral therapy: the challenge of viral persistence. Aids 2001;15:1201-11
64. Blankson JN, Persaud D and Siliciano RF. The challenge of viral reservoirs in HIV-1 infection. Annu Rev Med 2002;53:557-93
65. Perelson AS, Neumann AU, Markowitz M, Leonard JM and Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science 1996;271:1582-6
66. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995;373:117-22

67. Markowitz M, Louie M, Hurley A, et al. A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. J Virol 2003;77:5037-8
68. Zhang Z, Schuler T, Zupancic M, et al. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. Science 1999;286:1353-7
69. Notermans DW, Goudsmit J, Danner SA, de Wolf F, Perelson AS and Mittler J. Rate of HIV-1 decline following antiretroviral therapy is related to viral load at baseline and drug regimen. Aids 1998;12:1483-90
70. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 1997;387:188-91
71. Hlavacek WS, Stilianakis NI, Notermans DW, Danner SA and Perelson AS. Influence of follicular dendritic cells on decay of HIV during antiretroviral therapy. Proc Natl Acad Sci U S A 2000;97:10966-71
72. Keele BF, Tazi L, Gartner S, et al. Characterization of the follicular dendritic cell reservoir of human immunodeficiency virus type 1. J Virol 2008;82:5548-61
73. Smith BA, Gartner S, Liu Y, et al. Persistence of infectious HIV on follicular dendritic cells. J Immunol 2001;166:690-6
74. Zhang L, Ramratnam B, Tenner-Racz K, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. N Engl J Med 1999;340:1605-13
75. Di Mascio M, Dornadula G, Zhang H, et al. In a subset of subjects on highly active antiretroviral therapy, human immunodeficiency virus type 1 RNA in plasma decays from 50 to <5 copies per milliliter, with a half-life of 6 months. J Virol 2003;77:2271-5
76. Dornadula G, Zhang H, VanUitert B, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. Jama 1999;282:1627-32
77. Finzi D, Blankson J, Siliciano JD, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med 1999;5:512-7
78. Palmer S, Wiegand AP, Maldarelli F, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol 2003;41:4531-6
79. Palmer S, Maldarelli F, Wiegand A, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proc Natl Acad Sci U S A 2008;105:3879-84
80. Siliciano JD, Kajdas J, Finzi D, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med 2003;9:727-8
81. Bailey JR, Sedaghat AR, Kieffer T, et al. Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral

therapy is dominated by a small number of invariant clones rarely found in circulating CD4+ T cells. J Virol 2006;80:6441-57
82. Shen L, Siliciano RF. Viral reservoirs, residual viremia, and the potential of highly active antiretroviral therapy to eradicate HIV infection. J Allergy Clin Immunol 2008;122:22-8
83. McCune JM. Viral latency in HIV disease. Cell 1995;82:183-8
84. Pierson TC, Zhou Y, Kieffer TL, Ruff CT, Buck C and Siliciano RF. Molecular characterization of preintegration latency in human immunodeficiency virus type 1 infection. J Virol 2002;76:8518-31
85. Siliciano JD, Siliciano RF. A long-term latent reservoir for HIV-1: discovery and clinical implications. J Antimicrob Chemother 2004;54:6-9
86. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A and Chen IS. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell 1990;61:213-22
87. Koelsch KK, Liu L, Haubrich R, et al. Dynamics of total, linear nonintegrated, and integrated HIV-1 DNA in vivo and in vitro. J Infect Dis 2008;197:411-9
88. Zhou Y, Zhang H, Siliciano JD and Siliciano RF. Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells. J Virol 2005;79:2199-210
89. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D and Siliciano RF. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nat Med 1995;1:1284-90
90. Chun TW, Justement JS, Lempicki RA, et al. Gene expression and viral prodution in latently infected, resting CD4+ T cells in viremic versus aviremic HIV-infected individuals. Proc Natl Acad Sci U S A 2003;100:1908-13
91. Chun TW, Engel D, Berrey MM, Shea T, Corey L and Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc Natl Acad Sci U S A 1998;95:8869-73
92. Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 1997;387:183-8
93. Carter CC, Onafuwa-Nuga A, McNamara LA, et al. HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. Nat Med 2010;16:446-51
94. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med 2009;15:893-900
95. Tobin NH, Learn GH, Holte SE, et al. Evidence that low-level viremias during effective highly active antiretroviral therapy result from two processes: expression of archival virus and replication of virus. J Virol 2005;79:9625-34
96. Shiu C, Cunningham CK, Greenough T, et al. Identification of ongoing human immunodeficiency virus type 1 (HIV-1) replication in residual

viremia during recombinant HIV-1 poxvirus immunizations in patients with clinically undetectable viral loads on durable suppressive highly active antiretroviral therapy. J Virol 2009;83:9731-42
97. Hermankova M, Ray SC, Ruff C, et al. HIV-1 drug resistance profiles in children and adults with viral load of <50 copies/ml receiving combination therapy. Jama 2001;286:196-207
98. Kieffer TL, Finucane MM, Nettles RE, et al. Genotypic analysis of HIV-1 drug resistance at the limit of detection: virus production without evolution in treated adults with undetectable HIV loads. J Infect Dis 2004;189:1452-65
99. Nottet HS, van Dijk SJ, Fanoy EB, et al. HIV-1 can persist in aged memory CD4+ T lymphocytes with minimal signs of evolution after 8.3 years of effective highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2009;50:345-53
100. Persaud D, Siberry GK, Ahonkhai A, et al. Continued production of drug-sensitive human immunodeficiency virus type 1 in children on combination antiretroviral therapy who have undetectable viral loads. J Virol 2004;78:968-79
101. Joos B, Fischer M, Kuster H, et al. HIV rebounds from latently infected cells, rather than from continuing low-level replication. Proc Natl Acad Sci U S A 2008;105:16725-30
102. Nettles RE, Kieffer TL, Kwon P, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. Jama 2005;293:817-29
103. Chun TW, Nickle DC, Justement JS, et al. HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. J Clin Invest 2005;115:3250-5
104. Ramratnam B, Mittler JE, Zhang L, et al. The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. Nat Med 2000;6:82-5
105. Ramratnam B, Ribeiro R, He T, et al. Intensification of antiretroviral therapy accelerates the decay of the HIV-1 latent reservoir and decreases, but does not eliminate, ongoing virus replication. J Acquir Immune Defic Syndr 2004;35:33-7
106. Zhang L, Chung C, Hu BS, et al. Genetic characterization of rebounding HIV-1 after cessation of highly active antiretroviral therapy. J Clin Invest 2000;106:839-45
107. Sedaghat AR, Siliciano JD, Brennan TP, Wilke CO and Siliciano RF. Limits on replenishment of the resting CD4+ T cell reservoir for HIV in patients on HAART. PLoS Pathog 2007;3:e122
108. Bonora S, Nicastri E, Calcagno A, et al. Ultrasensitive assessment of residual HIV viraemia in HAART-treated patients with persistently undetectable plasma HIV-RNA: a cross-sectional evaluation. J Med Virol 2009;81:400-5

109. Dinoso JB, Kim SY, Wiegand AM, et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. Proc Natl Acad Sci U S A 2009;106:9403-8
110. Gandhi RT, Bosch RJ, Aga E, et al. No evidence for decay of the latent reservoir in HIV-1-infected patients receiving intensive enfuvirtide-containing antiretroviral therapy. J Infect Dis 2010;201:293-6
111. Archin NM, Cheema M, Parker D, et al. Antiretroviral intensification and valproic acid lack sustained effect on residual HIV-1 viremia or resting CD4+ cell infection. PLoS One 2010;5:e9390
112. Wilkin TJ, McKinnon JE, DiRienzo AG, et al. Regimen simplification to atazanavir-ritonavir alone as maintenance antiretroviral therapy: final 48-week clinical and virologic outcomes. J Infect Dis 2009;199:866-71
113. Buzon MJ, Massanella M, Llibre JM, et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. Nat Med 2010;16:460-5
