The Central Nervous System Manifestations of HIV-1 Infection in the Combination Antiretroviral Therapy Era

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Abstract

Following the introduction of effective therapies to treat human immunodeficiency virus (HIV-1) infection in the mid-1990s, a dramatic reduction in the incidence of severe HIV-1 associated brain disease was observed. In spite of such advances however, milder forms of HIV-1 associated neurocognitive disorders (HAND) have become increasingly apparent in recent years and can reduce the cognitive function, quality of life and overall survival of those affected. Coinfection with viral hepatitis C (HCV) and poor central nervous system (CNS) penetration of antiretroviral drugs may be risk factors for this condition.

This thesis examines the following two hypotheses:

Use of antiretroviral drugs with greater CNS penetration is associated with greater improvements in cerebral function parameters in HIV-1 infected subjects

Acquisition of acute HCV coinfection is associated with a deterioration of cerebral function parameters in HIV-1 infected subjects

These hypotheses were tested in a series of clinical studies. First, retrospective data from the largest UK cohort study of adult HIV-1 infected subjects were analysed to assess the impact of antiretroviral therapy CNS penetration upon HIV-associated brain disease incidence and survival between 1996 and 2008. Second, prospective changes to cerebral function parameters were assessed in HIV-1 infected subjects switching to novel antiretroviral regimens with differing CNS penetration via the use of longitudinal cerebral proton spectroscopy and computerised cognitive assessments.

In order to investigate the second hypothesis, a cross-sectional study was performed to assess cognition in HIV-1 infected subjects, with and without acute HCV coinfection. Finally, the presence of neuronal damage, neuronal inflammation and *in vivo* microglial cell activation in individuals with chronic HIV-1 and acute HCV coinfection were investigated in case-control studies utilising cerebral proton spectroscopy and positron emission tomography.

Results demonstrated that in some controlled settings, novel antiretroviral switching strategies involving simplification to darunavir-containing triple or monotherapy or intensification with maraviroc, are associated with improvements to parameters of cerebral function. No evidence that

the widespread use of regimens with higher CSF penetration effectiveness scores is associated with reduced incidence of HIV-1 associated CNS opportunistic diseases was found. It was also demonstrated that cerebral disturbance and a deterioration of cerebral function parameters is associated with acute hepatitis C (HCV) coinfection via impairment of executive functioning and increased cerebral metabolites. No association between microglial cell activation and acute HCV/HIV coinfection was found.

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Declaration

The work in this thesis was completed by Dr Lucy Garvey under the supervision of Dr Alan Winston and Professor Simon Taylor-Robinson at Imperial College, London, UK. Statistical calculations were performed by Dr Lucy Garvey and (in Chapter 3 only) by Professor Caroline Sabin for the UKCHIC Study.

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Abbreviations

ABC	Abacavir
AIDS	Acquired Immune Deficiency Syndrome
ANI	Asymptomatic neurocognitive impairment
ALT	Alanine aminotransferase
ATV	Atazanavir
AZT	Zidovudine
BBB	Blood brain barrier
b.i.d.	Twice-daily
B2-M	β2-microglobulin
BP	^{11}C PK11195 Binding potential
cART	Combination antiretroviral therapy
CCR5	Cysteine cysteine receptor 5
Cho	Choline-containing compounds
CI	Confidence interval
CNS	Central nervous system
CPE	CNS Penetration Effectiveness Rank
Cr	Creatine
CrAg	Cryptococcal antigen
^{11}C	Radioisotope carbon 11
CRYPTO	Cryptococcal meningitis
CSF	Cerebrospinal fluid
CT	Computerised tomography
ddI	Didanosine
d4T	Stavudine
DRV	Darunavir
EFV	Efavirenz
FGM	Frontal grey matter
FWM	Frontal white matter
FTC	Emtricitabine
HAND	HIV-associated neurocognitive disorders
HCV	Hepatitis C virus

HIV-1	Human Immunodeficiency Virus type 1
HIV-E	HIV-associated encephalopathy
HDS	HIV Dementia Scale
IC_{50}	50 percent inhibitory concentration
IDV	Indinavir
IHDS	International HIV Dementia Scale
IQR	Interquartile range
JCV	JC virus
LG	Lucy Garvey
LP	Lumbar puncture
LPV	Lopinavir
MBq	Megabecquerel (SI unit of radioactivity)
mCi	Millicurie (non SI unit of radioactivity)
mI	myo-inositol
MND	Mild neurocognitive disorder
MR	Magnetic resonance
^{1}H-MRS	Proton magnetic resonance spectroscopy
mSv	Millisievert (tissue dose of radioactivity)
MVC	Maraviroc
NAA	N-acetyl aspartate
NCI	Neurocognitive impairment
NNRTI	Non nucleoside reverse transcriptase inhibitor
NP	Neuropsychological
NiP	Nicola Pavese
NVP	Nevirapine
PCR	Polymerase chain reaction
PET	Positron emission tomography
PI	Protease inhibitor
pK	Pharmacokinetic
PK11195	(1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-2-isoquinoline carboxamide
PML	Progressive multifocal leucoencephalopathy
PYFU	Person-years-of-follow-up
q.d.	Once daily

RBG	Right basal ganglia
RNA	Ribonucleic acid
RTG	Raltegravir
RTV	Ritonavir
SD	Standard deviation
TDF	Tenofovir
3TC	Lamivudine

CHAPTER 1

General Introduction

Chapter 1: General Introduction

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1.1 Hypotheses of thesis

Mild forms of HIV-associated neurocognitive disorders (HAND) have become increasingly apparent in recent years. Poor CNS penetration of antiretroviral drugs and acquisition of acute HCV infection may be risk factors for these disorders.

This thesis will examine the following hypotheses:

- **Use of antiretroviral drugs with greater CNS penetration is associated with greater improvements in cerebral function parameters in HIV-1 infected subjects**
- **Acquisition of acute HCV coinfection is associated with a deterioration of cerebral function parameters in HIV-1 infected subjects**

1.2 Cerebral manifestations of HIV-1 infection

1.2.1 Neuropathology

Identification of HIV-1 infection, a retrovirus with the capacity to replicate in CD4+ T-lymphocytes and cause progressive immune-deficiency, was first made over 25 years ago (Barre-Sinoussi, et al. 1983; Broder and Gallo 1984; Snider, et al. 1983). A deleterious effect of this virus upon the human CNS was also described early in the epidemic, characterised typically by a progressive, sub-cortical encephalopathy, with associated high mortality (Nurnberg, et al. 1984; Snider, et al. 1983).

HIV-1 enters the CNS early, within days of viral transmission, migrating across the blood-brain-barrier (BBB) via infected monocytes, differentiating into perivascular microglia and macrophages and establishing a viral reservoir. These cells then release viral proteins (including gp 120 and Tat) which can cause neuronal apoptosis and excitotoxicity. Inflammatory chemokines are also released which activate neighbouring astrocytes and BBB epithelium, thereby enhancing monocyte recruitment (Gartner 2000; Mattson, et al. 2005). Increased numbers of circulating monocytes expressing CD16+ (Gartner 2000) and production of monocyte chemoattractant protein-1 (MCP-1) (Asensio and Campbell 1999), further enhance neurological damage by increasing numbers of HIV-1 infected cells entering the CNS. Conversely, CD8+ T-lymphocyte production, may assist the control of HIV-1 replication within the CNS during chronic infection in some individuals (Sadagopal, et al. 2008).

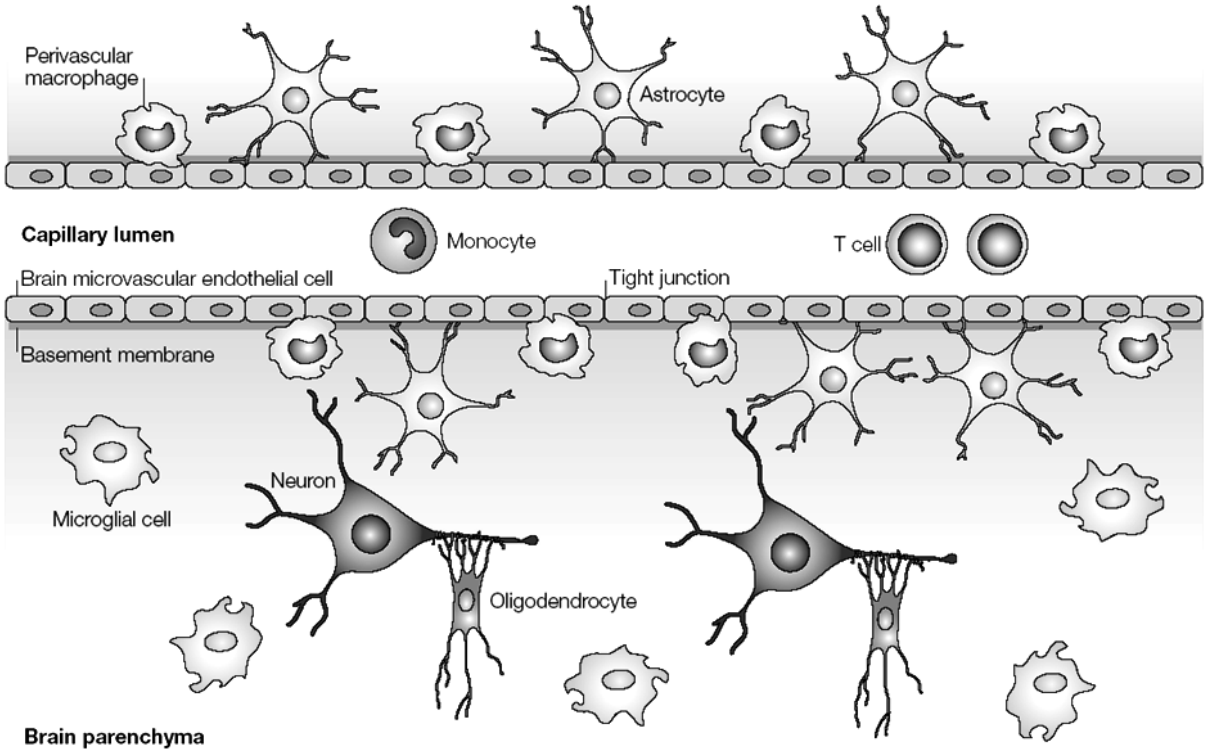


Figure 1.1: Diagram to represent the blood brain barrier. This structure protects the CNS from peripheral pathogens and toxins. It consists of a layer of tightly linked endothelial cells. Migration of HIV-1 infection into the CNS occurs via the activation of infected monocytes and perivascular macrophages [adapted from (Gonzalez-Scarano and Martin-Garcia 2005)]

In the early HIV-1 epidemic of the 1980s, prior to the introduction of effective combination antiretroviral therapy (cART), severe neurological complications were frequently observed clinical events. Autopsy series of patients with AIDS demonstrated CNS pathology in over 80% of cases (Navia, et al. 1986). These diseases were either opportunistic infections due to advanced immune suppression and depletion of CD4+ lymphocytes, or a spectrum of HIV-associated neurocognitive disorders (HAND), primarily caused by direct HIV-1 infection of the CNS, the most severe form being HIV encephalopathy (HIV-E, also known as HIV-associated dementia or AIDS dementia complex).

1.2.2 Central nervous system opportunistic infections

The CNS opportunistic infections progressive multifocal leucoencephalopathy (PML), cerebral toxoplasmosis (TOXO) and cryptococcal meningitis (CRYPTO) were devastating clinical presentations in the pre-cART era (Snider, et al. 1983). Fortunately, in the modern day, these conditions are now rare in resource-rich settings and are most frequently diagnosed in individuals who present to

healthcare providers with very advanced HIV-1 disease (usually accompanied by a CD4+ cell count below 200 cells/uL) or in those unable to commence or adhere to cART as recommended by clinical guidelines. Opportunistic infections of the CNS continue to carry the greatest morbidity and mortality of all systems. Radiological examination and cerebrospinal fluid (CSF) evaluation are often essential in determining diagnosis and appropriate management. Advances in magnetic resonance (MR) imaging and CSF nucleic acid amplification techniques have refined the diagnostic process making invasive procedures, such as brain biopsy, less frequently required. Early introduction of cART is vital in reducing the morbidity and mortality associated with these conditions (Zolopa, et al. 2009).

1.2.2.1 Progressive multifocal leucoencephalopathy (PML)

PML is a progressive, demyelinating neurological disease which arises due to reactivation of JC virus (JCV), a polyoma virus, in immunocompromised hosts. Primary infection with JCV most commonly occurs in childhood before establishing latency. Prevalence is estimated at over 70% (Stolt, et al. 2003). During reactivation, the virus replicates and is transported to the CNS by B-lymphocytes where it infects oligodendrocytes causing focal demyelination, most commonly in cerebral white matter, but which can also occur in the cerebellum and occasionally grey matter (Berenguer, et al. 2003). Clinical symptoms develop over weeks and months and reflect the location of demyelination, with focal signs, ataxia and seizures being observed. Typically, MR imaging demonstrates bilateral, asymmetrical and non-enhancing T_2-hyperintense and T_1-hypointense lesions, largely affecting white matter and without cerebral oedema. CSF detection of JCV by PCR techniques may assist diagnosis, with reported sensitivity of over 72% and specificity of 92-100% in the pre-cART era. In more recent years, such rates of detection have fallen due to decreased levels of JCV CSF clearance in subjects with prior antiretroviral exposure (Marzocchetti, et al. 2005). Where a diagnosis remains uncertain, stereotactic brain biopsy should be pursued. The recommended treatment for PML is prompt initiation of cART, with evidence from randomized clinical trials lacking for other agents with activity against JCV *in vitro*. Rarely PML may arise *de novo* after cART initiation as part of an immune reconstitution syndrome. In the cART era, survival rates following PML have increased, but less dramatically than following other opportunistic infections. It is estimated that approximately 50% of patients with PML are alive at 5 years (Antinori, et al. 2003).

1.2.2.2 Cerebral toxoplasmosis (TOXO)

TOXO arises due to reactivation of latent infection with *Toxoplasma gondii,* a protozoan which can infect birds, mammals or humans. Prevalence varies and in some parts of Europe approaches 90%. Reactivation rarely occurs in individuals with a CD4+ cell count above 100 cells/uL (Porter and Sande 1992). TOXO has an affinity for the CNS and is the most common cause of cerebral mass lesions in HIV-1 infected subjects. Typically, patients present with focal signs which may be associated with fever. Without treatment, intracranial pressure can rise and patients rapidly progress to seizures, coma and death. Diagnosis is usually made on the basis of clinical and radiological findings. Multiple ring-enhancing lesions, associated with cerebral oedema and mass effect, occur at the interface of grey/white matter and in the deep grey matter of the basal ganglia or thalamus (Offiah and Turnbull 2006; Steinmetz, et al. 1995). Where no contraindication exists, CSF PCR tests for *T.gondii* may be performed, but are of limited sensitivity (Schoondermark-van de Ven, et al. 1993). Positive serology (IgG) indicates only past infection, and is also, therefore of limited diagnostic utility, but the diagnosis is rare in sero-negative individuals. First-line therapy for TOXO is with pyrimethamine and sulphadiazine for 6 weeks followed by maintenance therapy (Katlama, et al. 1996; Luft, et al. 1993). Approximately 90% of patients will show a clinical and radiological response at 2 weeks of therapy (Luft, et al. 1993).

1.2.2.3 Cryptococcal meningitis (CRYPTO)

This CNS opportunistic infection is caused by the encapsulated yeast, *Cryptococcus neoformans* which is primarily acquired via inhalation. In patients with advanced immunosuppression (usually with a CD4+ cell count below 50 cells/uL), haematogenous dissemination to the CNS occurs, which if untreated, is fatal. The condition causes headaches with fever and as intracranial pressure rises, vomiting, seizures, confusion, blurred vision and coma can ensue. Simultaneous manifestations of pulmonary or skin disease may be present. Diagnosis is made using a serum CrAg test, which if negative excludes CRYPTO in the majority of cases (Nelson, et al. 1990). All patients should undergo CSF evaluation after CT or MR imaging and manometry must always be performed to exclude raised intracranial pressure. Positive CSF CrAg, Indian ink stain or cryptococcus culture confirms the condition. Treatment is with usually with amphoterecin B which may be combined with flucytosine (Brouwer, et al. 2004). Repeat assessments of CSF manometry and therapeutic CSF drainage may be required for persistently elevated intracranial pressure.

1.2.3 Clinical features of HIV-encephalopathy (HIV-E)

It is estimated that without antiretrovirals, approximately 15-20% of individuals with HIV-1 infection would develop HIV-E. This dementing illness typically occurs at the later stages of the HIV-1 infection when there is advanced immune-suppression (CD4+ cell count below 200/uL). Early reports from the pre-cART era describe a subcortical dementia with a progressive cognitive and motor dysfunction in individuals with AIDS (Fischer and Enzensberger 1988). Ataxia, loss of fine-motor skills, tremor and personality change (apathy and irritability) were also observed. In severe cases, paraplegia and mutism ensued and mortality was high (Anders, et al. 1986; Nurnberg, et al. 1984).

Initial clinical response of the condition to zidovudine (AZT) (Schmitt, et al. 1988) demonstrated a potential for treating HIV-E with antiretroviral agents, but clinical relapse typically followed as virological control was lost. With availability of more effective therapies in the cART era, HIV-E incidence fell dramatically and survival improved, albeit at a lesser rate than other opportunistic diseases (d'Arminio Monforte, et al. 2004; Dore, et al. 1999).

Assessment of subjects with suspected HIV-E typically reveals non-specific abnormalities and other CNS opportunistic infections must first be excluded. Findings may include global slowing of basal activity on electroencephalogram (EEG), generalised cortical atrophy, diffuse areas of altered signal intensity in white matter (leucoencephalopathy) and enlarged ventricles on radiological imaging (Balakrishnan, et al. 1990). Pathological findings at autopsy include multinucleated giant cells, myelin damage, amyloid plaques and microglial nodules (Grassi, et al. 2002). However these do not always correlate with clinical disease severity.

Currently, HIV-E is diagnosed where marked cognitive deficits impede an individual's ability to perform activities of daily living (Antinori, et al. 2007). Risk factors for the condition include lower CD4+ cell count, prior AIDS-defining illness, longer duration of HIV-1 infection and older age at seroconversion (Bhaskaran, et al. 2008). Owing to increased life expectancy and larger numbers of individuals living with chronic HIV-1 infection, the prevalence of HIV-E in recent years has actually increased (Dore, et al. 2003). Interestingly, some clinical features of HIV-E may have changed in recent years, with features of cortical dysfunction now being identified in some cohorts (Brew 2004; Cysique, et al. 2004). Cases have also been reported recently, whereby HIV-E has arisen, despite suppression of HIV in plasma by cART, but where viral replication is ongoing in CSF, suggesting compartmentalisation of viral strains due to selective drug pressure (Canestri, et al. 2010).

1.2.4 Clinical features of other HIV-1 associated neurocognitive disorders (HAND)

Less severe forms of cognitive deficits affecting HIV-1 infected subjects have become increasingly apparent in recent years, despite access to effective cART (McArthur, et al. 2004). Revised proposed diagnostic criteria of HAND recommend using detailed neuropsychological assessments assessing a minimum of 5 cognitive domains (including attention-information processing, language, abstraction and executive function, simple and complex perceptual motor skills, memory and sensory perceptual abilities). Where performance falls more than one SD below age-matched population norms in at least 2 domains, a diagnosis of HAND is made (Antinori, et al. 2007). Classification is then completed according to the presence or absence of symptoms including disturbed concentration, forgetfulness and emotional lability and the terms mild neurocognitive disorder (MND) or asymptomatic neurocognitive impairment (ANI) are used respectively.

Rates and features of HAND have now been examined in a variety of clinical and geographical research settings, although routine screening is not yet common place. Prevalence of cognitive deficits in HIV-infected cohorts are consistently higher than the general population (Ferrando, et al. 1998; Joska, et al. 2010; Njamnshi, et al. 2009; Robertson, et al. 2007) and have exceeded 50% of subjects assessed in some cohorts (Simioni, et al. 2010). Deficits most frequently affect executive function (including decision making and thought processing) and learning ability rather than cortical memory loss. Concern exists as some affected individuals demonstrate progressive neurocognitive deterioration on longitudinal assessment. Furthermore the condition has been associated with difficulty achieving employment, adhering to medication and with shorter survival (Albert SM 1999; Ellis, et al. 1997; Tozzi, et al. 2005b).

Reported risk factors for HAND include lower nadir CD4+ cell count and advanced clinical stage (Joska, et al. 2010), older age (Valcour, et al. 2004), insulin resistance (Valcour, et al. 2006) or previous cardiovascular disease (Wright, et al. 2010). Some cohorts, in addition, report association with chronic HCV coinfection (Ryan, et al. 2004).

In the current day, many other co-morbidities may cause CNS pathology in HIV-1 infected subjects. Conditions including neurological malignancies, neurovascular disease and other opportunistic infections such as cerebral tuberculosis continue to be observed, however are not discussed further in this thesis.

1.3 Investigation of cerebral function parameters in HIV-1 infection

1.3.1 Different methods of cognitive assessment

Different testing methods have been used to investigate the rate and features of cognitive deficits arising in HIV-1 infection. These include formal neuropsychological assessments, computerised tests and brief screening tools. Effect of practice and learning, confounding factors such as depression, alcohol or recreational drug misuse and range of cognitive domains assessed should be considered when interpreting results.

1.3.1.1 Formal neuropsychological (NP) assessment

Traditional NP tests, although the gold standard, take several hours to perform and require highly trained staff. They typically assess a wide range of cognitive domains including orientation, memory and new-learning, language, intelligence, visual perception and executive functioning. They incorporate assessments such as the Trailmaking A and B, Symbol-Digit Modalities and Grooved Pegboard tests (Selnes, et al. 1991). Psychological and personal information can also be incorporated and results compared to normative population data, stratified by age and frequently also ethnicity and education level.

1.3.1.2 Computerised cognitive tests

Computerised cognitive tests have now been developed as an alternative tool for the identification and progression of neurological deficits and are faster than traditional, formal assessments. This reduction in time and complexity of assessment is advantageous for patients with attention or concentration deficits, for those requiring multiple longitudinal assessments and reduces expense (Maruff, et al. 2009). Some have shown strong correlation with formal neuropsychological (NP) tests and high sensitivity for detecting cognitive deficits, including the subtle, sub-cortical features of HAND (including processing speed, working memory and learning)(Collie, et al. 2001; Mollica, et al. 2005). Tests can be designed to investigate the cerebral domains of interest within different neurological diseases and are now being more widely used for assessing cohorts of HIV-1 infected subjects (Boivin, et al. 2010; Cysique, et al. 2006).

1.3.1.3 Rapid screening assessments

The HIV Dementia Scale (HDS)

The HDS is a brief screening tool which was devised for the assessment of cognitive disorders in patients with HIV-1 in the pre-cART era. It is a face-to-face assessment which does not require equipment or extensive training (Table 1.1). Memory registration of four words is first performed (*dog, hat, green, peach*) and subjects are asked to recall of the words at the end of the assessment. Attention is assessed by performing 20 anti-saccadic eye movements and recording the error rate. Psychomotor speed is assessed by measuring the time taken to write all letters of the alphabet horizontally across a page. Finally, constructional skill is assessed by asking the subject to copy a simple diagram of a 3-dimensional cube and recording the time taken. This assessment has a maximum score of 16. Using a cut-off score of 10 or less, it has sensitivity of 80%, specificity of 91% and a positive predictive value of 78% for identifying subjects with HIV-E (Power, et al. 1995). This tool has, however, performed poorly as a screening tool for milder forms of HAND when using a cut-off score of 10 or less (Bottiggi, et al. 2007)and a recent study proposed a higher cut-off of 14 yielded a positive predictive value for HAND of between 82 and 92% according to presence of symptoms (Simioni, et al. 2010).

The International HIV Dementia Scale (IHDS)

This quick, face-to-face test assesses motor speed, psychomotor speed, and memory recall using 3 simple tasks (Table 1.2). Firstly memory registration of four words (*dog, hat, bean, red*) is performed. Motor speed is then assessed by instructing the subject to tap the first two fingers of their non-dominant hand on a flat surface, as widely and quickly as possible for 5 seconds. Psychomotor speed is then assessed using a sequential movement task of the non-dominant hand clenched in a fist, then flat on a surface and then perpendicular to the surface on the side of the 5^{th} digit. Finally memory recall is tested by asking the patient to say the 4 words given at the start of the test. Overall score for the IHDS ranges between 0 (worst performance) and 12 (best performance). The tool has been designed as a rapid assessment to identify subjects with HIV-associated cerebral function impairment and it is recommended any subject with a score of 10 or below warrants further investigation for neurocognitive problems. It has been used to assess HIV-infected cohorts in a variety of settings including the Africa and the United States (Maruff, et al. 2009; Patel, et al. 2007; Sacktor, et al. 2005b; Waldrop-Valverde, et al. 2010).

1.3.2 Cerebral imaging techniques used in HIV-1 disease

Cerebral imaging methods are available in most large treatment-centres in resource-rich settings and are widely used in the investigation of CNS opportunistic infections and HAND. In recent years, researchers have developed additional radiological techniques which show promise as alternative methods of investigating CNS disorders, but as yet, are not routinely used in clinical practice.

1.3.2.1 Computerised tomography and magnetic resonance imaging

In early and asymptomatic HIV-1 disease, cerebral abnormalities are difficult to detect using these imaging techniques and examination are normal in the majority of subjects. Small foci of demyelination within the white matter, commonly the frontal lobes, may be detected using T_2-weighted MR images in early stages of infection. Use of the fluid attenuated inversion recovery (FLAIR) sequence can aid detection of small lesions and those located at the cortical/subcortical interface and in the deep white matter (Thurnher, et al. 1997). With advancing HIV-1 disease, or in subjects with HAND, cerebral atrophy becomes more generalised and enlarged ventricles can be visualised (Balakrishnan, et al. 1990).

Demyelination due to PML appears as multifocal, asymmetrical hypodense lesions on CT images and hyperintense white matter lesions on T_2-weighted MR sequences. There is usually a lack of cerebral oedema or contrast enhancement and central areas of hyperintensity (on T_2-weighted MR) may represent neuronal necrosis (Thurnher, et al. 1997).

Toxoplasmosis cerebral abscesses appear as (usually multiple) hypodense lesions with ring-like or solid enhancement on CT imaging. Areas of cerebral oedema and mass effect may also be seen. Typical positioning of lesions includes the deep grey matter of the basal ganglia or thalamus (up to 75%), the interface of grey/white matter (corticomedullary junction) and posterior fossa (Offiah and Turnbull 2006; Steinmetz, et al. 1995). Such abscesses are usually hyperintense on T_2-weighted MR images and typically show regression of size, enhancement and associated oedema after 2 weeks of appropriate therapy.

Cerebral imaging of subjects with CRYPTO is typically non-specific, however mild dilation of ventricles and meningeal enhancement maybe observed after administration of contrast using MR-imaging. Rare findings include high signal intensity cystic lesions (containing fungi) in the basal ganglia on T_2-weighted MR images and cryptococcomas (solid or ring-like masses) which display contrast-enhancement in the choroid plexus (Tien, et al. 1991).

1.3.2.2 Magnetic resonance spectroscopy (^{1}H-MRS)

In recent years, cerebral ^{1}H-MRS has been used increasingly to investigate the cerebral effects of HIV-1 infection. This non-invasive tool is an objective and quantifiable radiological technique which uses hydrogen-1 (^{1}H) isotope to quantify the cerebral metabolites N-acetyl aspartate (NAA), Creatine (Cr), Choline-containing compounds (Cho) and myo-inositol (mI). NAA is located almost exclusively in neurones and is a marker of structural and functional neuronal integrity. mI and Cho are indicators of glial proliferation and cellular injury and increase with neuroinflammation. The Cr resonance is a marker of intracellular energy stores, since it contains a contribution from phosphocreatine and is often used as a reference for metabolite levels as levels are similar in neuronal and non-neuronal cell types (Grover, et al. 2006).

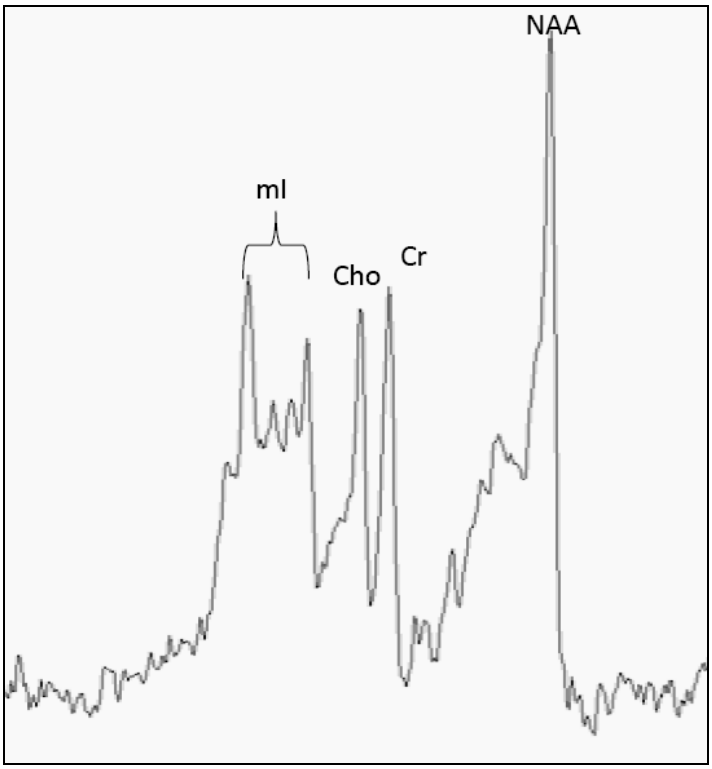


Figure 1.2: Example of cerebral proton magnetic resonance spectroscopy in frontal grey matter

[Legend: NAA= N-acetyl aspartate; Cr = creatine; Cho = Choline; mI=myo-inositol]

MRS changes reported in advanced HIV infection and HIV-E (summary Table 1.3) include reduced NAA/Cr of frontal white matter and thalamus (Lee, et al. 2003) (Stankoff, et al. 2001) and increased mI/Cr and Cho/Cr have also been reported in individuals with asymptomatic HIV-1 infection (Wilkinson, et al. 1997) (Suwanwelaa, et al. 2000) .

The effect of antiretroviral agents upon cerebral metabolites in HIV-E was initially encouraging, with increases of NAA described after high-dose AZT monotherapy (Salvan, et al. 1997). Subsequent work through the era of cART has, however, had variable results following antiretroviral agent administration. Decreased mI/Cr and Cho/Cr (Chang, et al. 1999) and increased NAA/Cr (Stankoff, et al. 2001) following cART in subjects with and without HAND have been reported. However, in contrast, other authors report no improvements to metabolite ratios (Chang, et al. 2004a) and even increases in markers of inflammation following short-course cART (Chang, et al. 2003). Finally, MRS has also been used to demonstrate potential neurotoxicity of antiretrovirals, whereby individuals receiving nucleoside analogues (including ddI, ABC and d4T) exhibited lower levels of frontal white matter NAA and reductions correlated with duration of treatment exposure (Schweinsburg BC 2005).

1.3.2.3 Positron Emission Tomography (PET)

PET scanning is a nuclear medicine technique, whereby positron emitting radionuclides with short half-lives are used to label molecules of biological interest. Tracer quantities of these radioactive molecules are administered to subjects and distributed throughout the body, reflecting regional differences in receptor availability or cell metabolism. A positron is a particle of equal mass, but opposite charge to an electron. After emission from an unstable nucleus, it travels a short distance before colliding with a neighbouring electron. The product of this collision is a pair of gamma rays emitted at 180 degrees to each other. The PET camera records distribution and activity of the gamma rays using pairs of oppositely placed electronically-linked scintillation detectors.

In recent years the study of microglial cell activity has become possible using such technology. Microglial cells are the intrinsic macrophage population of the brain, and can release excitatory amino acids, which induce neuronal apoptosis through a process known as excito-toxicity. They are also potent producers of neurotoxins such as nitric oxide, and release cytokines and chemokines, which have a neuro-modulatory role. Furthermore, activated microglia liberate neurosteroids, such as pregnenalone (as a result of peripheral benzodiazepine receptor stimulation) which may result in a reduction in NMDA glutamate excitation and increased neuro-inhibition via central GABA pathways (Tilleux, et al. 2007).

PK11195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-2-isoquinoline carboxamide) is a highly specific ligand for the peripheral benzodiazepine binding site (PBBS) on microglial cells (Banati, et al. 1997). Up-regulation of the PBBS occurs in activated microglia. In the normal brain, there is minimal binding of PK11195, but a significant increase of PBBS expression is seen after neuronal injury (Cagnin, et al. 2001). PK11195 can be labelled with ^{11}C and used as a non-invasive radiological marker of microglial activation using PET scanning. ^{11}C decays with a half-life of approximately 20.4 minutes and ^{11}C-labelled PK11195 has shown increased uptake in human subjects with AIDS-dementia (Hammoud, et al. 2005). Interestingly, no increase in uptake of this ligand has been reported in subjects with HIV-1 infection and minor cerebral function impairment, when compared to matched HIV-1 infected subjects without impairment, suggesting that microglial activation is an end-stage occurrence in HIV-1 monoinfection (Wiley, et al. 2006).

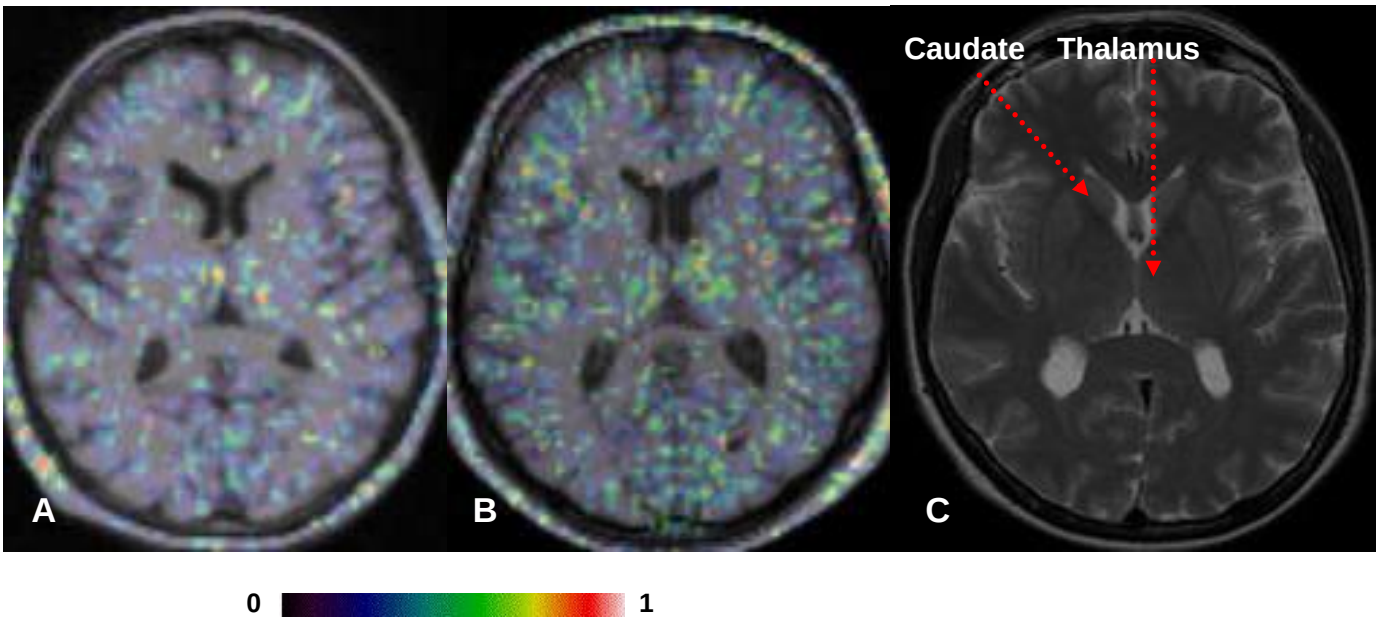


Figure 1.3: Axial PET image taken at the level of the basal ganglia from a healthy volunteer (A) and patient with mild chronic HCV (B). In the patient (B) binding is increased in the thalamus, while the healthy control (A) only shows constitutive PK11195 binding. Image C represents a T_2 MR image from the same axial level

1.3.3 Cerebrospinal fluid sampling (drug concentrations discussed further in section 1.4)

When investigating neurological symptoms in HIV-1 infected subjects, CSF sampling is frequently performed to assist the diagnostic process (if no radiological contraindications exist). CSF manometry is necessary when raised intracranial pressure is suspected (for example in CRYPTO). CSF

pleocytosis is a common finding in HIV-1 infected subjects and is associated with not taking antiretroviral therapy (Marra, et al. 2007). Increased CSF white cell count is also frequently present in CNS opportunistic infections, and if absent in CRYPTO is a poor prognostic indicator (Saag, et al. 1992). CSF protein levels are elevated in many CNS infections (including asymptomatic HIV-1 infection), however very high levels of CSF protein may be due to TB meningitis or CNS lymphoma. Additional CSF tests for the investigation of opportunistic infections include fungal culture and Indian Ink stain (CRYPTO), PCR techniques for *Toxoplasma gondii* (TOXO) and viruses including JCV (PML).

1.3.3.1 CSF HIV-1 replication

It was recognised in the early years of the epidemic, that replication of HIV-1 could be detected by viral culture in the CSF of subjects with HIV-E (Ho, et al. 1985; Levy, et al. 1985) and also asymptomatic HIV-disease (Sonnerborg, et al. 1988). Advances in PCR techniques in subsequent years have improved our understanding of viral kinetics within the CSF compartment (Shaunak, et al. 1990) and it is now possible to routinely quantify levels of replication (CSF HIV RNA level) and to perform genotypic sequencing to detect resistance associated mutations of viral species within the CSF (Antinori, et al. 2005). In the majority of individuals, levels of intrathecal HIV-1 detected are similar or lower than those in plasma (Robertson, et al. 1998). However higher levels of HIV RNA in CSF than plasma are reported in patients with neurological disorders (including HIV-E and HAND) (Letendre S 2010b), low CD4+ counts (below 200 cells/uL) and in subjects with higher plasma HIV RNA levels (Christo, et al. 2007). The genetic diversity of viral strains in CSF and plasma has recently been studied in patient with HIV-E. Interestingly, in patients with a suppressed plasma HIV RNA level, high viral diversity between CSF and plasma viral strains was observed, suggesting autonomous replication of HIV within the CNS. Conversely, in subjects without virological suppression in plasma, much lower diversity of strains was witnessed, suggesting movement of viral species between the plasma and CSF takes place in the absence of selective drug pressure (Soulie 2010).

Following initiation of cART, the dynamics of viral replication in CSF can differ from plasma and slower rates of viral decay are observed (Staprans, et al. 1999). In recent years it has been recognised that in neuroasymptomatic HIV-1 infected subjects receiving cART, replication of HIV-1 can be demonstrated in CSF, despite viral suppression in plasma. Reported rates of such 'CSF viral escape' are between 4 and 10% (Eden A 2010; Letendre 2010). In rare cases, individuals with neurological symptoms have also been found to have distinct genotypic patterns of resistance-associated mutations in viral strains isolated from CSF and plasma (Canestri, et al. 2010).

1.3.4 Biomarkers

Although not currently used in routine clinical practice, researchers have identified a series of immunological markers which can be quantified in CSF samples and that, in future, may aid diagnosis and monitoring of HAND.

Even before quantification of CSF HIV replication was being widely performed, identification of elevated levels of biomarkers of within CSF had been made in individuals with HIV-E (Brew, et al. 1990; Brew, et al. 1992). These include the products of immune activation CSF neopterin and MCP-1, β2-microglobulin (B2M) and quinolinic acid.

Neopterin, a pteridine metabolite, is a biochemical product released by activated macrophages (Price, et al. 2007). Intra-thecal levels are frequently elevated in patients with untreated HIV-1 and increase with progressive CD4+ lymphocyte cell count decline. Particularly high levels are observed in individuals with HIV-E where some association with elevated CSF HIV RNA level, but not CSF white cell count is observed. Interestingly, elevation of CSF neopterin is also present in the CNS opportunistic infections cerebral lymphoma, CRYPTO and cytomegalovirus encephalitis, however only modest elevations, similar to those of neuroasymptomatic HIV-1 untreated subjects, are found in TOXO and PML (Hagberg, et al. 2010). In neuroasymptomatic patients, following initiation of cART, it has been shown that while CSF and plasma viral replication become suppressed, neopterin levels remain mildly elevated, perhaps indicating ongoing intra-thecal immune activation (Abdulle, et al. 2002; Yilmaz, et al. 2006).

CSF levels of the inflammatory chemokine MCP-1 are also elevated in HIV-1 infected subjects and correlate both with CSF HIV RNA level and HIV-E severity (Kelder, et al. 1998). The presence of high CSF HIV RNA with either high MCP-1 or neopterin levels, may have additional clinical implications by demonstrating not only active viral replication, but also macrophage activation which may be necessary for the development of HIV-E and help distinguish from conditions such as vascular dementia.

B2-M is a protein component of MHC Class I molecules, present on the surface of virtually all cell types, with particularly high concentrations on activated T-cells and macrophages (Brew, et al. 1992). B2-M is not usually found within the CNS in the absence of disease, however in the pre-cART era, high CSF concentrations were consistently found in individuals with HIV-E (McArthur, et al. 1992). CSF B2-M concentrations increase as CD4+ cell counts decline in untreated subjects (Lucey, et al. 1993). Strong correlations between B2-M concentration and severity of dementia are also

described which are independent of CSF pleocytosis. CSF B2-M concentrations decrease following initiation of cART, frequently to normal levels (Abdulle, et al. 2002).

Finally, quinolinic acid, a product of tryptophan metabolism, has also been studied as a potential CSF biomarker in HIV-1 infected subjects. Quinolinic acid is released by activated macrophages in the CNS and is an excitotoxic metabolite (Valle, et al. 2004). Elevated levels have been found in CSF at all stages of HIV-1 infection, however levels become dramatically increased in advanced HIV-disease and show strong correlation with severity of NP deficits and dementia (Heyes, et al. 1991). Reductions of CSF quinolinic acid occur with treatment and reflect clinical improvement.

1.4 Pharmacology of antiretrovirals in the CNS

1.4.1. Factors affecting antiretroviral CNS penetration

The distribution of a drug into body compartments such as the genital tract and CNS is influenced by a multitude of factors including intrinsic pharmacological properties and host pathology. Pharmacological properties, including molecular weight, degree of protein-binding and lipophilicity will influence the concentration of a drug able to cross the BBB. Generally, drugs of low molecular weight, with a low degree of protein-binding and which are highly lipophilic will be delivered to the CSF more easily.

A large number of protein transporters are expressed at the BBB and blood-CSF barrier which, in addition to the tight junctions of epithelial cells, serve as a protective mechanism to inhibit or enhance drug delivery to the CNS. These transporters are classified into 2 main groups – the ATP-binding cassette (ABC) and the solute carrier (SLC) transporters (Strazielle and Ghersi-Egea 2005). P-glycoprotein is the most widely-studied ABC transporter and is expressed throughout a variety of body tissues, including the BBB, where it mainly functions to protect the brain from hydrophobic drugs. As the HIV-protease inhibitors are substrates of P-glycoprotein, they are likely to be actively removed from the CSF, via an efflux mechanism, thus limiting their CNS entry (Polli, et al. 1999). A more recently discovered group of ABC transporters includes the multi drug resistance-associated proteins (MRPs), also known as the ABCC family. These behave as efflux transporters to drug compounds containing glucoronide or sulphate and therefore may contribute to the low affinity of both protease inhibitors and NRTIs into the brain capillary endothelium (Varatharajan and Thomas 2009).

The SLC family of transporters, containing organic anion transporting polypeptides (OATPs), organic anion transporters (OATs) and organic cation transporters (OCTs) are also expressed in brain capillary endothelium and may influence drug delivery to the CNS in HIV-1 infected subjects. While some OATPs have been associated with removal of ARVs from the BBB (zalcitabine, ddC (Gibbs and Thomas 2002)), others including OATP2, have been implicated in enhancing the drug delivery of the NRTI 3TC into the choroid plexus (Gibbs, et al. 2003). Similarly OATS have been implicated in animal models, as potential influx enhancers of AZT and ddC. Transporters within the SLC29 group have also been shown to increase the drug delivery of the NRTIs AZT, ddI and ddC to the CNS in human and animal models (Baldwin, et al. 2004). Further work in this area of pharmacology and proteomics is needed.

In addition to the role of drug transporters, clinical or pathological factors may cause disruption to the integrity of the BBB. These include the presence of HIV-1 replication, which is associated with altered morphology of BBB endothelial cells, plus additional factors including sepsis or release of inflammatory mediators during CNS infections, which may also alter BBB integrity and thereby enhance CSF drug delivery (Roberts, et al. 2009).

How well a drug penetrates the CNS could theoretically be evaluated *in vivo* via direct sampling of brain tissue or indirectly by injection and imaging of radio-labelled drug. As neither method is realistically practical, due to associated risk and feasibility, such direct assessments of CNS penetration are not performed. At present, antiretroviral CNS penetration is usually estimated, based upon a drug's pharmacological properties, data from CSF drug concentration studies (relative to unbound plasma fraction and median IC_{50}), results of CSF HIV RNA analysis in exposed subjects and where available from clinical outcome data (including changes to NP performance or incidence of HAND). Use of CSF drug concentration data to evaluate CNS penetration may be criticised as it is known that the distribution of drug throughout the lumbar and ventricular CSF may differ widely, as demonstrated following administration of 3TC to macaques (Blaney, et al. 1995) and simultaneous sampling of brain tissue and CSF has, in animal models, shown wide variations in drug exposure (Shen, et al. 2004; Thomas and Segal 1998).

1.4.2 Estimation of antiretroviral CNS penetration using CSF exposure (summary Table 1.4)

In general, the nucleoside analogues have favourable properties for CNS penetration including low molecular weight and low protein-binding. AZT has demonstrated ability to cross the BBB (Burger, et al. 1993), reduce CSF HIV replication (Foudraine, et al. 1998; Gisslen, et al. 1997) and is also associated with clinical improvement of subjects with HIV-E (Sidtis, et al. 1993; Tozzi, et al. 1993).

3TC and d4T have been detected at CSF concentrations which appear adequate for suppression of viral replication (Foudraine, et al. 1998) and ABC has demonstrated high CSF/plasma ratios of 36% (Capparelli, et al. 2005b). No reduction in CSF HIV RNA level, or CSF biomarkers of immune activation was found in a series of subjects exposed to ddI (Gisslen, et al. 1997) and low CSF/plasma ratios of the nucleotide analogue TDF have been reported (4%) with CSF concentrations falling below the IC_{50} for wild-type viruses (Brookie Best 2008).

The CSF exposure of NVP has also been evaluated and CSF/plasma concentration ratios of 63% were observed in one study (Antinori, et al. 2005). Reports of EFV CSF exposure are variable, ranging from undetectable concentrations in one study (Antinori, et al. 2005) to CSF/plasma ratios of 0.61% in separate work when individuals were receiving EFV with either two nucleosides, or IDV (Tashima, et al. 1999). Despite HIV-1 PIs being lipophilic agents, they are highly protein bound and as a result are considered to penetrate the CNS poorly. Furthermore, they are substrates of P-glycoprotein, a trans-membrane efflux transporter and can therefore be actively removed from the CNS. This effect has been well described *in vitro* in animal models (Polli, et al. 1999). The exception to this is IDV which has been reported to penetrate the CNS effectively, probably due to lower protein binding than other protease inhibitors (Haas, et al. 2003). In current clinical practice, IDV is seldom used due to renal toxicity. The RTV-boosted PIs LPV and DRV are currently used more frequently and detectable CSF drug concentrations have been reported in small studies (Capparelli, et al. 2005a; DiFrancesco, et al. 2007; Yilmaz A 2009). Undetectable CSF HIV RNA levels have also been demonstrated in subjects receiving LPV/RTV monotherapy in one small series, supporting its anti-viral effect in the CSF (Letendre, et al. 2007b).

The CNS penetration of the recently licensed antiretroviral agents MVC and RTG has recently been estimated by researchers within small patient cohorts. MVC has been readily detected in the CSF and concentrations have exceeded the IC_{90} for unbound drug in the majority of subjects (Tiraboschi, et al.; Yilmaz, et al. 2009b). High MVC CSF/plasma ratios were also reported in neurologically symptomatic subjects (Melica, et al. 2010). Similarly detectable RTG CSF concentrations have been shown to exceed the IC_{50} for wild-type virus in the majority (but not all) of subjects in 2 recently published series (Croteau, et al.; Croteau, et al. 2010; Yilmaz, et al. 2009a).

1.4.2. Systems for ranking antiretroviral CNS penetration

There is no universally accepted approach for estimating the CNS penetration of an antiretroviral regimen and different methods have been utilised by researchers. In early work, the effects of antiretroviral therapy were analysed simply by drug class or by the number of drugs considered to

cross the BBB. In retrospective work between 1994 and 2002, authors selected all available NRTIs, NNRTIs and IDV as the drugs which have capacity to cross the BBB (d'Arminio Monforte, et al. 2004). Using this approach, the effect of CNS penetration upon CNS opportunistic infections was evaluated.