114. Svarovskaia ES, Barr R, Zhang X, et al. Azido-containing diketo acid derivatives inhibit human immunodeficiency virus type 1 integrase in vivo and influence the frequency of deletions at two-long-terminal-repeat-circle junctions. J Virol 2004;78:3210-22
115. McMahon D, Jones J, Wiegand A, et al. Short-course raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression during receipt of combination antiretroviral therapy. Clin Infect Dis 2010;50:912-9
116. Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 1993;362:355-8
117. Brenchley JM, Douek DC. HIV infection and the gastrointestinal immune system. Mucosal Immunol 2008;1:23-30
118. Chun TW, Nickle DC, Justement JS, et al. Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. J Infect Dis 2008;197:714-20
119. Bagasra O, Farzadegan H, Seshamma T, Oakes JW, Saah A and Pomerantz RJ. Detection of HIV-1 proviral DNA in sperm from HIV-1-infected men. Aids 1994;8:1669-74
120. Quayle AJ, Xu C, Mayer KH and Anderson DJ. T lymphocytes and macrophages, but not motile spermatozoa, are a significant source of human immunodeficiency virus in semen. J Infect Dis 1997;176:960-8
121. Zhu T, Wang N, Carr A, et al. Genetic characterization of human immunodeficiency virus type 1 in blood and genital secretions: evidence for viral compartmentalization and selection during sexual transmission. J Virol 1996;70:3098-107
122. Taylor S, Reynolds H, Sabin CA, et al. Penetration of efavirenz into the male genital tract: drug concentrations and antiviral activity in semen and blood of HIV-1-infected men. Aids 2001;15:2051-3

123. van Leeuwen E, Ter Heine R, van der Veen F, Repping S, Beijnen JH and Prins JM. Penetration of atazanavir in seminal plasma of men infected with human immunodeficiency virus type 1. Antimicrob Agents Chemother 2007;51:335-7
124. Gunthard HF, Havlir DV, Fiscus S, et al. Residual human immunodeficiency virus (HIV) Type 1 RNA and DNA in lymph nodes and HIV RNA in genital secretions and in cerebrospinal fluid after suppression of viremia for 2 years. J Infect Dis 2001;183:1318-27
125. Sheth PM, Kovacs C, Kemal KS, et al. Persistent HIV RNA shedding in semen despite effective antiretroviral therapy. Aids 2009;23:2050-4
126. Zhang H, Dornadula G, Beumont M, et al. Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. N Engl J Med 1998;339:1803-9
127. Davis LE, Hjelle BL, Miller VE, et al. Early viral brain invasion in iatrogenic human immunodeficiency virus infection. Neurology 1992;42:1736-9
128. Pilcher CD, Shugars DC, Fiscus SA, et al. HIV in body fluids during primary HIV infection: implications for pathogenesis, treatment and public health. AIDS 2001;15:837-45
129. Ellis RJ, Hsia K, Spector SA, et al. Cerebrospinal fluid human immunodeficiency virus type 1 RNA levels are elevated in neurocognitively impaired individuals with acquired immunodeficiency syndrome. HIV Neurobehavioral Research Center Group. Ann Neurol 1997;42:679-88
130. Gisslen M, Fuchs D, Svennerholm B and Hagberg L. Cerebrospinal fluid viral load, intrathecal immunoactivation, and cerebrospinal fluid monocytic cell count in HIV-1 infection. J Acquir Immune Defic Syndr 1999;21:271-6
131. Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC and Cleary P. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science 1988;239:586-92
132. Williams KC, Hickey WF. Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. Annu Rev Neurosci 2002;25:537-62
133. Price RW. Neurological complications of HIV infection. Lancet 1996;348:445-52
134. Simpson DM, Tagliati M. Neurologic manifestations of HIV infection. Ann Intern Med 1994;121:769-85
135. Mamidi A, DeSimone JA and Pomerantz RJ. Central nervous system infections in individuals with HIV-1 infection. J Neurovirol 2002;8:158-67
136. Navia BA, Jordan BD and Price RW. The AIDS dementia complex: I. Clinical features. Ann Neurol 1986;19:517-24
137. Price RW, Brew BJ. The AIDS dementia complex. J Infect Dis 1988;158:1079-83
138. Antinori A, Arendt G, Becker JT, et al. Updated research nosology for HIV-associated neurocognitive disorders. Neurology 2007;69:1789-99

139. d'Arminio Monforte A, Cinque P, Mocroft A, et al. Changing incidence of central nervous system diseases in the EuroSIDA cohort. Ann Neurol 2004;55:320-8
140. Price RW, Spudich S. Antiretroviral therapy and central nervous system HIV type 1 infection. J Infect Dis 2008;197 Suppl 3:S294-306
141. Cysique LA, Maruff P and Brew BJ. Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre- and post-highly active antiretroviral therapy eras: a combined study of two cohorts. J Neurovirol 2004;10:350-7
142. Marra CM, Lockhart D, Zunt JR, Perrin M, Coombs RW and Collier AC. Changes in CSF and plasma HIV-1 RNA and cognition after starting potent antiretroviral therapy. Neurology 2003;60:1388-90
143. Price RW, Yiannoutsos CT, Clifford DB, et al. Neurological outcomes in late HIV infection: adverse impact of neurological impairment on survival and protective effect of antiviral therapy. AIDS Clinical Trial Group and Neurological AIDS Research Consortium study team. Aids 1999;13:1677-85
144. Sidtis JJ, Gatsonis C, Price RW, et al. Zidovudine treatment of the AIDS dementia complex: results of a placebo-controlled trial. AIDS Clinical Trials Group. Ann Neurol 1993;33:343-9
145. Cysique LA, Brew BJ. Neuropsychological functioning and antiretroviral treatment in HIV/AIDS: a review. Neuropsychol Rev 2009;19:169-85
146. Robertson KR, Smurzynski M, Parsons TD, et al. The prevalence and incidence of neurocognitive impairment in the HAART era. AIDS 2007;21:1915-21
147. Gisslen M, Hagberg L, Rosengren L, et al. Defining and evaluating HIV-related neurodegenerative disease and its treatment targets: a combinatorial approach to use of cerebrospinal fluid molecular biomarkers. J Neuroimmune Pharmacol 2007;2:112-9
148. Price RW, Epstein LG, Becker JT, et al. Biomarkers of HIV-1 CNS infection and injury. Neurology 2007;69:1781-8
149. Spudich SS, Nilsson AC, Lollo ND, et al. Cerebrospinal fluid HIV infection and pleocytosis: relation to systemic infection and antiretroviral treatment. BMC Infect Dis 2005;5:98
150. Marra CM, Maxwell CL, Collier AC, Robertson KR and Imrie A. Interpreting cerebrospinal fluid pleocytosis in HIV in the era of potent antiretroviral therapy. BMC Infect Dis 2007;7:37
151. Conrad AJ, Schmid P, Syndulko K, et al. Quantifying HIV-1 RNA using the polymerase chain reaction on cerebrospinal fluid and serum of seropositive individuals with and without neurologic abnormalities. J Acquir Immune Defic Syndr Hum Retrovirol 1995;10:425-35
152. Brew BJ, Pemberton L, Cunningham P and Law MG. Levels of human immunodeficiency virus type 1 RNA in cerebrospinal fluid correlate with AIDS dementia stage. J Infect Dis 1997;175:963-6

153. McArthur JC, McClernon DR, Cronin MF, et al. Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain. Ann Neurol 1997;42:689-98
154. Tambussi G, Gori A, Capiluppi B, et al. Neurological symptoms during primary human immunodeficiency virus (HIV) infection correlate with high levels of HIV RNA in cerebrospinal fluid. Clin Infect Dis 2000;30:962-5
155. Mellgren A, Antinori A, Cinque P, et al. Cerebrospinal fluid HIV-1 infection usually responds well to antiretroviral treatment. Antivir Ther 2005;10:701-7
156. Elovaara I, Seppala I, Poutiainen E, Suni J and Valle SL. Intrathecal humoral immunologic response in neurologically symptomatic and asymptomatic patients with human immunodeficiency virus infection. Neurology 1988;38:1451-6
157. Martin C, Albert J, Hansson P, Pehrsson P, Link H and Sonnerborg A. Cerebrospinal fluid mononuclear cell counts influence CSF HIV-1 RNA levels. J Acquir Immune Defic Syndr Hum Retrovirol 1998;17:214-9
158. Elovaara I, Nykyri E, Poutiainen E, Hokkanen L, Raininko R and Suni J. CSF follow-up in HIV-1 infection: intrathecal production of HIV-specific and unspecific IGG, and beta-2-microglobulin increase with duration of HIV-1 infection. Acta Neurol Scand 1993;87:388-96
159. Gisslen M, Chiodi F, Fuchs D, et al. Markers of immune stimulation in the cerebrospinal fluid during HIV infection: a longitudinal study. Scand J Infect Dis 1994;26:523-33
160. Fuchs D, Spira TJ, Hausen A, et al. Neopterin as a predictive marker for disease progression in human immunodeficiency virus type 1 infection. Clin Chem 1989;35:1746-9