A similar technique was used for the CNS Penetration (CP) Reference Score (Tozzi, et al. 2009). This system assigned drugs considered to have 'high penetration' (AZT, 3TC, d4T, ABC, NVP, EFV and IDV) a score of '1' and all other drugs, a score of '0'. A total for an antiretroviral regimen was then calculated which could be incorporated into statistical analysis of clinical studies.

In recent years, the CNS penetration-effectiveness (CPE) ranking system has been devised by investigators and is increasingly being applied as a numerical tool in research settings to evaluate CNS penetration. The system is based upon an ongoing, extensive literature review which incorporates available CSF pK data, results of clinical studies and theoretical drug properties (Letendre, et al. 2008). Using these data, all licensed antiretroviral agents are assigned a rank by the authors (in the 2008 version each drug in a regimen scored 0, 0.5 or 1 with increasing CNS penetration) and a regimen total calculated. An updated version (Letendre 2010) has recently been presented whereby CPE scores of between 1 and 4 are assigned to antiretroviral agents [see Table 1.5]. A large cross-sectional analysis has demonstrated the use of drug regimens with higher scores is associated with increased probability of achieving undetectable CSF HIV-1 RNA levels (Letendre, et al. 2008).

When considering these systems for ranking CNS penetration, the wide inter-individual variability of ARV concentrations in plasma must be considered, as it is likely that similar variations are observed within the CSF. At a population level, such variability may have a significant influence on the true CSF concentrations achieved in some individuals which would be a major confounder of the actual antiviral CNS activity.

1.4.3. Evidence for an effect of antiretroviral therapy CNS penetration upon the cerebral manifestations of HIV-1 infection

1.4.3.1. CNS opportunistic infections

While there is now overwhelming evidence that rates of CNS opportunistic infections have fallen due to the availability of cART, the effect of antiretroviral CNS penetration upon these diseases has been infrequently studied. In recent years, data have been presented on two occasions, which describe the survival benefits following PML, HIV-E, CRYPTO and TOXO when antiretroviral regimens with higher CPE scores are prescribed (both in the pre-cART and the cART eras) (Gasnault J 2008; Lanoy E

2007). These data appear to show an association which is irrespective of the prescribing era, but to date, these remain unpublished. It is possible that prescribing bias, according to the clinical status of the subject at time of CNS disease diagnosis, may have influenced the choice of antiretroviral agents, and therefore the CPE score, confounding these outcome data.

1.4.3.2. HIV-E, HAND and NP performance

Several research groups have evaluated the effect of antiretroviral agents and CNS penetration upon clinical outcomes, either by comparing individual drugs or by using ranking systems including the CPE score (see section 1.4.2). The majority of these studies have been cross-sectional or observational in design, with only a small number of prospective studies to date and very few with randomised treatment arms in the post-cART era. Also of relevance are the diverse clinical endpoints and assessment tools used to evaluate NP performance.

Early studies to evaluate the effect of AZT on HIV-E enrolled large numbers of subjects and demonstrated improvements in cognition and clinical condition when compared to placebo (Schmitt, et al. 1988; Sidtis, et al. 1993). The clinical benefits of AZT were in some cases, not sustained after control of viral replication was lost with monotherapy (Tozzi, et al. 1993). Following this, a large observational study (where the majority of subjects had HAND), demonstrated greater cognitive improvements in patients receiving triple antiretroviral therapy, compared to either no therapy, or monotherapy (Sacktor, et al. 1999). More recently, in a large European cohort study, the nucleoside drug class has again been associated with reduced risk of HIV-E, irrespective of other concomitant drugs (d'Arminio Monforte, et al. 2004).

NVP was studied in an early randomized-controlled trial, when given in combination with AZT and ddI (and compared to dual nucleoside combinations). NP performance was significantly preserved or improved in subjects receiving NVP-containing treatment, suggesting a benefit from either being on triple-versus-dual therapy or NVP itself (Price, et al. 1999). A later small, prospective study assessed patients commencing triple-therapy and observed NP benefits at week 8 of therapy, particularly in those receiving IDV or AZT (Marra, et al. 2003).

The clinical effect of intensification with ABC, an antiretroviral predicted to have high CNS penetration, was assessed in patients with HIV-E in a phase 3 study which randomised 105 patients to receive ABC or placebo. No NP improvements were observed after 12 weeks. It was, however, retrospectively demonstrated that many patients randomised to the active-drug arm, had ABC

resistance-associated-mutations which may have negatively-impacted on this study's results (Brew, et al. 2007).

Later studies in the cART era, have demonstrated significant cognitive benefits associated with initiating therapy (Cysique, et al. 2004) and preservation of NP performance (including psychomotor function) for at least 5 years, while receiving cART (Cole, et al. 2007). The dynamics of NP change after therapy initiation have been explored in detail in patients with HAND (Cysique, et al. 2009). Rapid early improvements (by week 12) were only observed in a minority of subjects. However, over 40% improved significantly by week 48 (less than 5 % deteriorated). NP improvements were greatest in those with severe HAND at baseline.

Although NP performance improvements have been demonstrated upon commencing cART, the neurocognitive effects of cART interruption, although not usually clinically recommended, are unclear. In one small, prospective study evaluating this scenario, no NP deterioration occurred upon cessation of therapy despite CD4+ cell count decline, plus NP improvements were observed upon re-initiation of cART (Childers, et al. 2008). Of note, surprisingly, treatment interruption in patients with CD4+ cell counts of above 350 cells/uL resulted in NP improvements, leading authors to question potential neurotoxicity from the cART being taken (particularly EFV in this cohort) (Robertson, et al.).

As the majority of HIV-1 infected subjects now receive triple therapy, establishing any relationships between regimen CNS penetration and NP performance is of interest. The CP Reference and CPE Ranking systems were recently directly compared when evaluating patients commencing cART, with, or at risk of HAND. At follow up, a higher regimen CPE score (but not CP Reference score) showed a strong correlation with improved NP performance (Tozzi, et al. 2009).

Utilisation of antiretroviral regimens with higher CPE scores have also demonstrated greater improvements in neuropsychological performance in patients with HAND (Cysique, et al. 2009) and survival of perinatally-infected children and adolescents with HIV-E (Patel, et al. 2009). However, in a recent large prospective study, higher CPE scores were associated with control of CSF viral replication, but interestingly were actually associated with worse NP performance in subjects with advanced HIV disease in a recent, large prospective study (Marra, et al. 2009).

Finally, in one of the few randomised studies of the modern HIV-era, antiretroviral naïve subjects commencing three different treatment arms (TDF-FTC plus either EFV, ATV/RTV or AZT/ABC) were assessed after 48 weeks using ^{1}H-MRS and computerised cognitive assessments (Winston, et al. 2010). Greater improvements of cerebral metabolites were observed in the EFV treatment arm and

greater improvements of NP performance were observed in the AZT/ABC arm, leading authors to conclude that the CNS penetration of the quadruple nucleoside arm may aid NP recovery, but that NP improvements in the EFV arm may be negatively-affected by neuropsychiatric side effects.

1.5 Viral hepatitis C infection (HCV)

1.5.1 Epidemiology and clinical features

Chronic HCV is an important worldwide cause of cirrhosis and encephalopathy. It is estimated there are currently over 170 million adults with chronic HCV worldwide and in the UK, current prevalence is approximately 0.4% (http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HepatitisC/GeneralInformation/hepcGeneralInfo/ page updated June 2008, accessed 21 May 2010). Owing to similar modes of transmission, HCV is highly prevalent in HIV-1 infected subjects. HIV-1 and HCV coinfection exceeds 30% in some southern European settings and has been reported at 8.9% in UK clinics (Turner, et al. 2010), with particularly high rates being observed in injecting drug users and recipients of contaminated blood products (such as men with haemophilia).

Irrespective of transmission route, a diagnosis of HIV-1 and HCV coinfection has major health implications and liver disease has emerged as an important cause of death in the cART era (Rosenthal, et al. 2003). Individuals with coinfection are at risk of accelerated liver disease progression to fibrosis and cirrhosis, as well as an increased rate of hepatoma. In addition, they are at increased risk of drug hepatotoxicity which may complicate the successful administration of cART and other antimicrobial treatments. The Strategic Management of Antiretroviral Therapy (SMART) study recently demonstrated interrupting cART is associated with an increased incidence of clinical events and this risk is further enhanced for coinfected individuals (Tedaldi, et al. 2008).

1.5.2 Cerebral manifestations of HCV

Chronic HCV is associated with extrahepatic manifestations including cryoglobulinaemia, arthralgia and fatigue (Poynard, et al. 2002) and can be detected at extra-hepatic sites including the genital tract in HIV-1 and HCV coinfected women (Nowicki, et al. 2005). HCV is a flavivirus, a viral family of which other members exhibit neurovirulence (including West Nile and Japanese encephalitis viruses). Many subjects with chronic HCV report disturbance of concentration and mood, even in the

absence of significant liver disease and therefore a neuropathological mechanism has been explored in recent years.

HCV RNA has been identified in brain tissue (both post mortem and *in vivo*) using PCR techniques and levels can be quantified in plasma and CSF (Laskus, et al. 2002b; Murray, et al. 2008). Analysis of brain tissue at autopsy of 12 HIV-1 and HCV coinfected subjects revealed HCV sequences (NS5A antibody positive) in all subjects within the cortex, basal ganglia and white matter (Letendre, et al. 2007a). Detection of HCV in brain tissue has certainly been observed more frequently in subjects co-infected with HIV-1, particularly in those not receiving antiretroviral therapy, and in those with higher levels of plasma and CSF HIV-1 replication (Murray, et al. 2008). Detection of HCV in brain tissue appears unrelated to the level of plasma HCV viraemia. Interestingly, distinct sequences of HCV have been detected in brain and liver tissue specimens, suggesting CNS compartmentalisation of virus quasispecies can occur in some individuals (Fishman, et al. 2008; Forton, et al. 2004).

Using detailed neurocognitive assessments, subjects with chronic HCV monoinfection have previously demonstrated impairment in concentration and working memory domains (Forton, et al. 2002), attention and executive function (Weissenborn, et al. 2004) and in learning domains (McAndrews, et al. 2005), when compared with control subjects. These findings appear independent of recreational drug use and liver disease severity and of importance, improve after successful anti-HCV treatment, providing an additional incentive to attempt viral eradication with therapy (Thein, et al. 2007). Individuals with HIV-1 and HCV coinfection have repeatedly shown greater neurocognitive impairment than HIV-1 monoinfected subjects, with executive function impairments particularly observed (Letendre, et al. 2005; Murray, et al. 2008; Richardson, et al. 2005; Ryan, et al. 2004).

Metabolite changes consistent with cerebral inflammation have also been observed using MRS in subjects with histologically-mild chronic HCV (Table 1.6). Results have shown increased Cho/Cr ratios in frontal white matter and basal ganglia (Forton, et al. 2001), increased unadjusted Cho in central white matter (McAndrews, et al. 2005) and increased mI/Cr in frontal white matter (Forton, et al. 2008b). Other authors have demonstrated decreased neuronal activity in chronic HCV infection, with reduced NAA/Cr ratios demonstrated in cerebral grey matter (Weissenborn, et al. 2004).

The CNS cell-type(s) responsible for cerebral metabolite changes which occur in chronic HCV infection is (are) not yet established. Recent analysis of autopsy brain tissue with monoclonal antibody staining in 12 HCV infected subjects (6 with HIV-1 co-infection), detected HCV RNA in CD68+ positive cells (specific for macrophages/microglial cells) in 8/12 subjects, and also in GFAP mRNA+ cells (specific for astrocytes) in 4 of these subjects, suggesting HCV infection of these cell

types as the basis for cerebral disturbance, furthermore staining for neurons and oligodendrocytes was consistently negative (Wilkinson, et al. 2009).

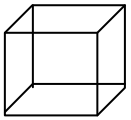
1.5.3 Current epidemic of acute HCV in homosexual men

Before 2003, identification of acute HCV was exceptionally rare, with occasional reports in subjects being monitored post-occupational needlestick exposure. Since 2003, an epidemic of sexually-transmitted acute HCV infection has been identified in HIV-1 infected homosexual men, in urban areas of Europe, North America and Australia (Browne, et al. 2004; Danta, et al. 2007). Behavioural studies have identified high risk sexual behaviour, concomitant sexually transmitted infections and recreational drug use (non-parenteral) as risk factors for acquiring acute HCV.

This acute phase of HCV replication is frequently asymptomatic and only detected by clinicians due to an asymptomatic elevation of serum transaminases, which are regularly monitored in HIV-1 infected subjects. This therefore provides a unique opportunity to investigate this very early phase of infection. HCV genotypes 1 and 4 have been most frequently observed to date (Vogel, et al. 2010). Unlike in chronic HCV monoinfection, HCV antibody seroconversion may be delayed for several months and therefore is not a recommended diagnostic tool (Thomson, et al. 2009), but rather PCR techniques should be utilised. As treatment outcome data has begun to emerge, it appears that higher rates of sustained HCV clearance occur if treatment is initiated within 24 weeks of diagnosis, than in subjects with chronic co-infection (Corey, et al. 2010). Whether CNS disturbance occurs during this acute phase of HCV in subjects with chronic HIV-1 infection is not yet known.

Table 1.1: The HIV Dementia Scale (HDS)

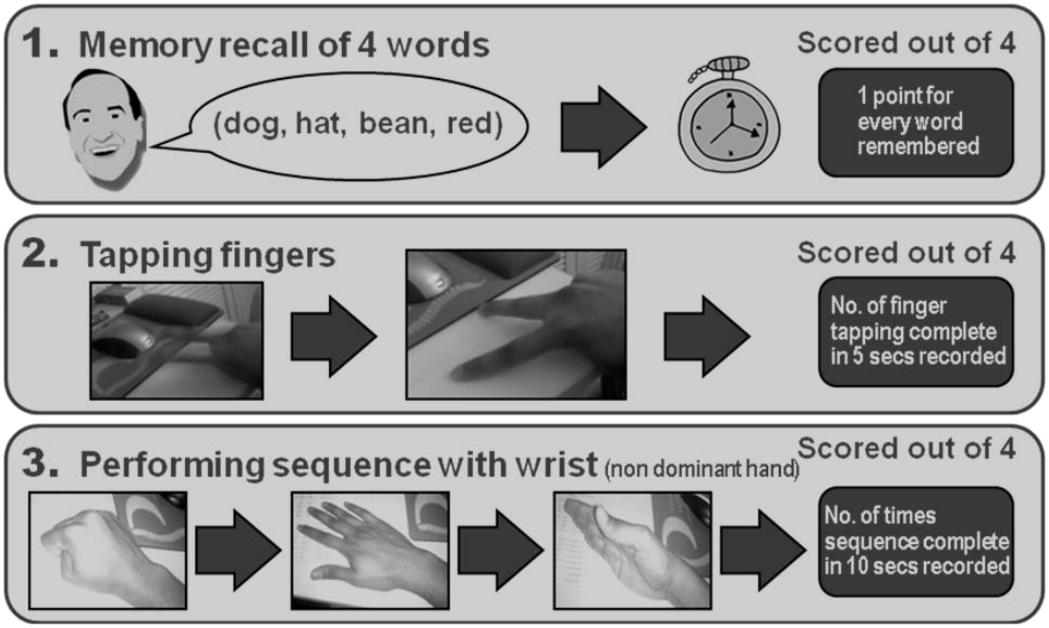
This is a brief screening tool devised to assess cognitive disorders in patients with HIV-1 in the pre-cART era. It is a face-to-face assessment which evaluates memory registration and recall, attention, psychomotor speed and constructional skill. Using a cut-off score of 10 or less, it has sensitivity of 80%, specificity of 91% and a positive predictive value of 78% for identifying subjects with HIV-E (Power, et al. 1995).

Memory-Registration Give four words to recall (dog, hat, green, peach) - 1 second to say each. Then ask the subject to repeat all 4 after you have said them	
4	**Attention*** Anti-saccadic eye movements: 20 commands. ____ errors of 20 trials *(≤3 errors = 4; 4 errors = 3; 5 errors = 2; 6 errors = 1; > 6 errors = 0)*
6	**Psychomotor Speed** Ask patient to write the alphabet in upper case letters horizontally across the page (use back of this form) and record time: ___seconds *(≤21 sec = 6; 21.1 - 24 sec = 5; 24.1 - 27 sec = 4; 27.1 - 30 sec = 3; 30.1 - 33 sec = 2; 33.1 - 36 sec = 1; > 36 sec = 0)*
4	**Memory - Recall** Ask for 4 words from Registration above. Give 1 point for each correct. For words not recalled, prompt with a "semantic" clue, as follows: animal (dog); piece of clothing (hat), color (green), fruit (peach) *Give 1/2 point for each correct after prompting*
2	**Construction** Copy the cube; record time: __ seconds. (< 25 sec = 2; 25 - 35 sec = 1; > 35 sec = 0) 
Total 16	

**Hold both hands up at patient's shoulder width and eye height, and ask patient to look at your nose. Move the index finger of one hand, and instruct patient to look at the finger that moves, then look back to your nose. Practice until patient is familiar with task. Then, instruct patient to look at the finger which is NOT moving. Practice until patient understands task. Perform 20 trials. An error is recorded when the patient looks towards the finger that is moving.*

Table 1.2: The International HIV Dementia Scale (IHDS)

This rapid screening tool assesses motor speed, psychomotor speed, and memory recall using 3 simple tasks (Sacktor, et al. 2005b).



Scoring of tasks	Score	Maximum
Memory recall of 4 words at end of test	1 point for each word recalled spontaneously 0.5 (if prompted)	4
Motor speed: number of finger taps in 5 seconds		4
15	4	
11-14	3	
7-10	2	
3-6	1	
0-2	0	
Psychomotor function: number of correct sequences performed in 10 seconds		4
4	4	
3	3	
2	2	
1	1	
Unable to perform	0	
Total		12

Scores can range between 0 (worst performance) and 12 (best performance). The tool has been designed as a rapid assessment to identify subjects with HIV-associated cerebral function impairment and it is recommended any subject with a score of 10 or below requires further investigation for the presence of HAND.

Table 1.3: Summary of studies evaluating cerebral proton magnetic resonance spectroscopy (^{1}H-MRS) in HIV-1 infected subjects

Author	HIV-1 infected cases	HIV negative controls	^{1}H-MRS changes in HIV-1 infected subjects	Comments
(Tracey, et al. 1996)	N=20, all clinical stages	N=10, age-matched	↓NAA/Cr in HIV-E and at CD4+cell counts below 200 cells/uL ↑Cho, all stages	
(Wilkinson, et al. 1997)	N=84, asymptomatic	N=77	↓NAA/Cho ↓NAA/Cr↓NAA(NAA+Cho+Cr) in FWM	
(Chang, et al. 1999)	N=16, with HAND, pre-ARV All re-assessed at 3/12 of ARV	N=15	↓NA, ↑Cho, ↑mI at baseline At 3/12 HIV-infected subjects had ↓Cho/Cr in BG and FGM, ↓Cho in FWM	Different ARV regimens commenced
(Meyerhoff, et al. 1999)	N=70, all clinical stages	N=30	↑Cho sub-cortex ↓NAA subcortex in HIV-E	↑Cho in thalamus associated with lower CD4+ cell count
(Suwanwelaa, et al. 2000)	N=30	N=13	↓NAA/Cr ↓NAA/Cho in thalamus and semi-ovale	Differences greater in white>grey matter
(Chang, et al. 2002)	N=45, ARV-naive, CD4+ below 500 cells/uL	N=25	↑Cho in FGM and FWM ↑mI in FWM	↑mI in FW associated with lower CD4+ cell count and higher plasma HIV RNA levels
(Lee, et al. 2003)	N=13, with HIV-E	N=18	↑Cho/Cr in BG, ↑mI/Cr in BG, ↓NAA/Cr in FWM, ↑mI/Cr in FWM	
(Chang, et al. 2004b)	N=61 with HIV-E N=39 asymptomatic	N=37	↑mI/Cr FWM in asymptomatic versus HIV-negative controls ↑mI/Cr and ↑Cho/Cr in FWM and BG in HIV-E versus HIV-negative controls ↓NAA/Cr in FWM in HIV-E versus asymptomatic HIV-infected	
(Sacktor, et al. 2005a)	N=20 with HIV-E	-	↑mI/Cr FWM and ↑Cho/Cr FGM in subjects with psychomotor slowing versus no psychomotor slowing ↓NAA/Cho in FGM in subjects with dementia	Used SV-MRS
(Schweinsburg BC 2005)	N=18 taking ddI and/or d4T N=14 taking AZT +3TC N=16 not on ARVs	N=17	↓NAA FWM ddI/d4T versus healthy controls ↓NAA FWM associated with longer ddI/d4T use	
(Paul, et al. 2007)	N=39 asymptomatic N=75 HIV-E	-	Association between ↑NAA/Cr in FWM and better cognitive performance Association between ↑mI/Cr in BG and lower cognitive performance	

[Table 1.3 legend: ARV=antiretrovirals; Cho=Choline-containing compounds; NAA=N-acetylaspartate; mI=myo-inositol; Cr=creatine; BG=basal ganglia; FWM= frontal white matter; FGM=frontal grey matter; ddI=didanosine; d4T=stavudine; AZT=zidovudine; 3TC=lamivudine; HIV-E=HIV-associated encephalopathy; SV-MRS=single voxel magnetic resonance spectroscopy; HIV-E=HIV-encephalopathy; HAND=HIV-associated neurocognitive disorders]

Table 1.4: Summary of studies investigating the CSF/plasma exposure of currently available antiretroviral agents

Antiretroviral agent	CSF/plasma (%)	Comments	Reference(s)
NRTI			
Emtricitabine	43		(Best 2009)
Abacavir	36		(Capparelli, et al. 2005b)
Zidovudine	61	doses ranged 200-500mg QD	(Burger, et al. 1993)
Lamivudine	22		(Antinori, et al. 2005)
Tenofovir	4		(Best 2008)
Didanosine	0-23	iv dosing used in one study	(Antinori, et al. 2005; Burger, et al. 1995; Yarchoan, et al. 1989)
NNRTI			
Nevirapine	63		(Antinori, et al. 2005)
Efavirenz	0-0.61		(Antinori, et al. 2005; Best 2009; Tashima, et al. 1999)
Etravarine	No data		
Ritonavir-Boosted PI			
Darunavir	0.9		(Yilmaz A 2009)
Lopinavir	0.23		(Capparelli, et al. 2005a)
Atazanavir	0.9		(Best, et al. 2009)
Entry/fusion inhibitor			
Maraviroc	2-3		(Tiraboschi, et al. 2010; Yilmaz, et al. 2009b)
Enfuvirtide	Not detected in CSF		(Price, et al. 2008)
Integrase inhibitor			
Raltegravir	3-5.8	Some CSF concentrations below IC_{95}	(Croteau, et al.; Yilmaz, et al. 2009a)

[Legend Table 1.4: NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI=protease inhibitor; CSF=cerebrospinal fluid; IC_{95}= 95% inhibitory concentration]

Table 1.5a: The CSF Penetration Effectiveness Score, presented 2008

	Increasing CSF penetration		
Antiretroviral class	**0**	**0.5**	**1**
NRTI	Didanosine Tenofovir Zalcitabine	Emtricitabine Lamivudine Stavudine	Abacavir Zidovudine
NNRTI		Efavirenz	Delavirdine Nevirapine
PI	Nelfinavir Ritonavir Saquinavir Saquinavir/r Tipranavir/r	Amprenavir Atazanavir Fosamprenavir Indinavir	Amprenavir/r Atazanavir/r Fosamprenavir/r Indinavir/r Lopinavir/r
Entry /fusion inhibitors	Enfuvirtide		

[Legend Table 1.5a: NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI= protease inhibitors; /r=ritonavir-boosted]

Table 1.5b: The CSF Penetration Effectiveness Score, presented 2010

	Increasing CSF penetration			
Antiretroviral class	**1**	**2**	**3**	**4**
NRTI	Tenofovir Zalcitabine	Didanosine Lamivudine Stavudine	Abacavir Emtricitabine	Zidovudine
NNRTI		Etravirine	Delavirdine Efavirenz	Nevirapine
PI	Nelfinavir Ritonavir Saquinavir Saquinavir/r Tipranavir/r	Atazanavir Atazanavir/r Fosamprenavir	Darunavir/r Fosamprenavir/r Indinavir Lopinavir/r	Indinavir/r
Entry/fusion inhibitors	Enfuvirtide		Maraviroc	
Integrase inhibitors			Raltegravir	

[Legend Table 1.5b: NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI= protease inhibitors; /r=ritonavir-boosted]

Table 1.6: Summary of studies evaluating cerebral proton magnetic resonance spectroscopy (^{1}H-MRS) in HCV-infected subjects

Author	Chronic HCV infected cases	HCV negative control subjects	^{1}H-MRS changes in HCV-infected subjects	Comments
(Forton, et al. 2001)	n=30, histologically-mild	[1] n=29 healthy controls [2] n=12 chronic HBV	↑Cho/Cr in WM and BG compared with both control groups	
(Forton, et al. 2002)	n=17	n=29	↑Cho/Cr in BG and WM	Higher Cho/Cr associated with cognitive impairment
(Weissenborn, et al. 2004)	n=30, normal liver function	n=15	↓NAA/Cr in cerebral cortex	MRS changes more marked in subjects with moderate fatigue
(McAndrews, et al. 2005)	n=37	n=46	↑Cho ↓NAA in central white matter	No correlation between MRS and severity of liver disease
(Forton, et al. 2008b)	n=25, histologically-mild	n=17	↑mI/Cr	Association between ↑ mI/Cr in FWM and prolonged working memory
(Bokemeyer, et al. 2011)	n=53, mild liver disease	n=23	↑Cho ↑Cr ↑NAA plus glutamate in BG ↑Cho in WM	Negative correlation between MRS changes and fatigue

[Legend Table 1.6: HCV=hepatitis C; HBV=hepatitis B; Cho=Choline-containing compounds; NAA=N-acetylaspartate; mI=myo-inositol; Cr=creatine; BG=basal ganglia; WM=white matter]

CHAPTER 2

Materials and Methods

Chapter 2: Materials and Methods

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2.1 Ethical approval and trial registration

Ethical approval was obtained prior to the conduct of this research via the UK National Research Ethics Service (NRES). Local approval was also obtained from the Research and Development Office at St Mary's Hospital, Imperial College NHS Healthcare Trust. All of the research was conducted according to the standards of the International Conference on Harmonisation for Good Clinical Practice (ICH-GCP). Ethical approval was granted via NRES to conduct the following studies presented in this thesis:

- **The UKCHIC Study *(Chapter 3)*, approval granted by NRES (reference number 00/7/47). According to UK regulations, no written informed consent from patients was required for this cohort study as only anonymised data collected routinely for other purposes were used**

- **The Darunavir Monotherapy Neurocognitive Study *(Chapter 4),* approval granted by NRES (reference number 07/MRE01/64)**

- **The Maraviroc CNS Study *(Chapter 5),* approval granted by NRES (reference number 09/H0707/35)**

- **Assessment of cerebral function parameters in HIV-1 monoinfected subjects and subjects with HIV-1 and HCV coinfection *(Chapters 6,7 and 8),* approval granted by NRES (reference numbers (07/H0803/128, 08/H0712/15 and 09/H0712/17)**

- **Permission to administer ^{11}C PK11195 to subjects was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC) of the UK**

2.2 Cohort development

2.2.1 The UK Collaborative HIV Cohort (UKCHIC) Study

The UKCHIC study is an observational cohort study, first established in 2001 and which currently collects data from 12 of the largest UK HIV clinical centres. Its primary purpose is to investigate the clinical outcomes, treatment response data and epidemic dynamics of HIV-1 in the UK. Data from 1996 have been collected including subject demographics (age, gender, risk-group for HIV

acquisition, ethnicity, date of HIV diagnosis), past and current antiretroviral exposure, laboratory test results and clinical parameters, clinician-reported AIDS diagnoses and death reports. Currently, the study contains more than 30,000 anonymous and de-linked records of subjects who have attended for care.

Description of UKCHIC cohort 1996 -2007

Between 1996 and 2007 the number of patients under follow-up at participating UKCHIC sites increased from 10,085 to 22,399 *(see Table 2.1*). Over this period the cohort demographics changed. The proportion of the cohort comprising of male subjects decreased from 84.5% to 76.3% and the proportion comprising of black African subjects increased from 11.6% to 23.6%. Between 1996/1997 and 2006/2007, the percentage of subjects exposed to HAART increased from 14.1% to 68.4%.

Permission to undertake study

A study design and proposal for the work using UKCHIC data (described in Chapter 2) was written by Dr Lucy Garvey and Dr Alan Winston and submitted to the UKCHIC Study Steering Committee who granted approval in 2008.

2.2.2 St Mary's Hospital HIV Outpatient Department, Imperial College Healthcare NHS Trust

Adult subjects attending the St Mary's Hospital HIV Outpatient Department were invited to participate in the clinical studies conducted within this thesis where they fulfilled eligibility criteria. In addition, subjects attending the HIV Outpatient Department at Chelsea and Westminster NHS Trust were also enrolled for 2 clinical studies *(Chapters 4 and 5).*

2.3 Subject selection

Cohort 1: Chronic HIV-1 mono-infection

Subjects were required to be HIV-1 antibody positive for a minimum of 6 months without evidence of HCV coinfection (negative HCV IgG or RNA level within 6 months and with normal liver function tests thereafter).

Cohort 2: Chronic HIV-1 infection and chronic HCV coinfection

Subjects were required to have evidence of chronic HCV as defined by HCV antibody positivity for a minimum of 12 months and detectable plasma HCV RNA on most recent testing.

Cohort 3: Chronic HIV-1 and acute HCV coinfection

Subjects were required to have acute HCV as defined by a new positive HCV RNA test within a maximum of 12 months of a negative HCV RNA test (for the work in Chapter 8, a maximum of 8 months elapsed since a negative HCV RNA test was required).

2.4 Assessment of cerebral function parameters

2.4.1 Cerebral proton magnetic resonance spectroscopy (^1H-MRS)

In this thesis, ^1H-MRS was performed on an Achieva™ 1.5 Tesla scanner (*Phillips NV, Best, Netherlands*) at the Robert Steiner Magnetic Resonance Unit, Hammersmith Hospital, London, UK. Examination included sagittal, coronal and axial T_1-weighted images of the brain to enable accurate positioning of the spectroscopy voxels and T_2-weighted axial double spin echo images. Cerebral ^1H-MRS was then performed in three voxel locations: right frontal white matter (FWM), mid-frontal grey matter (FGM) and the right basal ganglia (RBG) (*see Figure 2.1*), using a double spin echo point resolved spectroscopy (PRESS) sequence with the following settings: echo time (TE) 36 ms, repetition time (TR) 3000 ms, 2048 data points, spectral width of 2500 Hz and 128 data acquisitions. MR spectra were post-processed for automated water signal suppression and water shimming. Each examination lasted approximately 45 minutes.

Analysis of ^1H-MRS results

T_1 and T_2-weighted MR images were studied by a neuroradiologist. All spectra were analysed and quantified by one observer (LG) using a java-based version of the magnetic resonance user interface package (jMRUI Version Number: 3.0) (Naressi, et al. 2001), incorporating the AMARES algorithm (Kanowski, et al. 2004) and metabolites expressed as ratios to cerebral creatine (Cr).

As MR spectroscopy results can be affected by instrument and operator performance, voxel repositioning, patient motion, data analysis software and metabolic change (Brooks, et al. 1999), determining result reproducibility is essential for validity. Data analysis of spectra from 5 subjects (in 3 voxel locations) at different timepoints was therefore repeated 3 times by a single observer (LG) prior to undertaking formal analysis for this thesis (results shown Chapter 4).

Figure 2.1: Location of 3 voxels for cerebral proton spectroscopy

Figure 2.1a: Right frontal white matter (FWM)

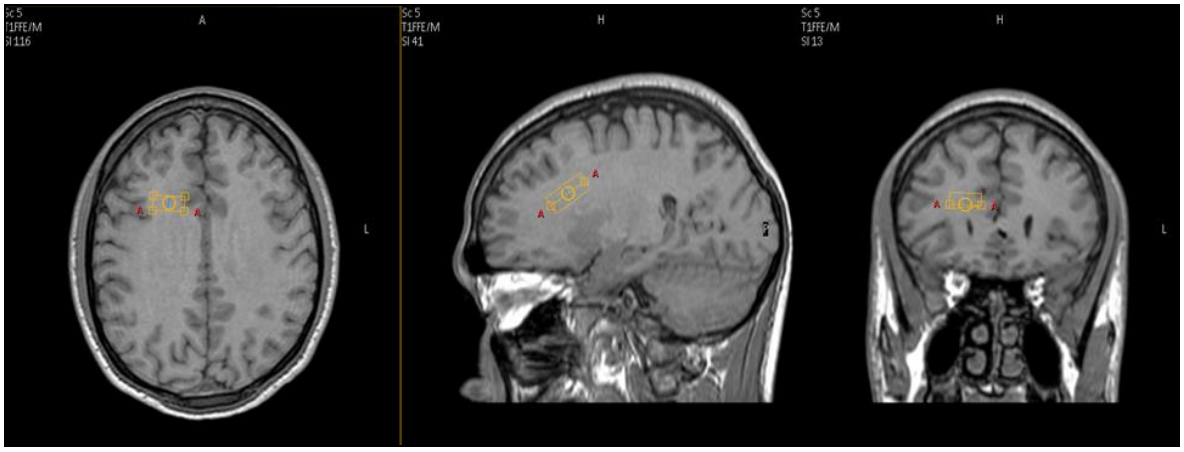


Figure 2.1b: Mid-frontal grey matter (FGM)

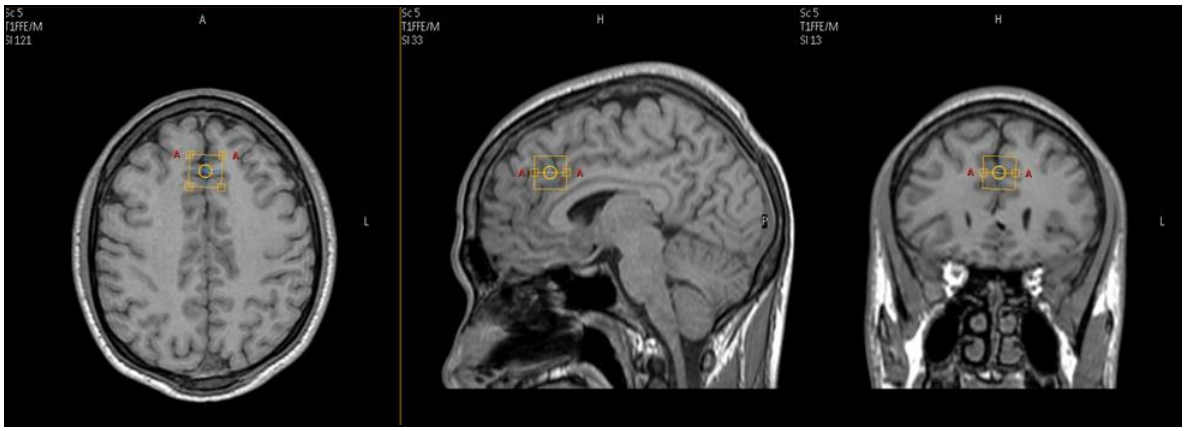
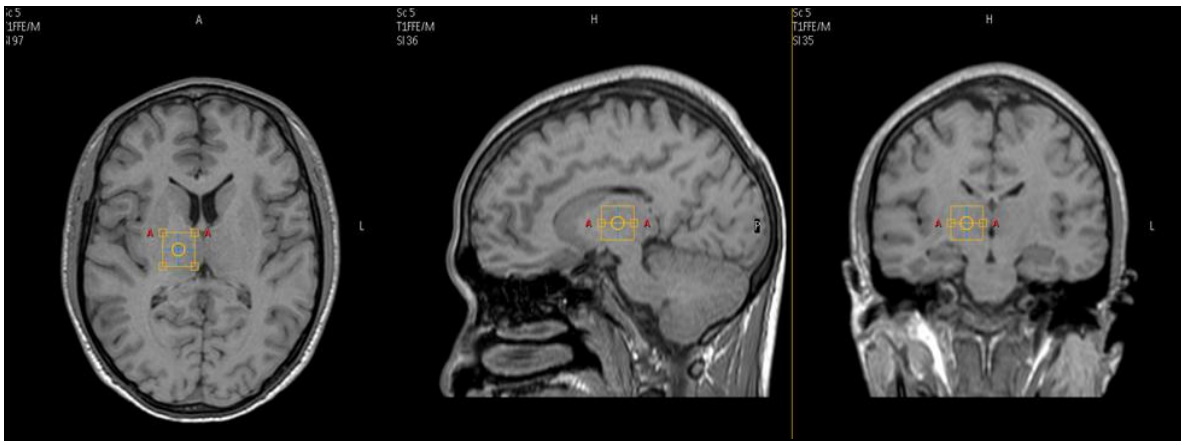


Figure 2.1c: Right basal ganglia (RBG)



2.4.2 PET with ^{11}C labelled PK11195 to assess microglial activity

For this work, PET scanning was performed on a PET-CT scanner (*GE Healthcare, Waukesha, Wisconsin*) at the MRC Cyclotron Building, Hammersmith Hospital, Imperial College, London. This scanner has axial and transaxial fields of view of 70.0 and 15.7cm respectively and a scatter fraction of 31.8% in 3D (Kemp, et al. 2006).

A transmission CT scan (5 min) was first performed followed by an emission scan (60min, 58 frames) with subjects lying in a partially-enclosed PET scanner. Subjects were monitored continuously for evidence of major movements. 30 seconds after the scan started, an injection of ^{11}C-labelled PK11195 radioactive ligand was given (by LG/NiP) via an intra-venous cannula as a smooth bolus by hand, followed by a 10 mL flush of normal saline over 20-30 seconds. The target quantity of PK11195 was 296 MBq (8.00mCi, approximately 1.7mSv tissue dose) with a minimum and maximum accepted radioactivity range of 185MBq (5mCi) and 325 MBq (8.78mCi) respectively. This radioactivity is the equivalent to approximately 8 months background radiation in the UK, where the average yearly exposure is 2.5mSv.This exposure falls easily within the ICRP62 category of between 1 and 10 mSv justified for research purposes. Radiochemical purity was required to exceed 96% and pH to be between 5 and 8 in order for the ligand to pass quality control prior to injection. Specific radioactivity and concentration of impurity at time of injection were recorded in accordance with ARSAC regulations.

PET images were co-registered with T_1-weighted magnetic resonance images performed at the Robert Steiner Magnetic Resonance Unit, Hammersmith Hospital, London, UK (see Figure 2.2).

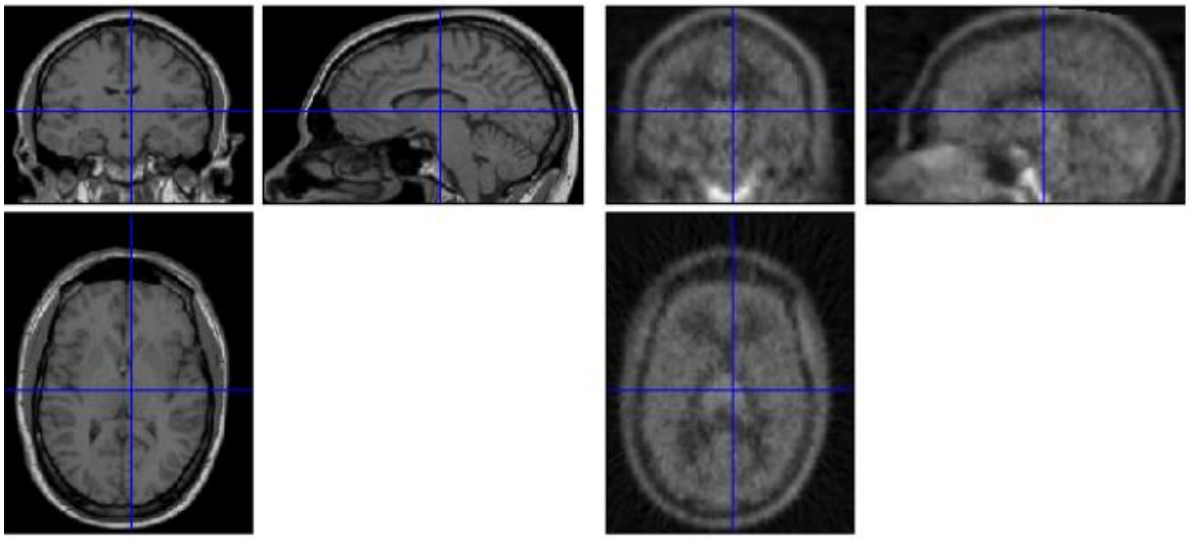


Figure 2.2: Example of co-registration of T1-weighted MR and PET-CT image

Co-registration was performed using the mutual information algorithm in the SPM'99 software package (*Wellcome Department of Cognitive Neuroscience, Institute of Neurology, London, UK*) implemented in Matlab5. Scans were then re-sliced separating the grey and white matter and CSF (see Figure 2.3).

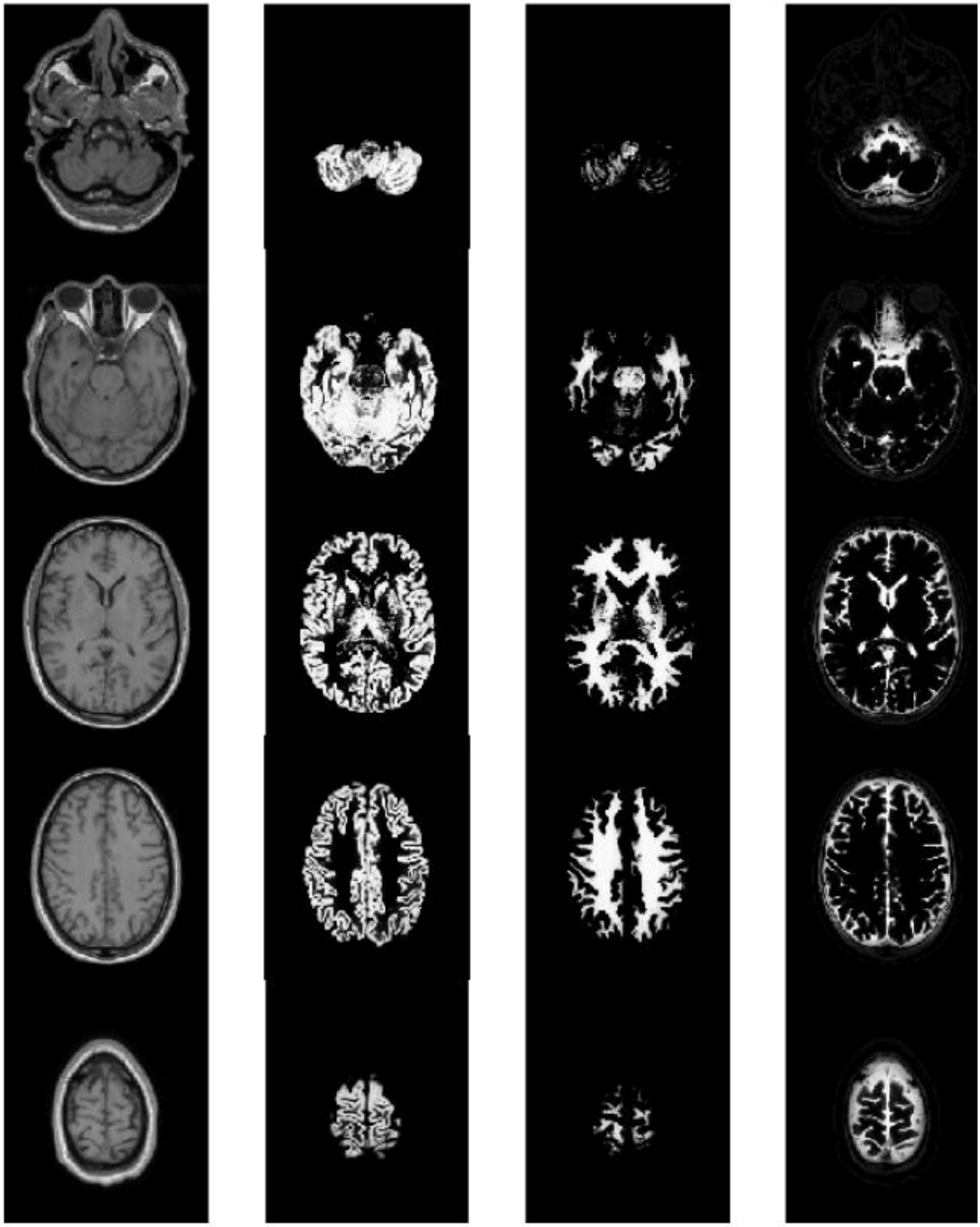


Figure 2.3: Example of MR segmentation into grey matter, white matter and CSF sections.

Images were then spatially normalised in order that standardised object maps (templates) could be applied (see Figure 2.4) using SPM'99 software.

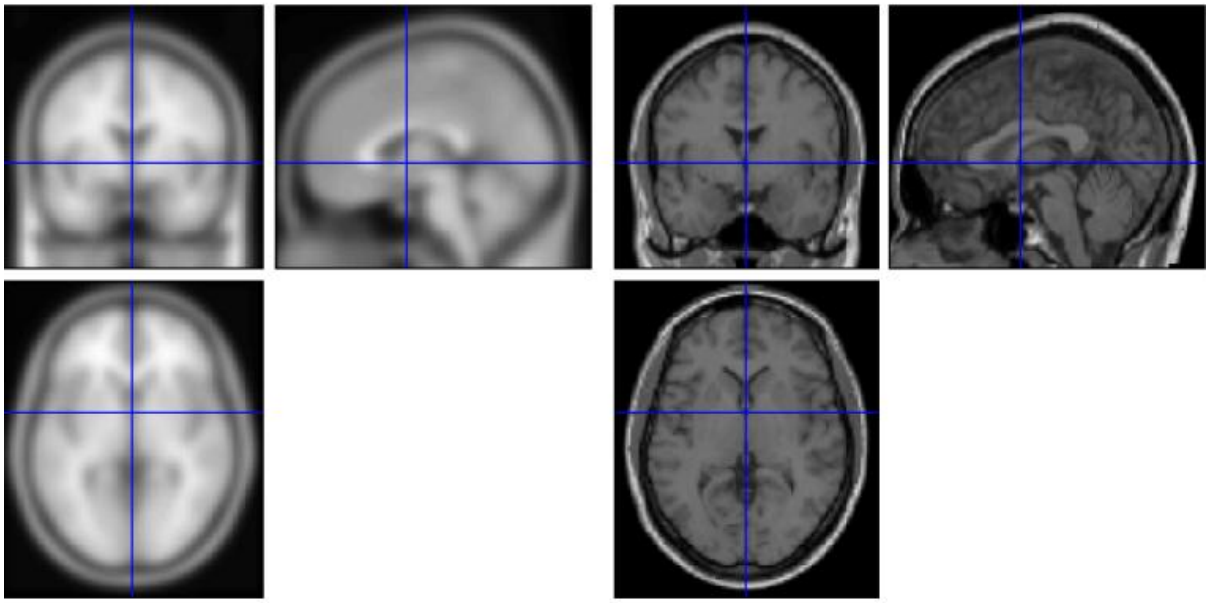


Figure 2.4: Example of spatial normalisation of MR and PET images in order to apply standardised sampling maps

Regional binding of ^{11}C PK11195, expressed as binding potential (BP), a measure of specific binding of the tracer, was calculated using a basis function implementation of a simplified reference tissue model (Lammertsma and Hume 1996). The BP represents B_{max}(receptor density of bound ligand) / K_o (ligand equilibrium dissociation constant), therefore it represents the ratio of specifically bound ligand to its maximum free concentration. Areas selected for BP assessment in this work were ventral striatum, caudate, putamen, and thalamus. Values from these areas were obtained using a standardised object map which defined regions of interest (ROIs) on MR images that were then used to sample the parametric images using Analyze software (*Analyze AVW, Mayo Clinic, US*). For each patient, mean (SD) total values for left and right striatum, caudate, putamen and thalamus BPs were calculated (Figure 2.5).