161. Hoffmann G, Wirleitner B and Fuchs D. Potential role of immune system activation-associated production of neopterin derivatives in humans. Inflamm Res 2003;52:313-21
162. Fuchs D, Chiodi F, Albert J, et al. Neopterin concentrations in cerebrospinal fluid and serum of individuals infected with HIV-1. Aids 1989;3:285-8
163. Hagberg L, Dotevall L, Norkrans G, Larsson M, Wachter H and Fuchs D. Cerebrospinal fluid neopterin concentrations in central nervous system infection. J Infect Dis 1993;168:1285-8
164. Hagberg L, Cinque P, Gisslen M, et al. Cerebrospinal Fluid Neopterin: An Informative Biomarker of Central Nervous System Immune Activation in HIV-1 Infection. Submitted 2010
165. Abdulle S, Hagberg L, Svennerholm B, Fuchs D and Gisslen M. Continuing intrathecal immunoactivation despite two years of effective antiretroviral therapy against HIV-1 infection. Aids 2002;16:2145-9
166. Albright AV, Soldan SS and Gonzalez-Scarano F. Pathogenesis of human immunodeficiency virus-induced neurological disease. J Neurovirol 2003;9:222-7
167. Gartner S. HIV infection and dementia. Science 2000;287:602-4

168. Liu Y, Tang XP, McArthur JC, Scott J and Gartner S. Analysis of human immunodeficiency virus type 1 gp160 sequences from a patient with HIV dementia: evidence for monocyte trafficking into brain. J Neurovirol 2000;6 Suppl 1:S70-81
169. Fischer-Smith T, Croul S, Sverstiuk AE, et al. CNS invasion by CD14+/CD16+ peripheral blood-derived monocytes in HIV dementia: perivascular accumulation and reservoir of HIV infection. J Neurovirol 2001;7:528-41
170. Gonzalez-Scarano F, Martin-Garcia J. The neuropathogenesis of AIDS. Nat Rev Immunol 2005;5:69-81
171. Ellis R, Langford D and Masliah E. HIV and antiretroviral therapy in the brain: neuronal injury and repair. Nat Rev Neurosci 2007;8:33-44
172. Kim WK, Avarez X and Williams K. The role of monocytes and perivascular macrophages in HIV and SIV neuropathogenesis: information from non-human primate models. Neurotox Res 2005;8:107-15
173. Kaul M, Lipton SA. Mechanisms of neuronal injury and death in HIV-1 associated dementia. Curr HIV Res 2006;4:307-18
174. Sinclair E, Ronquillo R, Lollo N, et al. Antiretroviral treatment effect on immune activation reduces cerebrospinal fluid HIV-1 infection. J Acquir Immune Defic Syndr 2008;47:544-52
175. Williams K, Alvarez X and Lackner AA. Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. Glia 2001;36:156-64
176. Dunfee RL, Thomas ER, Gorry PR, et al. The HIV Env variant N283 enhances macrophage tropism and is associated with brain infection and dementia. Proc Natl Acad Sci U S A 2006;103:15160-5
177. Ohagen A, Devitt A, Kunstman KJ, et al. Genetic and functional analysis of full-length human immunodeficiency virus type 1 env genes derived from brain and blood of patients with AIDS. J Virol 2003;77:12336-45
178. Power C, McArthur JC, Johnson RT, et al. Demented and nondemented patients with AIDS differ in brain-derived human immunodeficiency virus type 1 envelope sequences. J Virol 1994;68:4643-49
179. Ellis RJ, Gamst AC, Capparelli E, et al. Cerebrospinal fluid HIV RNA originates from both local CNS and systemic sources. Neurology 2000;54:927-36
180. Haas DW, Clough LA, Johnson BW, et al. Evidence of a source of HIV type 1 within the central nervous system by ultraintensive sampling of cerebrospinal fluid and plasma. AIDS Res Hum Retroviruses 2000;16:1491-502
181. Harrington PR, Haas DW, Ritola K and Swanstrom R. Compartmentalized human immunodeficiency virus type 1 present in cerebrospinal fluid is produced by short-lived cells. J Virol 2005;79:7959-66

182. Ritola K, Pilcher CD, Fiscus SA, et al. Multiple V1/V2 env variants are frequently present during primary infection with human immunodeficiency virus type 1. J Virol 2004;78:11208-18
183. Ritola K, Robertson K, Fiscus SA, Hall C and Swanstrom R. Increased human immunodeficiency virus type 1 (HIV-1) env compartmentalization in the presence of HIV-1-associated dementia. J Virol 2005;79:10830-4
184. Harrington PR, Schnell G, Letendre SL, et al. Cross-sectional characterization of HIV-1 env compartmentalization in cerebrospinal fluid over the full disease course. AIDS 2009;23:907-15
185. Schnell G, Spudich S, Harrington P, Price RW and Swanstrom R. Compartmentalized human immunodeficiency virus type 1 originates from long-lived cells in some subjects with HIV-1-associated dementia. PLoS Pathog 2009;5:e1000395
186. Cunningham PH, Smith DG, Satchell C, Cooper DA and Brew B. Evidence for independent development of resistance to HIV-1 reverse transcriptase inhibitors in the cerebrospinal fluid. Aids 2000;14:1949-54
187. Lanier ER, Sturge G, McClernon D, et al. HIV-1 reverse transcriptase sequence in plasma and cerebrospinal fluid of patients with AIDS dementia complex treated with Abacavir. Aids 2001;15:747-51
188. Spudich S, Lollo N, Liegler T, Deeks SG and Price RW. Treatment benefit on cerebrospinal fluid HIV-1 levels in the setting of systemic virological suppression and failure. J Infect Dis 2006;194:1686-96
189. Stingele K, Haas J, Zimmermann T, et al. Independent HIV replication in paired CSF and blood viral isolates during antiretroviral therapy. Neurology 2001;56:355-61
190. Maslin CL, Kedzierska K, Webster NL, Muller WA and Crowe SM. Transendothelial migration of monocytes: the underlying molecular mechanisms and consequences of HIV-1 infection. Curr HIV Res 2005;3:303-17
191. Enting RH, Hoetelmans RM, Lange JM, Burger DM, Beijnen JH and Portegies P. Antiretroviral drugs and the central nervous system. Aids 1998;12:1941-55
192. Best BM, Letendre SL, Brigid E, et al. Low atazanavir concentrations in cerebrospinal fluid. AIDS 2009;23:83-7
193. Foudraine NA, Hoetelmans RM, Lange JM, et al. Cerebrospinal-fluid HIV-1 RNA and drug concentrations after treatment with lamivudine plus zidovudine or stavudine. Lancet 1998;351:1547-51
194. Letendre S, Marquie-Beck J, Capparelli E, et al. Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system. Arch Neurol 2008;65:65-70
195. Tashima KT, Caliendo AM, Ahmad M, et al. Cerebrospinal fluid human immunodeficiency virus type 1 (HIV-1) suppression and efavirenz drug concentrations in HIV-1-infected patients receiving combination therapy. J Infect Dis 1999;180:862-4

196. Yilmaz A, Gisslen M, Spudich S, et al. Raltegravir cerebrospinal fluid concentrations in HIV-1 infection. PLoS One 2009;4:e6877
197. Yilmaz A, Stahle L, Hagberg L, Svennerholm B, Fuchs D and Gisslen M. Cerebrospinal fluid and plasma HIV-1 RNA levels and lopinavir concentrations following lopinavir/ritonavir regimen. Scand J Infect Dis 2004;36:823-8
198. Yilmaz A, Watson V, Else L and Gisslen M. Cerebrospinal fluid maraviroc concentrations in HIV-1 infected patients. Aids 2009;23:2537-40
199. Varatharajan L, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary of current knowledge and recommendations for further research. Antiviral Res 2009;82:A99-109
200. Perno CF, Svicher V, Schols D, Pollicita M, Balzarini J and Aquaro S. Therapeutic strategies towards HIV-1 infection in macrophages. Antiviral Res 2006;71:293-300
201. Polis MA, Suzman DL, Yoder CP, et al. Suppression of cerebrospinal fluid HIV burden in antiretroviral naive patients on a potent four-drug antiretroviral regimen. AIDS 2003;17:1167-72
202. Yilmaz A, Svennerholm B, Hagberg L and Gisslen M. Cerebrospinal fluid viral loads reach less than 2 copies/ml in HIV-1-infected patients with effective antiretroviral therapy. Antivir Ther 2006;11:833-7
203. Markowitz M, Perelson AS. HIV-1 viral dynamics studies in the setting of clinical trials--A window of opportunity. J Infect Dis 2007;195:1087-8
204. Boyd MA, Dixit NM, Siangphoe U, et al. Viral decay dynamics in HIV-infected patients receiving ritonavir-boosted saquinavir and efavirenz with or without enfuvirtide: a randomized, controlled trial (HIV-NAT 012). J Infect Dis 2006;194:1319-22
205. Kuritzkes DR, Ribaudo HJ, Squires KE, et al. Plasma HIV-1 RNA dynamics in antiretroviral-naive subjects receiving either triple-nucleoside or efavirenz-containing regimens: ACTG A5166s. J Infect Dis 2007;195:1169-76