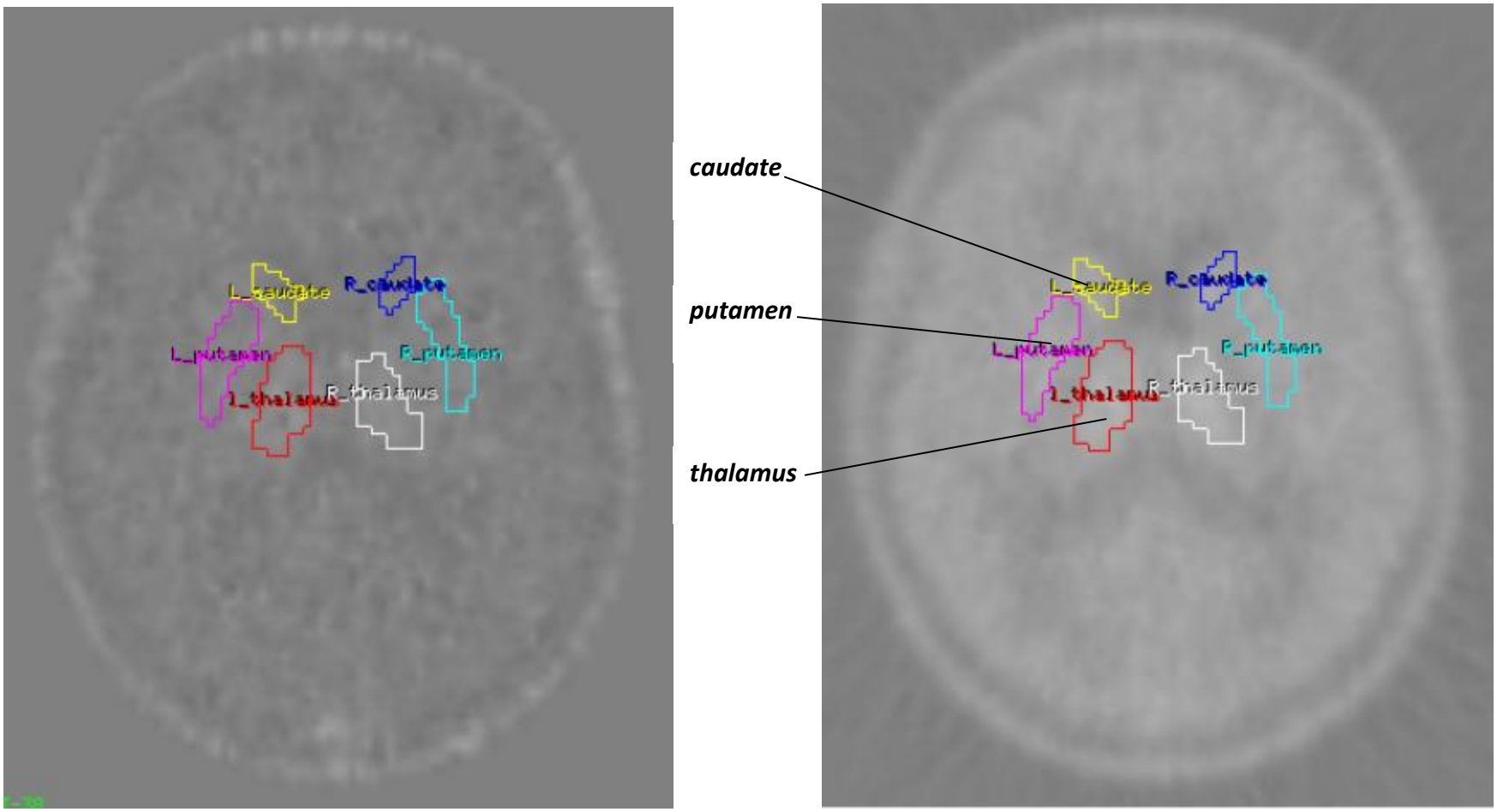


Figure 2.5: Example of standardised object maps applied to transverse PET images in order to assess the ^{11}C PK11195 Binding Potential within regions of interest (ROI) including the caudate, putamen, ventral striatum and thalamus

2.4.3 Computerised cognitive tests

The computerised cognitive assessment selected for use in this thesis was *Cogstate*^{TM}. This is a highly detailed computerised software programme, designed to measure cerebral function performance in HIV-1 infected subjects. This validated tool has previously demonstrated a positive predictive value of 81% for the detection of cognitive function impairment when compared to formal neuropsychological assessment in individuals with advanced HIV-1 disease (mean CD4+ lymphocyte cell count 338 cells/uL) and with HIV-E (mean CD4+ lymphocyte cell count 406 cells/uL)(Cysique, et al. 2006).

Testing was completed with a subject seated at a computer in a quiet room. Instructions were read from the screen and also given verbally in English by the test supervisor (LG). Eight individual tasks, in the form of card-games were completed, each with their own brief instructions (*see Table 2.2*). Participants respond to the tasks using the keyboard letters 'D' or 'K', indicating 'yes' or 'no'. A beep sound informs of an incorrect response. A practice session of all 8 tasks was completed for familiarisation and learning (approximately 20 minutes), followed by a baseline assessment of the same length in order to overcome a potential practice-effect bias (Collie, et al. 2003). Each task assessed a specific domain of cognitive function and reaction speeds, accuracy and error rates are captured electronically.

Analysis of cognitive task scores:

Average reaction times in each of four speed tasks were $\log_{10}$transformed and the proportion of correct responses in each of three accuracy tasks arcsine transformed to normalise data. For the set-shifting task, total error rate was used. Speed and accuracy composite scores were also calculated at each assessment. The composite speed score represents the sum of detection, identification, monitoring and congruent reaction times (a lower score indicates better performance) and the composite accuracy score represents the proportion of correct responses for one-card learning, one-back learning and associate learning (a higher score indicates better performance). For studies involving longitudinal assessment absolute changes to individual task measures and composite scores over time were then calculated. Age-matched normative population data were available for comparison of all raw scores. Neurocognitive impairment (NCI) was defined as a performance more than 1 SD below the age-matched population mean in at least 2 cognitive tasks.

2.4.4 Lumbar puncture and CSF samples

Lumbar punctures were performed (by LG) to obtain CSF samples under local anaesthesia (1-10mL of 1% lignocaine), using aseptic technique as a day-case procedure at St Mary's Hospital, London. Approximately 10mL of CSF was collected per subject and 500mL of normal saline was then administered intravenously in an attempt to reduce the incidence of post-procedure headache.

2.5 Laboratory techniques

2.5.1 Analysis of MVC and LPV concentration

Blood and CSF samples for MVC and LPV concentrations were centrifuged post-collection at approximately 1700g (~3000 rpm) for 10 minutes at 4˚c in a refrigerated centrifuge. After centrifugation, the upper plasma layer was transferred using a disposable pipette into a plasma storage tube. Plasma and CSF were stored within one hour of collection at -20˚c until shipment for analysis at the University of Liverpool, UK. Drug concentrations in plasma and CSF were established using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). The lower limits of quantification were 1.23 and 0.74 ng/mL and 8.26 and 5.65 ng/ml for MVC and LPV in plasma and CSF respectively. Intra- and inter-assay variability was less than 13% for each analyte at low, medium and high concentrations.

Analysis of CSF Tube 1 (Chapter 5) for CSF protein, glucose, microscopy and gram stain was performed in the Clinical Biochemistry and Microbiology Laboratories at St Mary's Hospital, London. Analysis of Tube 4 (CSF ultrasensitive HIV RNA level) was performed in the Jefferiss Trust laboratories, Imperial College, London by Dr Steve Kaye.

2.5.2 CSF HIV RNA analysis

CSF HIV RNA was quantified using an in-house ultra-sensitive RNA assay. Here, virus was pelleted by centrifugation and RNA extracted by the Qiagen MinElute method (*Qiagen, Crawley, UK*). The eluate was reverse transcribed and amplified for 20 cycles using the Invitrogen One-Step method (*Invitrogen, Paisley, UK*) and PCR products quantified in a real-time PCR using the Qiagen Probe PCR

method. A standard curve was generated from dilutions of the international working reagent WR1 (NIBSC, Potters Bar, UK). The lower limit of detection for this study was 10 copies/mL.

2.5.3 Analysis of plasma HIV RNA and CD4+cell count

Plasma HIV RNA assay

Quantification of plasma HIV RNA for the clinical studies within this thesis was measured at the Department of Virology, St Mary's hospital using the VERSANT HIV RNA 3.0 Assay (bDNA) kit (*Bayer Siemens, UK*). This has a lower limit of detection of 50 copies/mL and upper limit of 500,000 copies/mL.

CD4+cell count

CD4+ cell count was measured at the Department of Immunology, St Mary's Hospital. Samples were processed using an ST1000 automated processor and analysed by flow cytometry using SC500 or Navios flow cytometers. The Becton Coulter tetra CXP panel was used.

2.6 Statistical analysis

SPSS (*v18.0*), SAS (*v9.1*) and Microsoft Excel *(2007)* software were used for all statistical analysis in this thesis. Description of individual, statistical techniques are described in Chapters 3-8 as appropriate.

Table 2.1: Characteristics of patients under follow-up in UK CHIC in each calendar period

		Calendar year					
		1996/1997	**1998/1999**	**2000/2001**	**2002/2003**	**2004/2005**	**2006/2007**
Number of patients seen, n		10085	11896	14320	17105	20326	22399
Male gender, n (%)		8522 (84.5)	9786 (82.3)	11397 (79.6)	13280 (77.6)	15526 (76.4)	17079 (76.3)
Ethnicity, n(%)	**White**	7441 (73.8)	8469 (71.2)	9512 (66.4)	10686 (62.5)	12275 (60.4)	13360 (59.7)
	Black African	1165 (11.6)	1783 (15.0)	2716 (19.0)	3775 (22.1)	4817 (23.7)	5286 (23.6)
	Other	729 (7.2)	948 (8.0)	1297 (9.1)	1716 (10.0)	2203 (10.8)	2550 (11.4)
	Not known	750 (7.4)	696 (5.9)	795 (5.6)	928 (5.4)	1031 (5.1)	1203 (5.4)
HIV risk group, n (%)	**MSM**	6543 (64.9)	7538 (63.4)	8656 (60.5)	9950 (58.2)	11657 (57.4)	12554 (56.1)
	IDU	760 (7.5)	749 (6.3)	723 (5.1)	710 (4.2)	722 (3.6)	692 (3.1)
	Heterosexual	1856 (18.4)	2691 (22.6)	3930 (27.4)	5297 (31.0)	6617 (32.6)	7163 (32.0)
	Other/not known	926 (9.2)	918 (7.7)	1011 (7.1)	1148 (6.7)	1330 (6.5)	1990 (8.9)
Previous exposure* to:	**ART**	3711 (36.8)	6531 (54.9)	8452 (59.0)	10541 (61.6)	13155 (64.7)	15527 (69.3)
	cART	1421 (14.1)	5539 (46.6)	7941 (55.5)	10169 (59.5)	12871 (63.3)	15331 (68.4)
	PI	1190 (11.8)	4347 (36.5)	5134 (35.9)	5850 (34.2)	7116 (35.0)	8946 (39.9)
	NNRTI	299 (3.0)	2551 (21.4)	5705 (39.8)	7977 (46.6)	10528 (51.8)	12213 (54.5)
Age (years)*	**Median (IQR)**	34 (30, 40)	35 (31, 41)	36 (32, 42)	38 (33, 43)	39 (33, 44)	40 (34, 46)
Latest CD4 (cells/uL)*	**Median (IQR)**	275 (130, 450)	337 (198, 497)	377 (233, 552)	387 (250, 562)	413 (280, 581)	449 (310, 620)
Nadir CD4+ cell count (cells/uL)*	**Median (IQR)**	220 (80, 390)	267 (150, 409)	307 (180, 458)	330 (203, 490)	345 (228, 495)	380 (254, 531)
Latest viral load (log_{10} copies/mL)*	**Median (IQR)**	4.3 (3,3, 5.0)	3.5 (1.9, 4.5)	3.0 (1.7, 4.5)	2.3 (1.7, 4.3)	1.7 (1.7, 4.1)	1.7 (1.7, 3.7)

* Defined at mid-way through the calendar period (e.g. status at 1/1/97 for the period 1996/1997)

[Legend Table 2.1: MSM=Men-having-sex-with-men; IDU=injection drug use; cART=any drug regimen containing a protease inhibitor, non-nucleoside reverse transcriptase inhibitor, abacavir or an integrase / entry inhibitor, regardless of number of drugs in the combination; ART=any other antiretroviral therapy; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; IQR=inter-quartile range]

Table 2.2: Description of tasks performed during a computerised cognitive assessment

Task	Cognitive Domain	Task instruction	Primary outcome measure (unit)	Optimal test performance
Associate learning	Episodic, non-verbal learning	*"Does the card pair match?"*	Accuracy (Arcsine proportion correct)	Higher score
Detection	Psychomotor function (speed)	*"Has the card turned over?"*	Speed (Log_{10} milliseconds)	Lower score
Identification	Visual attention	*"Is the card red?"*	Speed (Log_{10} milliseconds)	Lower score
Congruent reaction time	Attention	*"Are the cards the same colour?"*	Speed (Log_{10} milliseconds)	Lower score
Monitoring	Divided attention	*"Has a card touched a white line?"*	Speed (Log_{10} milliseconds)	Lower score
One-card learning	Visual learning and memory	*"Have you seen this card before in this task?"*	Accuracy (Arcsine proportion correct)	Higher score
One-back learning	Working memory	*"Is the previous card the same?"*	Accuracy (Arcsine proportion correct)	Higher score
Set-shifting Tasks	Executive function	*"Is this a target card?"*	Accuracy (total number of errors)	Lower score

CHAPTER 3

A UK Cohort Study to Assess the Impact of Antiretroviral Therapy CNS Penetration upon the Incidence of HIV-1 Associated CNS Diseases and Overall Survival

Chapter 3: A UK Cohort Study to Assess the Impact of Antiretroviral Therapy CNS Penetration upon the Incidence of HIV-1 Associated CNS Diseases and Overall Survival

3.1 Introduction

HIV-1 associated CNS diseases including HIV-E, PML, TOXO and CRYTO were frequently observed prior to the cART era. These conditions typically progressed rapidly and were associated with high mortality (Fong and Toma 1995; Mocroft, et al. 1997). Following the widespread introduction of effective cART, a dramatic reduction in the incidence of such diseases was observed, as were improvements in survival (d'Arminio Monforte, et al. 2004; Grabar, et al. 2008).

Recently, a method of estimating the CNS penetration of antiretroviral regimens has been proposed (Letendre 2010; Letendre, et al. 2008), so called the CNS Penetration-Effectiveness (CPE) Rank. This system uses available pharmacokinetic data, results of clinical studies and/or theoretical drug properties to assigns a 'score' to currently licensed antiretroviral agents *(described section 1.4.2)*. Use of the CPE scoring system when applied in the research setting has had mixed results to date. Antiretroviral regimens with higher CPE scores were associated with improvements in neuropsychological performance (Cysique, et al. 2009; Tozzi, et al. 2009) and improved survival of HIV-1 infected adolescents with HIV-E (Patel, et al. 2009). However, in contrast, these were associated with poorer neurocognitive performance in a recent, prospective study (Marra, et al. 2009). Lastly, although currently unpublished, data have reported better survival following PML, HIV-E, CRYPTO and TOXO when antiretroviral regimens with higher CPE scores were used (Gasnault J 2008; Lanoy E 2007) in retrospective European studies.

The true impact, therefore, of antiretroviral CNS penetration upon the risk of developing or surviving a CNS disease remains unclear (d'Arminio Monforte, et al. 2004; Varatharajan and Thomas 2009; Winston, et al. 2010). In order to study the first hypothesis of this thesis, namely that antiretroviral agents with greater CNS penetration are associated with greater improvement of cerebral function parameters in HIV-1 infected subjects, this study aimed to investigate whether the use of cART regimens with higher CPE scores (and therefore greater CNS penetration) is associated with reduced incidence of CNS diseases and improved survival within a large UK Cohort between 1996 and 2008.

3.2 Methods

3.2.1 Data Collection

The data used in this study were obtained, with permission, from the UK Collaborative HIV Cohort (UKCHIC) Study Steering Committee. The UKCHIC study is an observational cohort study, first

established in 2001, which involves 12 of the largest UK HIV Centres and which has acquired prospective data from over 30,000 adults attending for care since 1996 (see Section 2.2.1).

3.2.2 Subject Selection

In order to investigate the hypothesis, eligibility criteria for this study required subjects to be HIV-1 infected adults (over 16 years old) who had started cART at any date between 1st January 1996 and 31st December 2008. All subjects required at least 1 day of follow-up data after starting cART available for analysis. In order to establish the effect of cART CNS penetration upon CNS diseases, any first clinical report of a diagnosis of HIV-E, PML, TOXO or CRYPTO occurring *after* commencing cART were included. Subjects experiencing a CNS disease prior to commencing cART were excluded, thus all reported events in this series occurred in subjects who were cART experienced.

3.2.3 Definition of cART

cART was defined as any antiretroviral regimen containing a PI, NNRTI, abacavir or an integrase / entry inhibitor, regardless of number of drugs in the combination (thus permitting PI monotherapy and some triple nucleoside regimens to be included). cART regimens were then scored using the CPE system and each individual's CPE score was updated each time a drug in the regimen was changed. According to Letendre *et al* (Letendre S 2010a), each antiretroviral drug was given a CPE score between 1 (low CNS penetration) and 4 (high CNS penetration) and a regimen total at each time-point was calculated.

3.2.4 Statistical analysis

Association of CPE score with demographics and clinical parameters

Demographic characteristics and clinical parameters were stratified according to the CPE score of the initial cART regimen (categorised as $\leq$4, 5-7, 8-9 and $\geq$10). Independent associations between clinical factors and an initial CPE score of $\leq$4 were investigated using multivariable logistic regression analysis, utilising a backwards selection process. Factors considered for these analyses were: gender and mode of HIV transmission (categorised as MSM, female heterosexuals, male heterosexuals, injection drug users (IDU), male other/unknown, female other/unknown), ethnicity (white, black African, other/unknown), age, CD4 count, plasma HIV RNA level, treatment status (antiretroviral-naïve or therapy-experienced) and calendar year at cART initiation.

Incidence of CNS diseases

The incidence of a first CNS disease (overall and stratified by CPE score) was calculated by dividing the number of events occurring by the total person-years of follow-up (PYFU) in the cohort. For these analyses, patient follow-up started on the date of cART initiation and ended on the date of the patient's first CNS disease. For those who remained free of CNS diseases at the end of the study period, follow-up was right-censored at the earliest of death, 31st December 2008 or three months after the patient's last clinic visit. The analysis was repeated separately for the combined endpoints of PML/HIV-E and TOXO/CRYPTO, given previously described similarities in the clinical presentations and clinical parameters of patients developing these events in this cohort (Garvey 2009). For these cause-specific analyses, follow-up of patients who experienced the other endpoint was right-censored at the time of that endpoint. Poisson regression models were used to describe any association between the CPE score and the development of each CNS disease, after adjustment for potential confounding factors. Two sets of analyses were performed: the first considered baseline covariates only (CPE score of initial regimen, sex/mode of transmission, age, ethnicity, CD4 count, HIV RNA, treatment status and calendar year at cART initiation), whereas the second incorporated changes in several of these covariates over time (latest CPE score, CD4 count and HIV RNA) through the use of time-dependent covariates – this latter model also incorporated a time-dependent covariate for time since initiation of cART.

Survival analysis

Similar analyses were performed for all-cause mortality; for these analyses, patient follow-up started on the date of cART initiation and ended at the earliest of death, 31st December 2008 or three months after the patient's last clinic visit. All statistical analyses for this study were supervised or performed by Professor Caroline Sabin at the Research Department of Infection and Population Health, UCL Medical School, London, UK. SAS software (v.9.1) was used. *P*-values below 0.05 were considered statistically significant.

Missing data

As with all cohort studies, within UKCHIC (1996 and 2007) there was a small amount of missing demographic or clinical data. Where age or gender data was lacking (<1% of subjects) subjects were excluded from all analyses. Where ethnicity (7.4% of subjects) and HIV-risk-group (9.2%) data were missing, a 'missing-indicator-variable' technique was used so that the full dataset could still be analysed (see Tables 3.1 and 3.3). Where baseline CD4+ cell count or plasma HIV RNA data were

missing (approximately 25% pre-cART), subjects were classified as having a missing value, however for time-updated analyses, most had complete datasets at later timepoints. In analyses where CD4+ cell count or HIV RNA were incorporated as continuous covariates, those with missing values were automatically excluded; for analyses where they were incorporated as categorical covariates, a category for 'not known' was incorporated therefore including all subjects.

3.3 RESULTS

3.3.1 Association between CPE score and demographics or clinical parameters

Over the study period 22,356 subjects with no history of prior CNS diseases commenced cART. Median (IQR) duration of follow-up of patients after starting cART was 4.6 (1.9, 8.5) years (118,123 PYFU in total), with 89.4% of this follow-up time being spent on cART, 1.1% on non-cART regimens and 9.5% off treatment. Median (IQR) age at cART initiation was 36 (31, 42) years, 16,704 (75%) of subjects were male and 5864 (26%) were of black African ethnicity (see Table 3.1). At the time of commencing cART, median (IQR) CD4+ lymphocyte count and plasma HIV RNA were 200 (100, 300) IU/mL and 4.8 (4.0, 5.3) $\log_{10}$ copies/mL respectively. Over the study period, median (IQR) CPE score for initial cART regimen initially increased from 7 (5, 8) in 1996/97 to 9 (8, 10) in 2000/01 and subsequently declined to 6 (5, 8) in 2006-2008. The majority of subjects (79.8%) were commenced on an initial cART regimen with a CPE score of 5-9. Differences in gender, HIV-risk group and ethnicity existed between CPE score strata. For instance, subjects with a baseline CPE score of $\leq$4 tended to be older, had lower CD4+ cell counts and were less likely to be treatment-naïve than those with higher baseline CPE scores.

Multivariable logistic regression analyses revealed that subjects who initiated cART while antiretroviral-naïve and those with a higher CD4 count were *less* likely to be prescribed an initial regimen with a low CPE score, whereas those of other or unknown ethnicities, older subjects and those who initiated cART from 1996-1999 or from 2004 onwards were *more* likely to be prescribed an initial regimen with a low CPE score (Table 3.2).

3.3.2 Incidence of CNS diseases

In total, 251 subjects experienced a new CNS disease during the study period (HIV-E: 80; TOXO: 59; CRYPTO: 56; PML: 54) over a total of 113,633 PYFU (censored at first event). Kaplan-Meier estimates of the proportions experiencing a CNS disease by 1, 2, 3, 4 and 5 years after initiation of cART were 0.6%, 0.8%, 1.0%, 1.1% and 1.2%, respectively. The overall CNS disease rate after starting cART was 2.2 (95%CI 1.9, 2.5)/1000 PYFU. At the time of CNS disease, median (IQR) CD4+ cell count was 103 (30,240) cells/uL, plasma HIV RNA was 4.4 (2.2, 5.5) $\log_{10}$ copies/mL. The median (IQR) time since cART initiation was 0.6 (0.1, 2.7) years and 40 (17.5%) of subjects had discontinued cART. Fifty (22.1%) subjects had an undetectable plasma HIV RNA level at the time of CNS disease and their median cART CPE score was 7.

When stratified according to the initial CPE score, CNS disease rates were highest in those subjects prescribed regimens with CPE scores of $\leq$4 and were lowest in subjects prescribed regimens with CPE scores of $\geq$10 (Figure 3.1), although these differences were non-significant, both before and after adjustment for potential confounders (Table 3.3). When considering baseline covariates only, predictors of a new CNS disease were heterosexual transmission, older age, a lower pre-treatment CD4+ cell count or higher plasma HIV RNA, and prior exposure to mono-or dual-NRTI therapy before starting cART. When changes in the CPE score over time were considered, scores of $\leq$4 were again associated with an increased risk of a new CNS disease. However, as with the baseline analysis, this association did not remain significant after adjustment for potential confounders. Similar associations were found between heterosexual transmission, older age, lower CD4 count and higher plasma HIV RNA and the risk of a new CNS disease, as with the baseline analysis. Time since initiation of cART was additionally identified as a strong predictor of a new CNS disease in these analyses, with the event rate being particularly high in the first 6 months. After controlling for this covariate, prior NRTI exposure was no longer associated with an increased risk of a new CNS disease.

New cases of PML and HIV-E occurred at a rate of 1.2/1000 PYFU (133 cases)**,** while new cases of TOXO and CRYPTO occurred at a rate of 1.1/1000 PYFU (119 cases). When stratified by initial CPE score, rates of PML and HIV-E were highest in those prescribed regimens with scores $\geq$10, whereas rates of TOXO and CRYPTO were highest in those with lower initial cART CPE scores (Figure 3.1). None of these associations were significant, either before or after adjustment for potential confounding factors (data not shown). A similar lack of association was found between each endpoint and the latest CPE score.

3.3.3 Survival analysis

During the study period, 1581 subjects died, giving an overall all-cause mortality rate of 13.8 /1000 PYFU. After stratifying according to the CPE score of the initial regimen, mortality was highest in those who were prescribed regimens with CPE scores $\leq$4 (21.0 deaths /1000 PYFU) and dropped as the CPE score increased (Table 3.4). Similarly after stratification by most recent CPE score, rates of death were highest in those with a CPE score $\leq$4 (16.5 deaths /1000 PYFU) and again dropped as the CPE score increased. A CPE score $\leq$4, whether based on the initial or latest cART regimen, was strongly associated with an increased mortality risk, although both associations were generally attenuated after adjustment for potential confounders. Of note, patients who had discontinued antiretroviral treatment had the highest mortality risk in time-updated analyses, supporting the hypothesis that treatment may have been selectively discontinued in those at terminal stages of disease.

3.4 Discussion

The relationship between antiretroviral CNS penetration and HIV-related CNS disease is of clinical importance, and may influence future antiretroviral selection in HIV-1 infected subjects. In order to investigate the first hypothesis of this thesis, the relationship between CPE score of different cART regimens and the incidence of CNS diseases and survival was retrospectively assessed within a large cohort study. Several interesting observations were made. Firstly, statistically significant changes in the median CPE score of initial cART have occurred between 1996 and 2008. This is likely to reflect changes in prescribing trends (represented in treatment guidelines) as new clinical evidence and newly-licensed antiretrovirals became available. Interestingly, there has been an overall reduction in CPE score in the most recent years studied. While the majority of individuals achieving full suppression of HIV replication in plasma will achieve similar results in cerebrospinal fluid and respond clinically (Antinori, et al. 2002), it is possible that a trend towards lower CPE scores may, in future, increase the risk of HIV replication in the CNS compartment, resulting in more cerebral sequelae, including cognitive problems and HIV-associated encephalopathy.