206. Gisslen M, Fredman P, Fuchs D, Lekman A and Rosengren L. Temporarily controlled HIV-1 replication after intravenous immunoglobulin treatment of Guillain-Barre syndrome. Scand J Infect Dis 2005;37:877-81
207. Polis MA, Sidorov IA, Yoder C, et al. Correlation between reduction in plasma HIV-1 RNA concentration 1 week after start of antiretroviral treatment and longer-term efficacy. Lancet 2001;358:1760-5
208. Wu H, Lathey J, Ruan P, et al. Relationship of plasma HIV-1 RNA dynamics to baseline factors and virological responses to highly active antiretroviral therapy in adolescents (aged 12-22 years) infected through high-risk behavior. J Infect Dis 2004;189:593-601
209. van Leth F, Huisamen CB, Badaro R, et al. Plasma HIV-1 RNA decline within the first two weeks of treatment is comparable for nevirapine, efavirenz, or both drugs combined and is not predictive of long-term virologic efficacy: A 2NN substudy. J Acquir Immune Defic Syndr 2005;38:296-300

210. Molina JM, Andrade-Villanueva J, Echevarria J, et al. Once-daily atazanavir/ritonavir compared with twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 96-week efficacy and safety results of the CASTLE study. J Acquir Immune Defic Syndr 2010;53:323-32
211. Riddler SA, Haubrich R, DiRienzo AG, et al. Class-sparing regimens for initial treatment of HIV-1 infection. N Engl J Med 2008;358:2095-106
212. Murray JM, Emery S, Kelleher AD, et al. Antiretroviral therapy with the integrase inhibitor raltegravir alters decay kinetics of HIV, significantly reducing the second phase. Aids 2007;21:2315-21
213. Sedaghat AR, Dinoso JB, Shen L, Wilke CO and Siliciano RF. Decay dynamics of HIV-1 depend on the inhibited stages of the viral life cycle. Proc Natl Acad Sci U S A 2008;105:4832-7
214. Shen L, Rabi SA and Siliciano RF. A novel method for determining the inhibitory potential of anti-HIV drugs. Trends Pharmacol Sci 2009;30:610-6
215. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. December 1, 2009; 1-161. Available at http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed february 2010., 2009
216. Hammer SM, Eron JJ, Jr., Reiss P, et al. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. Jama 2008;300:555-70
217. Chun TW, Engel D, Mizell SB, et al. Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. Nat Med 1999;5:651-5
218. Chun TW, Davey RT, Jr., Engel D, Lane HC and Fauci AS. Re-emergence of HIV after stopping therapy. Nature 1999;401:874-5
219. Davey RT, Jr., Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc Natl Acad Sci U S A 1999;96:15109-14
220. Stellbrink HJ, van Lunzen J, Westby M, et al. Effects of interleukin-2 plus highly active antiretroviral therapy on HIV-1 replication and proviral DNA (COSMIC trial). Aids 2002;16:1479-87
221. Fraser C, Ferguson NM, Ghani AC, et al. Reduction of the HIV-1-infected T-cell reservoir by immune activation treatment is dose-dependent and restricted by the potency of antiretroviral drugs. Aids 2000;14:659-69
222. Kulkosky J, Nunnari G, Otero M, et al. Intensification and stimulation therapy for human immunodeficiency virus type 1 reservoirs in infected persons receiving virally suppressive highly active antiretroviral therapy. J Infect Dis 2002;186:1403-11

223. Prins JM, Jurriaans S, van Praag RM, et al. Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. Aids 1999;13:2405-10
224. van Praag RM, Prins JM, Roos MT, et al. OKT3 and IL-2 treatment for purging of the latent HIV-1 reservoir in vivo results in selective long-lasting CD4+ T cell depletion. J Clin Immunol 2001;21:218-26
225. Bowman MC, Archin NM and Margolis DM. Pharmaceutical approaches to eradication of persistent HIV infection. Expert Rev Mol Med 2009;11:e6
226. Lehrman G, Hogue IB, Palmer S, et al. Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. Lancet 2005;366:549-55
227. Archin NM, Eron JJ, Palmer S, et al. Valproic acid without intensified antiviral therapy has limited impact on persistent HIV infection of resting CD4+ T cells. Aids 2008;22:1131-5
228. Sagot-Lerolle N, Lamine A, Chaix ML, et al. Prolonged valproic acid treatment does not reduce the size of latent HIV reservoir. Aids 2008;22:1125-9
229. Siliciano JD, Lai J, Callender M, et al. Stability of the latent reservoir for HIV-1 in patients receiving valproic acid. J Infect Dis 2007;195:833-6
230. Steel A, Clark S, Teo I, et al. No change to HIV-1 latency with valproate therapy. Aids 2006;20:1681-2
231. Wang FX, Xu Y, Sullivan J, et al. IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. J Clin Invest 2005;115:128-37
232. Sereti I, Dunham RM, Spritzler J, et al. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. Blood 2009;113:6304-14
233. Chi LJ, Wang HB, Zhang Y and Wang WZ. Abnormality of circulating CD4(+)CD25(+) regulatory T cell in patients with Guillain-Barre syndrome. J Neuroimmunol 2007;192:206-14
234. Ephrem A, Chamat S, Miquel C, et al. Expansion of CD4+CD25+ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. Blood 2008;111:715-22
235. Kumar V. Homeostatic control of immunity by TCR peptide-specific Tregs. J Clin Invest 2004;114:1222-6
236. Chase AJ, Yang HC, Zhang H, Blankson JN and Siliciano RF. Preservation of FoxP3+ regulatory T cells in the peripheral blood of human immunodeficiency virus type 1-infected elite suppressors correlates with low CD4+ T-cell activation. J Virol 2008;82:8307-15
237. Yilmaz A, Price RW, Spudich S, Fuchs D, Hagberg L and Gisslen M. Persistent intrathecal immune activation in HIV-1-infected individuals on antiretroviral therapy. J Acquir Immune Defic Syndr 2008;47:168-73
238. Hagberg L, Norkrans G, Zanetta JP, Lehmann S and Bergstrom T. Cerebrospinal fluid anti-cerebellar soluble lectin antibodies in human immunodeficiency virus type 1 infection. J Neuroimmunol 1992;36:245-9

239. Resnick L, diMarzo-Veronese F, Schupbach J, et al. Intra-blood-brain-barrier synthesis of HTLV-III-specific IgG in patients with neurologic symptoms associated with AIDS or AIDS-related complex. N Engl J Med 1985;313:1498-504
240. Karlstrom O, Stahle L, Perrin L, Tegude H and Sonnerborg A. Efficacy of nelfinavir-based treatment in the central nervous system of HIV-1 infected patients. Scand J Infect Dis 2006;38:371-4
241. Ho DD, Rota TR and Hirsch MS. Infection of monocyte/macrophages by human T lymphotropic virus type III. J Clin Invest 1986;77:1712-5
242. Swingler S, Mann AM, Zhou J, Swingler C and Stevenson M. Apoptotic killing of HIV-1-infected macrophages is subverted by the viral envelope glycoprotein. PLoS Pathog 2007;3:1281-90
243. Gisolf EH, Enting RH, Jurriaans S, et al. Cerebrospinal fluid HIV-1 RNA during treatment with ritonavir/saquinavir or ritonavir/saquinavir/stavudine. AIDS 2000;14:1583-9
244. Schrager LK, D'Souza MP. Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. Jama 1998;280:67-71
245. El-Sadr WM, Lundgren JD, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med 2006;355:2283-96
246. Gisslen M, Rosengren L, Hagberg L, Deeks SG and Price RW. Cerebrospinal fluid signs of neuronal damage after antiretroviral treatment interruption in HIV-1 infection. AIDS Res Ther 2005;2:6
247. Podsadecki TJ, Vrijens BC, Tousset EP, Rode RA and Hanna GJ. Decreased adherence to antiretroviral therapy observed prior to transient human immunodeficiency virus type 1 viremia. J Infect Dis 2007;196:1773-8
248. Letendre S, FitzSimons C, Ellis R, et al. Correlates of CSF Viral Loads in 1221 Volunteers of the CHARTER Cohort. 17th Conference on Retroviruses and Opportunistic Infections. San Francisco, CA, US, 2010
249. Canestri A, Lescure FX, Jaureguiberry S, et al. Discordance between cerebral spinal fluid and plasma HIV replication in patients with neurological symptoms who are receiving suppressive antiretroviral therapy. Clin Infect Dis 2010;50:773-8
250. de Truchis P, Mathez D, Abe E, et al. Cerebrospinal fluid HIV-1 virological escape with lymphocytic meningitis under lopinavir/ritonavir monotherapy. Aids 2010;24:1235-6
251. Garvey LJ, Everitt A, Winston A, Mackie NE and Benzie A. Detectable cerebrospinal fluid HIV RNA with associated neurological deficits, despite suppression of HIV replication in the plasma compartment. AIDS 2009;23:1443-4