A statistically significant association was observed between initial cART CPE score and subject demographics (gender, HIV-risk group), calendar year of treatment initiation and clinical parameters (including CD4 cell count and plasma HIV RNA). This novel finding suggests that, according to a subject's clinical status, treatment is tailored or individualised, particularly in those with advanced HIV disease. Such individuals in this cohort were more likely to be prescribed regimens with very low

(≤4) CPE scores. To my knowledge, this association has not previously been reported and may reflect a sub-group of individuals, with high levels of co-morbidity, opportunistic disease or with predicted or known poor adherence. Such confounders are important considerations when the CPE score is utilised as a research tool in retrospective analyses, as bias in outcome data would be expected.

Low rates of CNS diseases were observed overall in this cohort, with low CD4+ cell count, high plasma HIV RNA and short time-elapsed since cART initiation, all statistically significantly associated with increased event risk, rather than CPE score. This suggests that strategies to support adherence to effective therapies, particularly in early months of treatment, are more important than selection of antiretroviral agents with greater CNS penetration. A minority of subjects, however, did experience CNS diseases while plasma HIV RNA was fully suppressed. In these subjects, it is possible viral replication within the CNS compartment and a modest CPE score of cART (median 7) may have influenced the pathogenesis of these events, but, owing to small numbers, it is not possible to draw further conclusions.

In this study, an independent association between lower CPE scores (initial and most recent) and higher all-cause mortality was observed, after adjustment for relevant clinical factors. This finding may reflect the process of altering cART due to a subject's clinical parameters (as earlier described) with those deteriorating clinically or with advanced disease being prescribed regimens with lower scores than asymptomatic individuals. Alternatively, it maybe hypothesised that an association between low CPE score and poorer survival (despite the absence of relationship with our selected CNS diseases) is in fact related to cognitive impairment (for which no data were collected) occurring on cART regimens with low CNS penetration. Cognitive impairment has previously been associated with increased mortality in HIV-1 infected adults (Ellis, et al. 1997; Mayeux, et al. 1993) and improved survival outcomes in perinatally-infected adolescents with higher CPE scoring regimens have also been reported (Patel, et al. 2009). Nevertheless, without randomised, prospective data, this explanation remains speculative.

The data analysed in this chapter successfully examined the hypothesis that antiretroviral regimens with greater CNS penetration are associated with improved cerebral function parameters and found that higher CPE scores were not significantly associated with reduced incidence of CNS diseases, but were associated with improved overall survival. Limitations of our data include the absence of information regarding adherence to therapy and clinic attendance. Furthermore, all CNS diseases were reports of clinical diagnoses rather than strict protocol-defined criteria, and data regarding

cognitive impairment are not collected. Reporting of events may therefore have varied by clinician, centre, and over the study period. While the study size is large, CNS diseases occur relatively infrequently, limiting the power to perform more detailed analyses. Finally, analyses of the changing CPE score over time may be affected by time-varying confounding, in that treatments may be selectively switched in those already showing early signs of CNS problems; thus, it is difficult to assess the causal association between regimens with different CPE scores and the development of these events. While novel statistical methods exist for the evaluation of causal effects (subject to the assumption of no unmeasured confounding), their application requires large study sizes. As such, collaborative efforts are ongoing between several large HIV cohorts to apply these methods to this question, with results anticipated within the next 1-2 years. Nevertheless, this study adds important information to our understanding of the relationship between CPE score, CNS diseases and survival. The study highlights, in particular, that clinical status at time of commencing cART influences antiretroviral selection and CPE score. This should be considered when utilising CPE scores for future retrospective cohort analyses.

Table 3.1: Baseline characteristics of patients, overall and stratified by central nervous system penetration effectiveness (CPE) rank of initial combination antiretroviral therapy (cART)

			CPE score of initial cART			
		Total	**≤4**	**5-7**	**8-9**	**≥10**
Number of patients	**N (%)**	22356 (100.0)	1248 (5.6)	9918 (44.4)	7906 (35.4)	3284 (14.7)
Sex/mode of transmission	**MSM**	11873 (53.1)	671 (53.8)	5748 (58.0)	4021 (50.9)	1433 (43.6)
	Male heterosexual	2932 (13.1)	191 (15.3)	1142 (11.5)	1198 (15.2)	401 (12.2)
	Female heterosexual	4688 (21.0)	221 (17.7)	1673 (16.9)	1704 (21.6)	1090 (33.2)
	IDU	790 (3.5)	44 (3.5)	355 (3.6)	281 (3.6)	110 (3.4)
	Male other/unknown	1371 (6.1)	88 (7.1)	696 (7.0)	448 (5.7)	139 (4.2)
	Female other/unknown	702 (3.1)	33 (2.6)	304 (3.1)	254 (3.2)	111 (3.4)
Ethnicity	**White**	12853 (57.5)	726 (58.2)	6185 (62.4)	4384 (55.5)	1558 (47.4)
	Black African	5864 (26.2)	285 (22.8)	2076 (20.9)	2316 (29.3)	1187 (36.1)
	Other/unknown	3639 (16.3)	237 (19.0)	1657 (16.7)	1206 (15.3)	539 (16.4)
Age (years)	**Median (IQR)**	36 (31, 42)	37 (32, 44)	36 (31, 42)	36 (31, 42)	34 (30, 40)
Pre-treatment CD4 (cells/μL)	**Median (IQR)**	200 (100, 300)	170 (75, 299)	200 (94, 300)	200 (102, 297)	202 (114, 307)
Pre-treatment plasma HIV RNA (log_{10} copies/mL)	**Median (IQR)**	4.8 (4.0, 5.3)	4.7 (4.0, 5.2)	4.8 (4.1, 5.3)	4.8 (4.1, 5.3)	4.7 (3.9, 5.2)
Treatment-naive	**N (%)**	18204 (81.4)	889 (71.2)	7819 (78.8)	6796 (86.0)	2700 (82.2)
Follow-up (yrs)	**Median (IQR)**	4.6 (1.9, 8.5)	3.4 (1.6, 9.5)	3.6 (1.3, 8.9)	5.1 (2.3, 8.0)	6.6 (3.9, 8.8)
Calendar year	**1996/1997**	3751 (16.8)	355 (28.5)	2126 (21.4)	904 (11.4)	366 (11.1)
	1998/1999	3531 (15.8)	146 (11.7)	1418 (14.3)	1254 (15.9)	714 (21.7)
	2000/2001	2877 (12.9)	59 (4.7)	598 (6.0)	1330 (16.8)	890 (27.1)
	2002/2003	3193 (14.3)	61 (4.9)	761 (7.7)	1599 (20.2)	772 (23.5)
	2004/2005	3496 (15.6)	205 (16.4)	1737 (17.5)	1186 (15.0)	368 (11.2)
	2006/2007/2008	5508 (24.6)	422 (33.8)	3278 (33.1)	1634 (20.7)	174 (5.3)
Initial regimen	**PI-based**	8396 (37.6)	871 (69.8)	5363 (54.1)	1925 (24.4)	237 (7.2)
	NNRTI-based	12783 (57.2)	332 (26.6)	4273 (43.1)	5286 (66.9)	2892 (88.1)
	PI- + NNRTI-based	470 (2.1)	6 (0.5)	175 (1.8)	145 (1.8)	144 (4.4)
	Other cART	707 (3.2)	39 (3.1)	107 (1.1)	550 (7.0)	11 (0.3)

[Table 3.1 legend: CPE = central nervous system penetration effectiveness rank ; cART= combination antiretroviral therapy; MSM = men-having-sex-with-men; IDU = injecting drug user, IQR= interquartile range; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor]

Table 3.2: Results from multivariable logistic regression analysis of factors associated with a low (≤4) baseline central nervous system penetration effectiveness (CPE) rank

		Odds Ratio	95% Confidence Interval	p-value
Ethnicity	**White**	1	-	**0.008**
	Black African	0.93	0.78, 1.10	
	Other/unknown	1.26	1.06, 1.50	
Age	**Per 10 years older**	1.14	1.07, 1.23	**0.0002**
Treatment status	**Antiretroviral-naive**	0.59	0.49, 0.71	**0.0001**
Calendar year	**1996/1997**	4.83	3.34, 6.99	**0.0001**
	1998/1999	2.28	1.55, 3.35	
	2000/2001	1	-	
	2002/2003	0.96	0.61, 1.53	
	2004/2005	3.79	2.62, 5.47	
	2006/2007/2008	5.27	3.72, 7.46	
Pre-treatment CD4+ cell count	**Per 50 cells/μL higher**	0.98	0.96, 0.99	**0.0001**

Table 3.3: Results from adjusted Poisson regression analyses of factors associated with the development of a new central nervous system (CNS) event over study period

		Baseline (fixed) covariates only			Latest (time-updated) covariates		
		RR	**95% CI**	**p-value**	**RR**	**95% CI**	**p-value**
CPE score	**≤4**	1	-	0.97	1	-	0.66
	5-7	0.95	(0.57, 1.60)		0.96	(0.56, 1.65)	
	8-9	1.02	(0.59, 1.74)		1.20	(0.69, 2.07)	
	≥10	0.95	(0.53, 1.72)		1.03	(0.56, 1.89)	
	Off treatment	n/a			1.20	(0.66, 2.17)	
Time since initiation of cART (months)	**0-6**	n/a			3.37	(2.32, 4.89)	**0.0001**
	7-12				1.28	(0.77, 2.12)	
	13-18				1.62	(0.98, 2.67)	
	19-24				1.13	(0.62, 2.08)	
	25-30				1.12	(0.58, 2.14)	
	31-36				1.05	(0.52, 2.12)	
	>36				1	-	
Sex/mode of transmission	**MSM**	1	-	**0.006**	1	-	0.11
	Male heterosexual	2.44	(1.34, 4.45)		1.46	(0.80, 2.67)	
	Female heterosexual	3.18	(1.54, 6.57)		1.98	(0.95, 4.10)	
	IDU	1.32	(0.90, 1.94)		1.15	(0.78, 1.69)	
	Male other/unknown	0.74	(0.35, 1.54)		0.54	(0.26, 1.12)	
	Female other/unknown	1.37	(0.65, 2.88)		1.18	(0.56, 2.49)	
Age (years)	**≤30**	1	-	0.30	1	-	0.15
	31-40	1.36	(0.94. 1.97)		1.42	(0.98, 2.07)	
	41-50	1.45	(0.95, 2.20)		1.57	(1.04, 2.39)	
	>50	1.40	(0.81, 2.43)		1.50	(0.87, 2.60)	
Ethnicity	**White**	1	-	0.11	1	-	0.06
	Black African	1.22	(0.80, 1.85)		1.08	(0.71, 1.65)	
	Other	1.47	(0.97, 2.24)		1.44	(0.95, 2.20)	
	Unknown	0.61	(0.28, 1.31)		0.51	(0.24, 1.09)	
CD4 count (cells/μL)	**0-50**	5.75	(3.60, 9.18)	**0.0001**	10.10	(6.55, 15.57)	**0.0001**
	50-199	2.02	(1.26, 3.23)		3.27	(2.19, 4.89)	
	200-349	1	-		1	-	
	350-499	1.46	(0.71, 2.98)		0.48	(0.25, 0.94)	
	≥500	1.22	(0.46, 3.21)		0.41	(0.20, 0.81)	
	Missing	2.57	(1.48, 4.46)		3.39	(1.80, 6.36)	
Plasma HIV RNA (copies/mL)	**≤50**	1	-	**0.0001**	1	-	**0.0001**
	>50, ≤10,000	1.91	(0.25, 14.91)		3.03	(1.93, 4.77)	
	>10,000, ≤100,000	4.94	(0.68, 36.15)		4.24	(2.60, 6.92)	
	>100,000	5.74	(0.79, 41.84)		5.35	(3.29, 8.69)	
	Missing	9.18	(1.25, 67.36)		6.36	(3.54, 11.45)	
Year of starting cART	**96/97**	0.71	(0.43, 1.17)	**0.02**	0.79	(0.49, 1.26)	0.11
	98/99	1.23	(0.81, 1.89)		1.14	(0.75, 1.74)	
	00/01	1	-		1	-	
	02/03	1.38	(0.87, 2.18)		1.27	(0.80, 2.01)	
	04/05	0.98	(0.56, 1.74)		0.73	(0.42, 1.29)	
	06/07/08	1.81	(1.02, 3.22)		0.78	(0.44, 1.39)	
Antiretroviral-naive		0.58	(0.41, 0.82)	**0.002**	0.89	(0.64, 1.24)	0.49

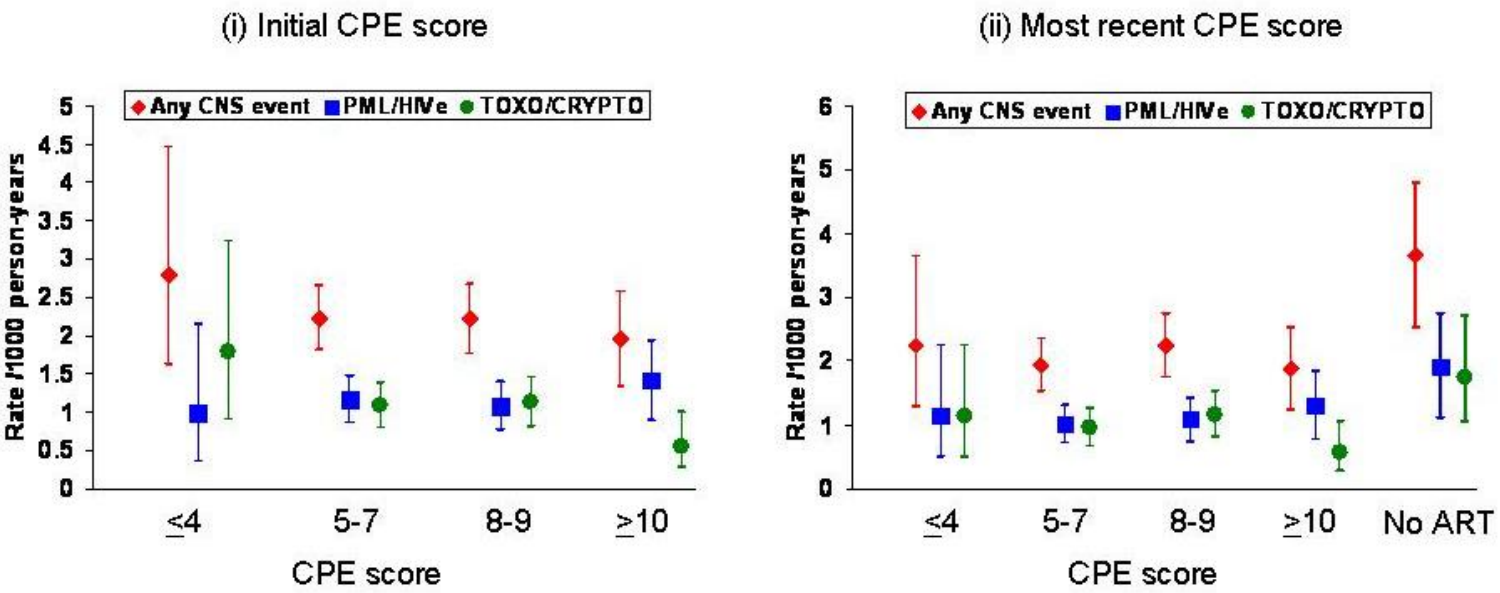
[Table 3.3 legend: CPE = central nervous system penetration effectiveness rank; cART= combination antiretroviral therapy; MSM = men-having-sex-with-men; IDU = injecting drug user; RR=relative rate; 95%CI= 95% confidence interval]

Table 3.4: Univariate and multivariable estimates of association between mortality and initial or most recent CPE score

	Initial CPE score				Most recent CPE score			
	Unadjusted		**Adjusted**		**Unadjusted**		**Adjusted**	
CPE Score	**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**
≤4	1	**0.0001**	1	**0.03**	1	**0.0001**	1	**0.0001**
5-7	0.72 (0.60, 0.87)		0.80 (0.66, 0.97)		0.71 (0.58, 0.87)		0.81 (0.66, 0.98)	
8-9	0.60 (0.50, 0.73)		0.78 (0.64, 0.95)		0.63 (0.50, 0.76)		0.80 (0.64, 0.98)	
≥10	0.50 (0.40, 0.62)		0.71 (0.56, 0.89)		0.65 (0.51, 0.81)		0.83 (0.66, 1.04)	
Off treatment	n/a				2.21 (1.80, 2.72)		1.73 (1.40, 2.14)	

[Table 3.4 legend: CPE = central nervous system penetration effectiveness rank; RR= relative rate; 95% CI=95% confidence interval]

Figure 3.1: CNS disease (event) rates for a new (i) CNS (diamond), (ii) PML/HIV-E (square) or (iii) TOXO/CRYPTO (circle) event stratified by initial and most recent central nervous penetration effectiveness (CPE) scores



[Figure 3.1 legend: CPE score = central nervous system penetration effectiveness rank; HIVe = HIV-associated encephalopathy, PML = progressive multifocal leucoencephalopathy, TOXO = cerebral toxoplasmosis, CRYP = cryptococcal meningitis, ART=antiretroviral therapy]

Chapter 4

A Prospective, Randomised-Controlled Study to Assess Changes to Cerebral function parameters in Stable HIV-1 Infected Subjects Switching Antiretroviral Therapy to Darunavir/Ritonavir Alone or Darunavir/Ritonavir with Nucleoside Analogues

Chapter 4: A Proscpective, Randomised-Controlled Study to Assess Changes to Cerebral Function Parameters in Stable, HIV-1 Infected Subjects Switching Antiretroviral Therapy to Darunavir/Ritonavir alone or Darunavir/Ritonavir with Nucleoside Analogues

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4.1 Introduction

With improved life expectancy for HIV-1 infected individuals, there is a growing need to manage chronic HIV-associated morbidities in the modern era. One such problem is the ongoing high prevalence of HAND (Tozzi, et al. 2007; Tozzi, et al. 2005a) which can reduce an individual's ability to adhere to therapy, quality of life and overall survival (Albert SM 1999; Ellis, et al. 1997; Tozzi, et al. 2005b). While improvements in the cognition of therapy-naive subjects can be demonstrated after commencing cART (Cysique, et al. 2009), the optimal antiretrovirals for this purpose are not currently known and only one small study has prospectively addressed this question using randomised therapy-arms in the post-cART era (Winston, et al. 2010).

Antiretroviral drugs vary in their ability to cross the BBB, due to factors including drug size and protein-binding (Schweinsburg BC 2005; Strazielle and Ghersi-Egea 2005) and some nucleoside analogues (with favourable properties for CNS affinity) have been associated with neurotoxicity (Schweinsburg BC 2005) and less neuronal recovery than other drug classes (Winston, et al. 2010). Despite being lipophilic molecules, the PI drug class, due to the generally high level of protein-binding and activity as substrates of trans-membrane transporters including P-glycoprotein, are considered to have less CNS affinity than some other antiretroviral drug classes. The most-recently licensed drug from this class, ritonavir-boosted DRV, is approximately 95% protein bound (primarily to plasma alpha ⁻1 ⁻acid glycoprotein), yet in small studies, favourable DRV concentrations have been demonstrated in the cerebrospinal fluid, suggesting it may contribute significantly to suppression of HIV-1 activity within the CNS. (http://www.medicines.org.uk/EMC/medicine/22152/SPC/Prezista+75+mg%2c+150+mg%2c+400+mg%2c+600+mg+film-coated+tablets/; Letendre S September 12-15, 2009. ; Yilmaz A 2009)

In recent years, researchers have investigated clinical outcomes when using novel, nucleoside-sparing treatment simplification in patients established on cART, in an attempt to reduce therapy-related toxicities. It is postulated that utilisation of such strategies, including PI-monotherapy, may result in more cognitive sequelae due to reduced CNS affinity of the total regimen. In order to investigate the first hypothesis of this thesis, that use of antiretroviral agents with greater antiretroviral CNS penetration is associated with improved cerebral function parameters, this study aimed to assess prospective changes to cerebral function parameters in HIV-1 infected subjects switching antiretroviral therapy to DRV/RTV alone (DRV*mono*) or DRV/RTV with nucleoside analogues (DRV*nrti*).

4.2 Methods

4.2.1 Subject Selection

This 48 week prospective, randomized-controlled study was performed as a sub-study within the international 'MONET' clinical trial (Arribas, et al. 2010). The study was conducted at St Mary's Hospital, Imperial College, London and Chelsea and Westminster Hospital NHS Trust, London between September 2007 and September 2009. Ethical approval was obtained as described in section 2.1. Eligibility criteria required subjects to be HIV-1 infected adults, proficient in English, receiving stable cART (including 2 NRTIs + a boosted PI) and with a plasma HIV RNA level below 50 copies/mL for at least six months prior to study entry. Exclusion criteria included any active neurological disease, current major depression or psychosis, recent head injury, current use of recreational drugs or alcohol abuse.

After providing written, informed consent and completing screening procedures, subjects were randomised one-to-one at baseline, in an open-label fashion, to receive DRV 800mg q.d., RTV 100mg q.d. (arm 1: DRV*mono*) or DRV 800mg q.d., RTV 100mg q.d. plus any 2 nucleoside analogues (arm 2: DRV*nrti*). Patients were then followed prospectively with a medical review at 3-monthly intervals to assess therapy compliance, use of recreational drugs, adverse events, physical assessment and measurements of plasma HIV RNA level, CD4+ lymphocyte cell count, haematology and biochemistry markers.

4.2.2 Computerised neurocognitive assessment

Changes to cognitive function were measured using the computerised cognitive assessment *Cogstate*^{TM} (described in section 2.4.3). A full practice session was completed before a formal assessment at baseline and week 48.

4.2.3 Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

In this work, cerebral ^{1}H-MRS was performed (as described in section 2.4.1) in the FGM, FWM and RBG at baseline (prior to commencing study medication) and week 48. These voxels locations were selected according to previous studies and historical postmortem data analysing areas of cerebral damage in HIV-1 infected individuals. While reducing the number of voxels selected would reduce time required to perform ^{1}H-MRS assessments (with potential cost-saving implications), important data may not be captured as changes in metabolites occur at different speeds in different locations throughout the course of a cerebral disease and may vary between individuals. In order to first ensure validity of the ^{1}H-MRS data used throughout this thesis, it was necessary to ensure

reproducility of the technique. Therefore spectra from 5 subjects (in 3 voxels) at different timepoints throughout the study were analysed 3 times by a single observer (LG). MRS reproducibility data will thus also be presented within the results section of this chapter.

4.2.4 Data analysis

Computerised neurocognitive assessment

Data were analysed as described in section 2.4.3. Average reaction times in four speed tasks and the proportion of correct responses in each of three accuracy tasks were calculated and transformed to normalise the data. Composite speed and accuracy scores were also calculated at each assessment. To evaluate longitudinal changes, within-subject absolute and percentage changes to individual task scores and composite scores by week 48 were then calculated. Significant changes to scores between baseline and week 48 were evaluated using a paired samples *t*-test. Associations between changes to cognitive assessment scores and clinical parameters were investigated using univariate linear regression.

^{1}H-MRS

Data were analysed as described in section 2.4.1. For the reproducibility analysis following thrice-repeated analysis of spectra from 5 examinations (in 3 voxel locations), the coefficient of variation (CV) was calculated (SD/mean). Individual subject mean, SD and CV percentage (SD/mean x 100) for each cerebral metabolite peak area was measured and then group averages calculated.

Cerebral metabolites and metabolite ratios at each assessment and absolute changes between baseline and week 48 were then calculated. Significant changes to scores between baseline and week 48 were evaluated using a paired samples *t*-test. Associations between composite cognitive scores or cerebral metabolite ratios with clinical parameters (including treatment arm) were evaluated using linear regression.

Finally the presence of association between changes to composite cognitive assessment scores and cerebral metabolite ratios were investigated using univariate linear regression. SPSS software (version 18.0; SPSS Inc., Chicago, Illinois, USA) was used for all analysis. *p*-values below 0.05 were considered significant.

4.3 Results

6 subjects were enrolled and 5 completed all study procedures. Mean age was 44 years (SD 5) and 80% were male. Mean baseline CD4+ cell count was 535 cells/uL (SD 172). All subjects were naive to DRV at study entry. Patient demographics at baseline are shown in Table 4.1. Three subjects were randomised to each treatment arm and details of their treatment at study entry and following randomisation are described in Table 4.2.).

4.3.1 Computerised cognitive assessment

Results of the cognitive assessments at baseline and week 48 are shown in Table 4.3a and 4.3b respectively. Over the study period, mean score improvements were observed in 6 out of 8 cognitive tasks assessed. These included identification speed (0.7 $\log_{10}$ms faster), visual learning (accuracy increased by 0.23 arc. proportion correct) and executive function (error rate reduced from 45 to 34). In the 2 remaining tasks, slowing of simple reaction time (0.03 $\log_{10}$ms slower) and divided attention speed (0.01 $\log_{10}$ms) were observed.

4.3.2 ^{1}H-MRS

Reproducibility analysis

The results of cerebral ^{1}H-MRS reproducibility are shown in Table 4.4. The spectra quantification in several subjects was identical on each of the 3 occasions (due to spectra clarity) giving a SD (and therefore CV) of 0. The CV for all metabolites and cerebral metabolite ratios in each voxel location was below 1%.

Cerebral metabolite ratios

Analysis of ^{1}H-MRS cerebral metabolite ratios revealed overall reductions in markers of inflammation (Cho/Cr and mI/Cr ratios) in FGM, FWM and RBG (maximum reduction of mI/Cr ratio in FGM between baseline and week 48, see Table 4.5). NAA/Cr, a marker of neuronal integrity, increased in the RBG by 0.05, but decreased in both FGM and FWM regions (by 0.06 and 0.13, respectively).

4.3.3 Association between cerebral parameter improvements and patient demographics or study treatment arm

No association between study treatment arm and improvements to cognitive assessment composite scores or cerebral metabolite ratios was observed (*p-value*>0.13, see Table 4.6 and 4.7). In addition, no significant association between clinical parameters including subject age, time elapsed since HIV-1 diagnosis or CD4+lymphocyte count and improvements to neurocognitive test scores were observed (*p*> 0.06 all values). Associations between longer-time elapsed since HIV diagnosis and increase in NAA:Cr (neuronal recovery) in the RBG (*p*-value=0.03, 95%CI 0.05, 0.45) was observed.

4.3.4 Association between cerebral metabolite ratios and neurocognitive composite scores

Improvement in composite speed score during the study period was significantly associated with increased NAA/Cr (neuronal recovery) in the FWM (p=0.01, 95%CI -0.25, -0.08). No other significant associations between cognitive scores and MRS results were observed (see Table 4.7).

4.4 Discussion

In order to investigate the first hypothesis of this thesis, this prospective study investigated changes to cerebral function parameters in subjects switching therapy to either DRV*mono* or *DRVnrti* – novel antiretroviral regimens with differing CNS penetration and CPE scores. It is one of the only randomised, prospective studies to investigate cerebral effects in the modern era. Overall, improvements in cerebral function parameters were observed in all subjects. Features of this cognitive improvement included improvements in cognitive speed, learning, accuracy and executive functioning. Additionally, within 3 cerebral locations, reductions in markers of cerebral inflammation (mI and Cho) were observed between baseline and week 48 using the objective radiological tool, ^{1}H-MRS. Improved neurocognitive performance and improvements to cerebral metabolite ratios after commencing cART have previously been described in therapy-naive subjects (Cysique, et al. 2009; Winston, et al. 2010), however to my knowledge these have not previously been evaluated when utilising nucleoside-sparing cART regimens.

The cerebral metabolites Cho and mI are present in glial cells and become elevated upon cell membrane injury or with glial activation and increased concentrations of these compounds correlate with advanced HIV-1 disease and dementia (Chang L 2002). It is therefore reassuring, that following enrolment to this study, favourable Cho and mI metabolite shifts were observed, representing reductions in cerebral inflammation. In this study, I observed only small increases in NAA/Cr in the RBG and decreases of this metabolite ratio in other cerebral locations. NAA represents neuronal

integrity and is responsible for the processing of cognition into motor activity (Ross and Bluml 2001). Reduced NAA/Cr has been observed in advanced disease stages, including AIDS-dementia complex and severe neurocognitive impairment (Meyerhoff, et al. 1999; Paul, et al. 2007). Increases in NAA/Cr following initiation of cART have previously been described in antiretroviral-naive individuals (Winston, et al. 2010). It is likely, however, the small changes to this ratio observed in my study, were due a higher mean CD4+cell count (535 cells/uL) and lengthy duration of prior virological than in this previous study.

Improvements in neurocognitive test scores may be attributed to a learning effect, whereby a subject performance improves on re-testing. In my study, subjects underwent a full practice test prior to study entry, in order to minimise this effect. Furthermore, as simultaneous improvements in metabolite markers of cerebral inflammation were also observed in my study, it is likely the changes described represent real cerebral function improvements, rather than a learning effect.

The major limitation to the significance of this study is the very small sample size. It was therefore not possible to elucidate significant differences between study groups, and meaningfully evaluate the differing antiretroviral regimen CNS penetration to definitively address the first thesis hypothesis. Reassuringly, however, despite a reduction in overall CPE score for half of the enrolled subjects, we observed small improvements in cerebral function parameters across the group. This raises the possibility that predicting poor sanctuary site penetration and the development of subsequent CNS deficits in individuals starting or switching therapy, remains more complex than consideration of the CPE score alone. The small size of the study underpowers any ability to elicit subtle differences between treatment arms which may occur. Unfortunately, despite 256 subjects being enrolled within the parent MONET study, owing to the time taken to gain regulatory approval for this sub-study in the UK, and competitive recruitment around Europe, only very few subjects were able to participate. Nevertheless, this work demonstrates as a proof-of-principle, that objective, non-invasive tools can practically be administered and prospectively assess cerebral function in HIV-1-infeceted individuals and should be considered for utilisation in future HIV-1 treatment trials.

Table 4.1: Subject demographic factors and clinical parameters at study entry

Parameter	**Mean (SD) unless otherwise stated**
Number of participants	5
Age (years)	44 (5)
Male, n (%)	4 (80%)
CD4+ cell count, cells/uL	535 (172)
Ethnicity, n (%)	
White	2 (40%)
Black	1 (20%)
Asian	1 (20%)
Other	1 (20%)
Years elapsed since HIV diagnosis	12.5 (7)
HIV RNA less than 50 copies/mL for >3 months, n (%)	5 (100)

Table 4.2: Antiretroviral therapy received by subjects prior to study and following randomisation

		Prior to study entry	Randomisation
Subject number	**1**	TDF, ddI, ATV/RTV	TDF, FTC, DRV/RTV
	2	TDF, FTC, LPV/RTV	DRV/RTV
	3	ABC, 3TC, ATV/RTV	DRV/RTV
	4	TDF, FTC, LPV/RTV	TDF, FTC, DRV/RTV
	5	ABC, 3TC, SQV/RTV	DRV/RTV
Mean CPE score 2010		6.2	4.3

[Legend Table 4.2: TDF=tenofovir; FTC=emtricitabine; ddI=didanosine; ATV/RTV=atazanavir/ritonavir; DRV/RTV=darunavir/ritonavir; LPV/RTV=lopinavir/ritonavir; ABC=abacavir; 3TC= lamivudine; SQV/RTV = saquinavir/ritonavir]

Table 4.3a: Neurocognitive assessment scores at baseline and week 48

Cognitive task	**Simple reaction speed**	**Identification speed**	**Divided attention speed**	**Complex reaction speed**	**Associate, non visual learning**	**Visual learning and memory**	**Working memory**	**Executive function**	**Composite speed score**	**Composite accuracy score**
Unit of measure	***log_{10}ms***	***log_{10}ms***	***log_{10}ms***	***log_{10}ms***	***arcsine proportion correct***	***arcsine proportion correct***	***arcsine proportion correct***	***error rate***	***log_{10}ms***	***arcsine proportion correct***
Mean (SD) at baseline	2.47 (0.12)	2.71 (0.07)	2.60 (0.15)	2.82 (0.08)	0.87 (0.28)	0.73 (0.22)	1.21 (0.25)	45.00 (23.07)	10.61 (0.37)	2.81 (0.73)
Mean (SD) at week 48	2.51 (0.14)	2.65 (0.05)	2.61 (0.14)	2.80 (0.08)	0.92 (0.22)	0.96 (0.15)	1.23 (0.09)	34.00 (19.04)	10.56 (0.36)	3.12 (0.43)

[Legend Table 4.3a: ms=millisecond; SD=standard deviation]

Table 4.3b: Absolute changes to mean neurocognitive task scores between baseline and week 48

Cognitive task	**Simple reaction speed**	**Identification speed**	**Divided attention speed**	**Complex reaction speed**	**Associate, non visual learning**	**Visual learning and memory**	**Working memory**	**Executive function**	**Composite speed score**	**Composite accuracy score**
Unit of measure	***log_{10}ms***	***log_{10}ms***	***log_{10}ms***	***log_{10}ms***	***arcsine proportion correct***	***arcsine proportion correct***	***arcsine proportion correct***	***error rate***	***log_{10}ms***	***arcsine proportion correct***
Mean (SD) absolute change by week 48	0.03 (0.06)	**-0.07 (0.06)**	0.01 (0.01)	**-0.03 (0.05)**	**0.06 (0.20)**	**0.23 (0.25)**	**0.01 (0.26)**	**-11.00 (21.22)**	**-0.05 (0.08)**	**0.30 (0.67)**
***p*-value for change [95%CI]**	0.31	0.07	0.18	0.29	0.57	0.11	0.92	0.31	0.24	0.37
	[-0.1, 0.1]	[-0.0,0.1]	[-0.0,0.1]	[-0.0,0.1]	[-0.3,0.2]	[-0.5, 0.1]	[-0.3, 0.3]	[-15.0,37.1]	[-0.1, 0.2]	[-1.1, 0.5]

*[Legend Table 4.3b: **bold font indicates improved score;** ms=millisecond; SD=standard deviation; 95%CI=95% confidence interval]*

T***able 4.4: Cerebral proton spectroscopy reproducibility analysis in 5 subjects***

		Frontal Grey Matter				Frontal White Matter				Right basal ganglia			
		NAA/Cr	**Cho/Cr**	**mI1/Cr**	**Total mI/Cr**	**NAA/Cr**	**Cho/Cr**	**mI1/Cr**	**Total mI/Cr**	**NAA/Cr**	**Cho/Cr**	**mI1/Cr**	**Total mI/Cr**
Subject 1	**Mean**	1.41	0.60	0.45	2.12	2.55	1.45	1.71	4.97	2.00	1.04	1.24	2.27
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CV (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Subject 2	**Mean**	1.45	0.59	0.76	3.15	1.52	1.16	1.45	3.71	1.36	0.79	1.04	2.44
	SD	0.00	0.01	0.01	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CV (%)	0.00	0.10	0.23	1.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Subject 3	**Mean**	1.36	0.74	0.35	1.59	1.82	1.22	1.05	4.28	2.45	0.98	0.69	2.73
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CV (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Subject 4	**Mean**	2.3	0.84	0.63	4.94	2.05	0.94	0.32	5.51	1.56	0.79	1.03	3.28
	SD	0.29	0.01	0.01	0.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CV (%)	1.25	0.78	1.77	1.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Subject 5	**Mean**	1.41	0.6	0.61	2.34	2.04	1.04	0.86	4.09	2.12	0.94	0.22	4.85
	SD	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01
	CV (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.12	0.53	0.24
Overall group mean CV (%)		0.25	0.18	0.40	0.62	0.00	0.00	0.00	0.00	0.05	0.02	0.11	0.05

[Legend Table 4.4: SD=standard deviation; CV=coefficient of variation; NAA=N-acetyl aspartate; Cr= creatine; Cho = choline; mi1=first myo-inositol peak; Total mI= total of myo-inositol peaks]

Table 4.5a: Results of cerebral proton spectroscopy at baseline and week 48 in all subjects

Cerebral metabolite ratio	Frontal grey matter				Frontal white matter				Right basal ganglia			
	NAA/Cr	**Cho/Cr**	**MI1/Cr**	**TOTAL MI/Cr**	**NAA/Cr**	**Cho/Cr**	**MI1/Cr**	**TOTAL MI/Cr**	**NAA/Cr**	**Cho/Cr**	**MI1/Cr**	**TOTAL MI/Cr**
Mean (SD) cerebral metabolite ratio at baseline	1.54 (0.29)	0.69 (0.11)	0.88 (0.23)	3.23 (0.59)	1.66 (0.50)	1.06 (0.14)	0.89 (0.29)	3.35 (0.55)	1.62 (0.10)	0.89 (0.13)	0.83 (0.22)	2.48 (0.52)
Mean (SD) cerebral metabolite ratio at week 48	1.39 (0.21)	0.55 (0.06)	0.60 (0.12)	2.67 (0.40)	1.52 (0.11)	0.98 (0.15)	0.88 (0.35)	2.91 (0.84)	1.67 (0.20)	0.84 (0.02)	0.67 (0.14)	2.56 (0.41)

[Legend Table 4.5a: SD=standard deviation; NAA=N-acetyl aspartate; Cr= creatine; Cho = choline; mi1=first myo-inositol peak; Total mI= total of myo-inositol peaks]

Table 4.5b: Absolute changes to cerebral metabolite ratios over the study period (between baseline and week 48)

	Frontal grey matter				**Frontal white matter**				**Right basal ganglia**			
	NAA/Cr	**Cho/Cr**	**MI1/Cr**	**TOTAL MI/Cr**	**NAA/Cr**	**Cho/Cr**	**MI1/Cr**	**TOTAL MI/Cr**	**NAA/Cr**	**Cho/Cr**	**MI1/Cr**	**TOTAL MI/Cr**
Mean (SD) absolute change by week 48	-0.06 (0.21)	**-0.11 (0.17)**	**-0.22 (0.30)**	**-0.40 (0.62)**	-0.13 (0.47)	**-0.09 (0.17)**	**-0.01 (0.20)**	**-0.45 (0.47)**	**0.05 (0.20)**	**-0.05 (0.13)**	**-0.16 (0.18)**	0.08 (0.44)
p*-value [95%CI] for change between baseline and week 48	0.59 [-0.27, 0.40]	0.31 [-0.17, 0.38]	0.24 [-0.26, 0.70]	0.29 [-0.58, 1.39]	0.56 [-0.44, 0.71]	0.32 [-0.12, 0.30]	0.90 [-0.27, 0.26]	0.10 [-0.13, 1.02]	0.58 [-0.31, 0.20]	0.42 [-0.11, 0.22]	0.12 [-0.07, 0.38]	0.70 [-0.62, 0.46]

**using paired samples t-test*

*[Legend Table 4.5b: **bold font indicates improvement in ratio;** SD=standard deviation; 95%CI= 95% confidence interval; NAA=N-acetyl aspartate; Cr=creatine; Cho =choline; mi1=first myo-inositol peak; Total mI=total of myo-inositol peaks]*

Table 4.6: Results of univariate linear regression analysis to investigate the relationship between change to cognitive assessment composite scores over 48 weeks and clinical parameters

Clinical Parameter	**Executive function** ***p-value [95%CI]***	**Composite speed score** ***p-value [95%CI]***	**Composite accuracy score** ***p-value [95%CI]***
Age, per 10 year increase	0.44 [-50.64, 89.30]	0.35 [-0.16, 0.34]	0.58 [-1.87, 2.77]
Baseline CD4+ count, per 100 cell/uL increase	0.06 [-22.06, 0.71]	0.62 [-0.07, 0.10]	0.34 [-0.81, 0.39]
Years since HIV diagnosis, per 10 year increase	0.85 [-55.82, 48.93]	0.94 [-0.19, 0.20]	0.26 [-1.86, 0.73]
Treatment arm of study (ITT)	0.26 [-79.83, 31.49]	0.51 [-0.30, 0.19]	0.44 [-2.55, 1.44]

[Legend Table 4.6: 95% CI=95% confidence interval; ITT=intention to treat]

Table 4.7: Results of univariate linear regression analysis to investigate association between changes to cerebral metabolite ratios, cognitive assessment scores and clinical parameters

Parameter	Frontal grey matter *p*-value [95%CI]			Frontal white matter *p*-value [95%CI]			Right basal ganglia *p*-value [95%CI]		
	NAA/Cr	Cho/Cr	MI1/Cr	NAA/Cr	Cho/Cr	MI1/Cr	NAA/Cr	Cho/Cr	MI1/Cr
Change to composite speed score (log_{10}ms)	0.83 [-1.13, 1.27]	0.75 [-1.57, 1.32]	0.68 [-0.71, 0.89]	**0.01 [-0.25, -0.08]**	0.10 [-0.90, 0.14]	0.19 [-0.80, 0.24]	0.88 [-0.68, 0.76]	0.88 [-1.16, 1.05]	0.33 [-0.92, 0.43]
Change to composite accuracy score (arc.proportion correct)	0.89 [-3.86, 4.14]	0.77 [-5.16, 4.44]	0.19 [-2.36, 0.87]	0.58 [-2.96, 1.99]	0.07 [-7.17, 0.48]	0.69 [-5.12, 6.74]	0.49 [-6.86, 4.14]	0.99 [-9.22, 9.30]	0.93 [-6.99, 6.55]
Age, per 10 year increase	0.30 [-0.64, 1.25]	0.36 [-1.05, 0.60]	0.67 [-1.59, 2.01]	0.21 [-1.90, 0.62]	0.33 [-0.71, 0.33]	0.97 [-0.60, 0.73]	0.71 [-0.64, 0.82]	0.12 [-0.10, 0.52]	0.92 [-0.69, 0.64]
Baseline CD4+ count, per 100 cell/uL increase	0.77 [-0.46, 0.39]	0.87 [-0.34, 0.37]	0.75 [-0.56, 0.66]	0.75 [-0.54, 0.43]	0.76 [-0.16, 0.20]	0.05 [-0.21, 0.00]	0.86 [-0.20, 0.23]	0.26 [-0.16, 0.06]	0.31 [-0.22, 0.10]
Years since HIV diagnosis, per 10 year increase	0.91 [-0.81, 0.76]	0.82 [-0.60, 0.67]	0.09 [-0.13, 0.81]	0.64 [-1.29, 0.93]	0.48 [-0.29, 0.48]	0.61 [-0.56, 0.40]	**0.03 [0.05, 0.45]**	0.56 [-0.24, 0.37]	0.56 [-0.33, 0.51]
Treatment arm of study (ITT)	0.13 [-0.27, 0.99]	0.13 [-0.81, 0.21]	0.20 [-1.58, 0.60]	0.42 [-0.96, 1.78]	0.56 [-0.43, 0.64]	0.78 [-0.60, 0.73]	0.35 [-0.77, 0.38]	0.68 [-0.37, 0.49]	0.44 [-0.69, 0.39]

[Legend Table 4.7: 95% CI=95% confidence interval; NAA=N-acetyl aspartate; Cr=creatine; Cho =choline; mi1=first myo-inositol peak; ITT=intention to treat]

CHAPTER 5

A Prospective Study to Assess the Central Nervous System Effects of a CCR5-inhibitor in HIV-1 Infected Subjects on Stable Antiretroviral Therapy; A Pharmacokinetic and Cerebral Metabolite Study

Chapter 5: A Prospective Study to Assess the Central Nervous System Effects of a CCR5-Inhibitor in HIV-1 Infected Subjects on Stable Antiretroviral Therapy ; A Pharmacokinetic and Cerebral Metabolite Study

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5.1 Introduction

Maraviroc (MVC) is a recently-licensed antiretroviral with a novel mechanism of action. It selectively blocks the CCR5 chemokine-receptors, preventing HIV-1 entry. In clinical trials to date, virological efficacy has been described when MVC is administered as part of cART in both therapy-naive HIV-infected subjects (Sierra-Madero, et al. 2010) and in subjects harbouring HIV-viral strains with mutations associated with drug-resistance (Hardy, et al. 2010).

Several factors suggest MVC may have antiviral activity within the CNS. First, due to pharmacological properties, such as a relatively low degree of plasma-protein-binding (approximately 76%)(http://www.medicines.org.uk/emc/medicine/20386/SPC/Celsentri.), MVC may theoretically cross the BBB and gain exposure in the CSF at concentrations great enough to suppress HIV-viral replication. Second, as a predominance of CCR5-tropic HIV-virus has been described within the CNS (Spudich, et al. 2005), CCR5-inhibitors such as MVC may have profound antiviral activity within this compartment. Interestingly, higher concentrations of MVC and a slower elimination phase after dosing were observed in the female genital tract (which may have similar pK properties to CSF) when compared to plasma exposure and genital tract concentrations consistently exceeded the protein-free IC_{90} of 0.57ng/mL (Dumond, et al. 2009).

Estimations of MVC CNS exposure, via CSF exposure in HIV-1 infected subjects, have been reported. Yilmaz *et al.* sampled CSF and plasma from 7 neuro-asymptomatic HIV-1 infected subjects and reported a median MVC CSF/plasma ratio of 3% (range 1-10%) (Yilmaz, et al. 2009b). Similarly, when 12 subjects without neurological symptoms, but with advanced HIV disease (median CD4+ cell count 281/uL) were studied, variable CSF/plasma ratios (0.4 – 17%) were again reported (Tiraboschi, et al.). Finally in antiretroviral-naive subjects with neurocognitive impairment, greater CSF/plasma ratios (of up to 29%) have recently been reported (Melica, et al. 2010). Crucially, in all these reported series, background cART regimens and clinical indications for undertaking LP examinations varied which may confound their findings. Also, the direct cerebral effects of MVC therapy were not evaluated.

The aim of this study was investigate the first hypothesis of thesis and estimate the cerebral effects of MVC intensification in a population of neurologically-asymptomatic HIV-1 infected subjects receiving a standardised cART regimen. Cerebral effects were assessed via MVC CSF exposure and changes to cerebral metabolite ratios.

5.2 Methods

This phase I pharmacokinetic and magnetic resonance study was conducted at Imperial College Healthcare Trust at St Mary's Hospital, London, UK between August 2009 and August 2010. Ethics approval was attained as described in Section 2.1.

5.2.1 Subject selection

Eligible subjects were neuro-asymptomatic adults with chronic HIV-1 infection, receiving TDF (245mg *q.d.*), FTC (200mg *q.d.*) and LPV/RTV (400/100mg *b.i.d.*). All had a plasma HIV RNA level below 50 copies/mL (*Bayer Quantiplex assay*^{TM}) for at least 3 months prior to study entry. Exclusion criteria included a body mass index above 32 kg/m^2, any neurological disease or dementia, active hepatitis B or C infection, current AIDS-defining illness, a history of failure or resistance to PIs and alcohol or recreational drug misuse. Concomitant medication which may cause pK interactions with the study medication were prohibited and detailed in the study protocol. No alcohol or recreational drugs were permitted during the study.

5.2.2 Study procedures

At baseline MVC 150mg *b.i.d.* was introduced. Drug intake was witnessed after a standard breakfast containing 20g fat (600 kilocalories) on days 1, 7, 14 and 15. At each visit, subjects were questioned about adverse events, tolerability and concomitant medications. Adherence was assessed using a validated questionnaire (Chesney, et al. 2000) and urine screened for recreational drug use (*Williams Medical Supplies Ltd, Gwent, UK*). Routine biochemical and haematological tests, CD4+ cell count and plasma HIV RNA were performed on day 14 (see details Section 2.5.3).

5.2.3 Maraviroc and lopinavir plasma and CSF sampling

Plasma sampling:

On days 14 and 15, 4mL of blood was sampled pre-dose *(Ctrough)* for plasma MVC and LPV concentrations. Details of sample processing are described in Section 2.5.1. On day 15, in addition, 4mL of blood was collected at 4 or 6 hours post-dose (*C4h or C6h*), sequentially in enrolled subjects.

CSF sampling:

A lumbar puncture was performed either 4 or 6 hours after intake of medication, to obtain a paired CSF/plasma sample as described in Section 2.4.4. Approximately 10mL of CSF was collected in 4 tubes for the following analysis:

Tube 1 (1mL) CSF protein, glucose and microscopy

Tube 2 (3mL) MVC concentration

Tube 3 (3mL) LPV concentration

Tube 4 (3 mL) ultrasensitive HIV RNA level (lower limit of quantification 5 copies/mL)

Following the LP procedure, 500mL of normal saline was administered to subjects intravenously over 1 hour in an attempt to reduce the incidence of post-LP headache.

5.2.4 Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

During the study screening period, cerebral MR imaging was performed (T_1- and T_2-weighted images) and studied by a neuroradiologist to ensure no contra-indications to LP were present. ^{1}H-MRS was also performed as described in Chapter 2 (Section 2.4.1) during screening and day 14. On each occasion the cerebral metabolite ratios NAA/Cr, Cho/Cr and mI/Cr were calculated in the FGM, FWM and RBG.

5.2.5 Statistical analysis

MVC and LPV plasma and CSF pharmacokinetic results

Mean (SD) of MVC and LPV concentrations were determined including pre-dose plasma (*Ctrough*) and 4h (*C4h*) or 6h (*C6h*) post-dose for both CSF and plasma. CSF/plasma concentration ratio at *C4h* or *C6h* was calculated and expressed as a percentage. As MVC is approximately 76% bound to plasma proteins (http://www.medicines.org.uk/emc/medicine/20386/SPC/Celsentri.), the CSF/unbound plasma concentration was also calculated (24% of total plasma concentration). Associations between plasma and CSF concentrations and relationship to clinical parameters were performed using linear regression modelling or Spearman's rank test.

Absolute changes to cerebral metabolite ratios between baseline and day 14 were determined for each subject and evaluated using a paired samples *t*-test. Where *p*-values below 0.2 were observed, linear regression analysis was used to evaluate associations between changes to metabolite ratios over the study period and both clinical and pK parameters. *P*-values below 0.05 were considered statistically significant. SPSS (v18.0) software (*SPSS, Chicago, IL)* was used for all statistical analysis.

5.3 Results

13 subjects were enrolled and 12 completed all study procedures. 1 subject discontinued the study on day 14 due to recreational drug misuse. Mean (SD) age was 42 (8) years, 9 (75%) were male, and 7 (58%) were of black ethnicity. All had a plasma HIV RNA below 50 copies/mL and mean (SD) CD4+ cell count was 503 (199). Subject demographics and clinical parameters are shown in Table 5.1.

5.3.1 Pharmacokinetic and LP results

Adherence was reported at 100% for all subjects. Ten (83%) subjects had a CSF HIV RNA level below 10 copies/mL. In the remaining 2 subjects CSF HIV RNA was detectable at 63 and 190 copies/mL respectively. CSF protein ranged between 0.27-0.94 g/L (mean 0.46). Mean CSF protein was 0.46 (range 0.27-0.94) g/L, CSF glucose 3.57 (range 3.1-4.1) mmol/L, CSF WCC 2.25 (range 0-10) cells/uL and CSF RCC 478 (range 0-2650) cells/uL. Gram stain was negative for organisms in all samples. CSF TB culture was also performed in one subject where an elevated WCC of 10 was found, which was negative.

MVC and LPV plasma and CSF concentrations are shown for each subject in Table 5.2. Mean (SD) MVC plasma pre-dose (*Ctrough*) was 337 (74.5) ng/mL. Plasma MVC concentration at *C4h* was 842 (174) ng/mL and at *C6h* was 485 (100) ng/mL. Mean (SD) MVC CSF concentration was 7.54 (1.26) ng/mL at *C4h* and 5.10 (1.21) ng/mL at *C6h*. The mean overall MVC CSF:plasma ratio was 1.01% [range 0.57 - 1.61] and when studied by time of sampling, mean MVC CSF:plasma ratio was 0.93% [range 0.57 - 1.27] at *C4h* and 1.09% [range 0.71 - 1.61] at *C6h*. The mean overall MVC CSF:unbound plasma ratio was 4.20% [range 2.37 - 6.70].

Mean (SD) pre-dose LPV plasma concentration *(Ctrough)* was 6088 (1215) ng/mL and post-dose 9048 (870) ng/mL at *C4h* and 9253 (1441) ng/mL at *C6h*. Mean (SD) CSF concentration was 75.1 (45.0) at *C4h* and 76.8 (30.8) ng/mL at *C6h.* Mean overall LPV CSF:plasma ratio was 0.85 % [range 0.32-1.83].

5.3.2 ^{1}H-MRS metabolite ratios

No significant abnormalities of T_1- and T_2-weighted MR images were reported. Results of baseline and day 14 ^{1}H-MRS metabolite ratios are shown in Table 5.3. No significant changes to cerebral metabolite ratios in either the FGM or FWM occurred after MVC intensification during the study period (*p*-value>0.41 all values). Changes were observed in the RBG metabolites with absolute (%) increases in NAA/Cr ratios of +0.27 (14.8%, *p*-value=0.18), Cho/Cr +0.14 (17.9%, *p-value*=0.07) and mI/Cr +0.24 (34.8%, *p-value*=0.17).

5.3.3 Association between pharmacokinetic results, metabolite ratios and clinical parameters

Pharmacokinetic results and clinical parameters

No significant associations between plasma or CSF MVC parameters and patient demographics or clinical parameters (including CSF protein, RCC or WCC) were observed in this neuro-asymptomatic HIV-1 infected population (p-value>0.1 all observations, see Table 5.4). A trend towards higher plasma LPV concentration at C4-6h with younger age was observed (*p*=0.05 [95% CI -165, 0]. In addition, associations between higher CSF protein level and higher CSF LPV concentration (*p*=0.01, 95% CI 30,205) and also higher CSF/plasma ratios (p=0.02, 95%CI 0,3] were observed. No other associations between patient demographics and plasma or CSF LPV parameters were found.

Plasma and CSF correlation

A strong correlation between MVC CSF concentration and plasma *Ctrough* (*p*=0.009, *r*=0.71) and plasma C4-6h (*p*=0.007, *r*=0.73) was observed, see Figure 5.1. There was no relationship between LPV plasma and CSF exposure (p>0.62).

5.3.4 Correlation between pharmacokinetic results and changes to metabolite ratios in basal ganglia

Changes observed in the RBG were examined for correlation with MVC pK parameters as this was the only active study intervention. A significant correlation between higher MVC plasma *Ctrough* and increased NAA/Cr was observed (p=0.047, r=0.61, see Figure 5.2). No correlation with MVC CSF concentration, or any other metabolite ratios and MVC pK parameters was observed.

5.4 Discussion

In this study, which enrolled HIV-1 infected subjects on a standardised and stable antiretroviral regimen, a mean MVC CSF:plasma ratio of 1.01% was observed, together with changes in neuronal (NAA/Cr) cerebral metabolite ratios (indicating neuronal recovery) which are associated with MVC plasma exposure.

This is the first study to describe a cerebral effect of MVC, observed by measuring MR-visible cerebral metabolite ratios, and a relationship of this effect to MVC exposure. I made several interesting observations. First, unlike other cohorts, I observed MVC CSF concentrations greater than 5-fold the median protein-free IC_{90} (0.57ng/mL) in all subjects. Interestingly, however, CSF:plasma ratios [range 0.57-1.61%] were lower and less variable than previously described where CSF:plasma

ratios have ranged between 1-10 and 0.4-17% (Tiraboschi, et al. 2010). High CSF MVC concentrations have previously been described in individuals with neurological impairment and associated CSF pleocytosis (Melica, et al. 2010). This higher exposure may be related to BBB disruption from cerebral inflammation which can enhance drug-delivery to the CSF (Roberts, et al. 2009). It is therefore likely the lower MVC CSF:plasma ratios observed in this study, are due to the strict inclusion criteria requiring an absence of neurological symptoms and disease, supporting the BBB integrity hypothesis. Furthermore, the strict eligibility criteria, including standardised cART at study entry and undetectable plasma HIV RNA, may also have contributed to the lower observed CSF/plasma ratios than reported in other cohorts for the above reasons.

A strong correlation was observed between MVC CSF and plasma concentrations which has not previously been described (Melica, et al. 2010; Tiraboschi, et al. 2010; Yilmaz, et al. 2009b). Such associations may only be recognised within a formal study designed like this, where a lack of variability in clinical parameters such as BMI, cART regimen or concomitant medications, and standardised sampling times following fed-state dosing, allows such observations to become apparent.

Of interest, in this chapter, changes in the metabolite ratios of the RBG were demonstrated after only 14 days of MVC intensification. Changes were not, however, observed in the frontal white or grey matter of the cerebral cortex. I postulate these changes are related to the introduction of MVC for several reasons. First, a statistically significant association between increases in RBG NAA/Cr ratio and MVC plasma *Ctrough* concentration was observed, suggesting a direct relationship. Second, I observed metabolite changes in the RBG, but in no other cerebral location. The basal ganglia has a higher blood flow per unit volume (Kim, et al. 2008), compared to other cerebral locations, suggesting greater and earlier exposure of this part of the brain, which may explain why changes were observed here in this short study, but had not yet evolved in other cerebral locations. Lastly, very small absolute changes to metabolite ratios were observed in the frontal anatomical locations, which provide assuring data that the changes observed are not due to high intra-patient variability when undergoing *in vivo* cerebral ^{1}H-MRS on two occasions. Indeed, the intra-patient variability between scanning in the frontal anatomical voxels in our study, is within the lower variability range from previous published series, assessing such variability of sequential ^{1}H-MRS (Brooks, et al. 1999).

Two subjects had detectable CSF HIV RNA, despite plasma HIV RNA < 50 copies/mL at study entry. In one subject (subject 6), low level CSF HIV viraemia of 63 copies/mL was detected and in one subject (subject 9) CSF viraemia of 190 copies/mL was detected. MVC CSF concentration and CSF/plasma

ratio in subject 9 were below the study mean and not elevated as may be expected in neuro-symptomatic subjects (Melica, et al. 2010). Furthermore, the absolute change in the RBG NAA/Cr ratio during the study period in subject 9 was low (0.02) and below the cohort mean (0.27), making it unlikely that this subject could have influenced, or in any way driven, the findings of this study.

NAA is a marker of neuronal integrity and reductions in NAA/Cr are reported in advanced HIV-disease stages, including AIDS-dementia complex and severe neurocognitive impairment (Meyerhoff, et al. 1999; Paul, et al. 2007). Increases in NAA/Cr following initiation of cART have previously been described in antiretroviral-naive individuals (Winston, et al. 2010), but over much longer treatment programmes. Increases in mI/Cr and Cho/Cr ratios also occurred in the RBG. Such metabolites are osmolyte markers of glial cell metabolism and alter in the presence of neuroinflammation. Increases have previously been observed in AIDS dementia complex (Lee, et al. 2003), but changes occurring within an asymptomatic cohort and over such a short period of study are unlikely to represent disease progression. Osmosensitive glial markers, such as mI, play a crucial role in cell volume regulation(Haussinger, et al. 2000). Organic osmolytes such as mI are also rapidly released into the extracellular space in response to cell swelling via osmoregulated membrane channels (Burg 1995; Lang, et al. 1998). It is possible that the increase in mI/Cr ratio that I have observed may represent an initial immune response to the short-course of MVC.

Sampling CSF for LPV concentrations has in the past revealed inconsistent findings with undetectable CSF LPV concentrations being described in some adherent subjects, even in the cART era, although lower limits of LPV quantification have been variable (Capparelli, et al. 2005a; DiCenzo, et al. 2009; Lafeuillade, et al. 2002; Solas, et al. 2003). In this study, LPV was detected in CSF samples from all subjects and observed CSF/plasma ratios were higher than previously described. This consistent detection of CSF LPV may therefore represent enhanced sensitivity of this assay or strict inclusion criteria of our study. Alternatively, it may be secondary to a pharmacokinetic interaction when LPV and MVC are co-administered. Although MVC has no known effect on the plasma pharmacokinetic profile of LPV (Abel, et al. 2008), pharmacokinetic effects could be observed within the CSF compartment and as no previous study has assessed LPV CSF exposure in subjects also receiving MVC, such an interaction is plausible. Interestingly an association between increased CSF protein levels and CSF LPV concentration was observed, suggesting enhanced delivery of LPV to the CSF can occur via CNS-trapping of protein. BBB integrity was unfortunately not analysed in this study (via the use of plasma:CSF albumin ratios) and therefore further conclusions cannot be drawn.

In summary, this study supports the first hypothesis of this thesis and demonstrates that intensification with MVC, an antiretroviral agent with good CNS penetration, has a positive effect on cerebral function parameters (RBG cerebral metabolites) in neuro-asymptomatic HIV-1 infected subjects.

Table 5.1: Patient demographics and clinical parameters at study entry

Demographic or clinical parameter	**Mean(SD) unless otherwise stated**
Number of subjects	12
Age	42 (7.9)
Male gender, n (%)	9 (75)
Ethnicity, n (%)	
White	4 (33)
Black	7 (58)
Other (Brazil)	1 (8)
Years since HIV diagnosis	11 (4.6)
Past AIDS defining illness, n (%)	6 (50)
Baseline CD4 cell count (cells/uL)	503 (199)
Baseline HIV RNA level <50 copies/mL, n (%)	12 (100)
BMI	28 (3.5)
Current smoker, n (%)	6 (50)

[Table 5.1 legend: SD = standard deviation; BMI =body mass index]

Table 5.2a: Maraviroc pharmacokinetic and virological results of plasma and CSF samples by individual subject and overall

Subject	Time of CSF sample (hr post dose)	Plasma HIV RNA (copies/mL)	CSF HIV RNA (copies/mL)	MVC plasma *Ctrough* (ng/mL)	MVC plasma *C4 or 6h* (ng/mL)	MVC CSF *C4 or 6h* (ng/mL)	MVC CSF:plasma ratio (%)	MVC CSF:unbound plasma ratio (%)
1	4	<50	<10	474	995	8.04	0.81	3.37
2	6	<50	<10	272	304	4.90	1.61	6.70
3	6	<50	<10	328	534	6.04	1.13	4.71
4	4	<50	<10	377	898	5.11	0.57	2.37
5	4	<50	<10	452	1036	8.67	0.84	3.49
6	4	<50	63	369	696	8.06	1.16	4.82
7	6	<50	<10	224	607	6.11	1.01	4.19
8	4	<50	<10	341	582	7.41	1.27	5.30
9	6	<50	190	264	481	3.41	0.71	2.95
10	4	<50	<10	347	848	7.98	0.94	3.92
11	6	<50	<10	277	497	3.97	0.80	3.32
12	6	<50	<10	324	487	6.19	1.27	5.30
Mean				**337.49**	**663.79**	**6.32**	**1.01**	**4.20**
SD				74.55	230.74	1.73	0.29	1.22
CV(%)				22.09	34.76	27.37	28.92	28.92

[Legend Table 5.2a: CSF = cerebrospinal fluid; MVC =maraviroc; Ctrough=trough concentration immediately pre-dose; C4 or 6h = concentration 4 or 6 hours post-dosing; SD = standard deviation; CV=coefficient of variation]

Table 5.2b: Lopinavir pharmacokinetic and virological results of plasma and CSF in all subjects

Subject	Time of CSF sample (hr post dose)	Plasma HIV RNA (copies/mL)	CSF HIV RNA (copies/mL)	LPV plasma Ctrough concentration ng/mL	LPV plasma C4 or C6h concentration ng/mL	LPV CSF C4-6h concentration ng/mL	LPV CSF/ plasma ratio *100 (%)	LPV CSF/ unbound plasma ratio *100 (%)
1	4	≤50	<10	3332.40	8407.80	59.40	0.71	70.65
2	6	≤50	<10	5532.40	6448.30	117.81	1.83	182.70
3	6	≤50	<10	7762.20	9889.60	100.37	1.01	101.49
4	4	≤50	<10	6508.00	9532.00	72.74	0.76	76.31
5	4	≤50	<10	5659.00	9284.10	164.47	1.77	177.15
6	4	≤50	63	6393.10	8422.20	40.50	0.48	48.09
7	6	≤50	<10	6016.90	9695.30	61.99	0.64	63.94
8	4	≤50	<10	7113.40	8191.90	54.88	0.67	66.99
9	6	≤50	190	5042.00	9055.60	29.05	0.32	32.08
10	4	≤50	<10	7609.60	10450.90	58.77	0.56	56.24
11	6	≤50	<10	6603.70	10327.60	73.83	0.71	71.49
12	6	≤50	<10	5486.10	10104.30	77.78	0.77	76.98
Mean	**5**	**≤50**		**6088.23**	**9150.80**	**75.97**	**0.85**	**85.34**
SD	**1**	**0**		**1215.53**	**1140.36**	**36.76**	**0.47**	**47.29**
CV(%)	**20**	**0**		**19.97**	**12.46**	**48.39**	**12.46**	**12.46**

[Legend Table 5.2b: CSF = cerebrospinal fluid; LPV=lopinavir; Ctrough=trough concentration immediately pre-dose; C4 or 6h = concentration 4 or 6 hours post-dosing; SD = standard deviation; CV=coefficient of variation]

Table 5.3: Results of cerebral proton spectroscopy at study entry and day 14

Parameter	**Frontal grey matter**			**Frontal white matter**			**Right basal ganglia**		
Mean (SD)	**NAA/Cr**	**Cho/Cr**	**mI/Cr**	**NAA/Cr**	**Cho/Cr**	**mI/Cr**	**NAA/Cr**	**Cho/Cr**	**mI/Cr**
Day 0	1.75 [0.19]	0.66 [0.10]	0.64 [0.33]	1.76 [0.20]	1.05 [0.22]	1.09 [0.65]	1.82 [0.39]	0.78 [0.12]	0.69 [0.50]
Day 14	1.72 [0.20]	0.67 [0.08]	0.65 [0.47]	1.77 [0.19]	1.05 [0.18]	1.25 [0.55]	2.09 [0.31]	0.92 [0.24]	0.93 [0.48]
Change over study period	-0.02 [0.21]	0.02 [0.14]	0.01 [0.63]	0.00 [0.19]	0.00 [0.23]	0.17 [0.65]	0.27 [0.61]	0.14 [0.23]	0.24 [0.60]
***p*-valueof change to day 14 * [95%CI]**	0.74 [-0.13, 0.17]	0.73 [-0.12, 0.09]	0.96 [-0.46, 0.44]	0.99 [-0.13, 0.12]	0.98 [-0.16, 0.16]	0.41 [-0.60, 0.26]	0.18 [-0.68, 0.14]	0.07 [-0.29, 0.01]	0.17 [-0.67, 0.13]

* using paired samples t-test

[Legend Table 5.3: SD=standard deviation; 95% CI=95% confidence interval; NAA=N-acetyl aspartate; Cr=creatine; Cho =choline; mI=myo-inositol]

Table 5.4: Association between pharmacokinetic results and clinical parameters/demographic using univariate linear regression analysiss

	Plasma *Ctrough* (D14) *p*-value [95% CI]	Plasma C4 or 6h *p*-value [95% CI]	CSF C4 or 6h *p*-value [95% CI]	CSF/plasma ratio (%) *p*-value [95% CI]
MVC sampling				
Age, per year increase	0.86 [-6, 5]	0.88 [-22, 19]	0.89 [0, 0]	0.57 [0,0]
Male gender	0.15 [-143, 26]	0.44 [-474, 223]	0.08 [-4, 0]	0.65 [-1, 0]
BMI, per unit increase	0.93 [-12, 13]	0.73 [-39, 54]	0.78 [0, 0]	0.92 [0, 0]
Smoker	0.15 [-125, 21]	0.22 [-457, 118]	0.18 [-4, 1]	0.62 [0,0]
White ethnicity	0.09 [-12, 137]	0.23 [-130, 482]	0.07 [0, 4]	0.80 [0, 0]
Plasma CD4 cell count, per 100 cell/uL increase	0.77 [-0, 0]	0.77 [-0, 0]	0.72 [-1, 1]	0.37 [-3, 7]
CSF protein	-	-	0.19 [-2, 9]	0.59 [-1, 1]
CSF WCC, per 10 cell increase	-	-	0.13 [-7,1]	0.10 [-1, 0]
CSF RCC, per 1000 cell increase	-	-	0.82 [-1, 1]	0.21 [0, 0]
LPV sampling				
Age, per year increase	0.14 [-207, 35]	**0.05 [-165, 0]**	0.23 [-1, 5]	0.10 [0, 0]
Male gender	0.73 [-1974, 2737]	0.17 [-525, 2685]	0.20 [-20, 85]	0.35 [0, 1]
BMI, per unit increase	0.16 [-89, 467]	0.80 [-258, 203]	0.64 [-6, 9]	0.81 [0, 0]
Smoker	0.99 [-2068, 2038]	0.43 [-2038, 940]	0.41 [-66, 29]	0.72 [-1, 1]
White ethnicity	0.49 [-2811, 1435]	0.92 [-1560, 1703]	0.55 [-66, 37]	0.42 [-1, 0]
Plasma CD4 cell count, per 100 cell/uL increase	0.42 [-324, 719]	0.80 [-450, 356]	0.94 [-13, 13]	0.96 [0, 0]
CSF protein	-	-	**0.01 [30, 205]**	**0.02 [0, 3]**
CSF WCC, per 10 cell increase	-	-	0.57 [-120, 69]	0.57 [-2, 1]
CSF RCC, per 1000 cell increase	-	-	0.40 [-15, 34]	0.53 [0,0]

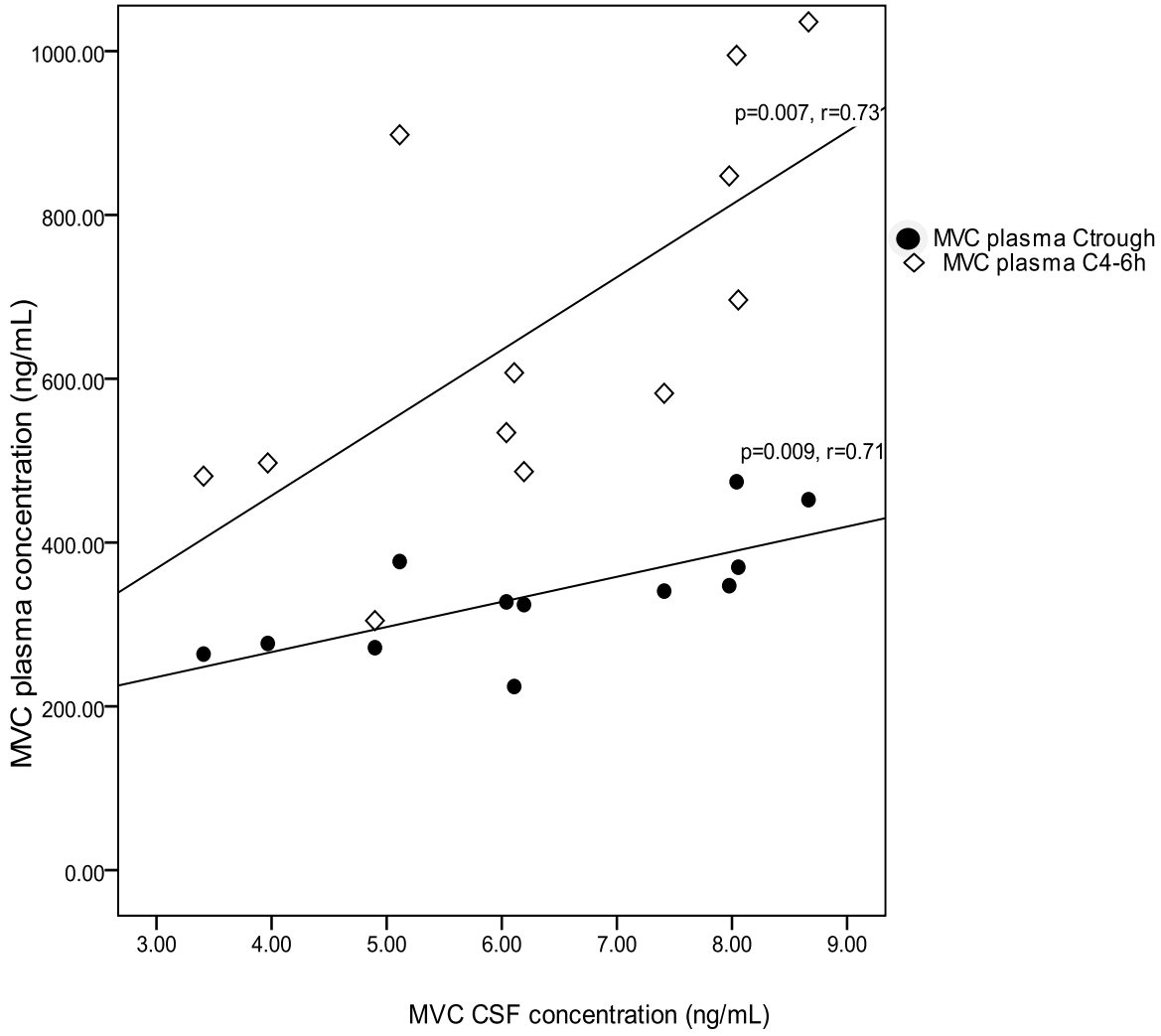
[Legend Table 5.4: CSF = cerebrospinal fluid; MVC = maraviroc; LPV=lopinavir; Ctrough=trough concentration immediately pre-dose; C4 or 6h = concentration 4 or 6 hours post-dosing; WCC= white cell count; RCC=red cell count]

Table 5.5: Correlation between observed changes to RBG cerebral metabolite ratios and MVC pharmacokinetic parameters

Cerebral metabolite ratio	RBG NAA/Cr	RBG Cho/Cr	RBG mI/Cr
Plasma C*trough* (ng/mL)	**p= 0.047, r = 0.61**	p=0.92, r=-0.04	p=0.69, r=0.14
Plasma *C4 or 6h* (ng/mL)	p=0.34, r = 0.318	p=0.24, r=-0.40	p=0.50, r=0.23
CSF *C4 or 6h* (ng/mL)	p=0.16, r =0.455	p=0.63, r=-0.16	p=0.63, r=0.16

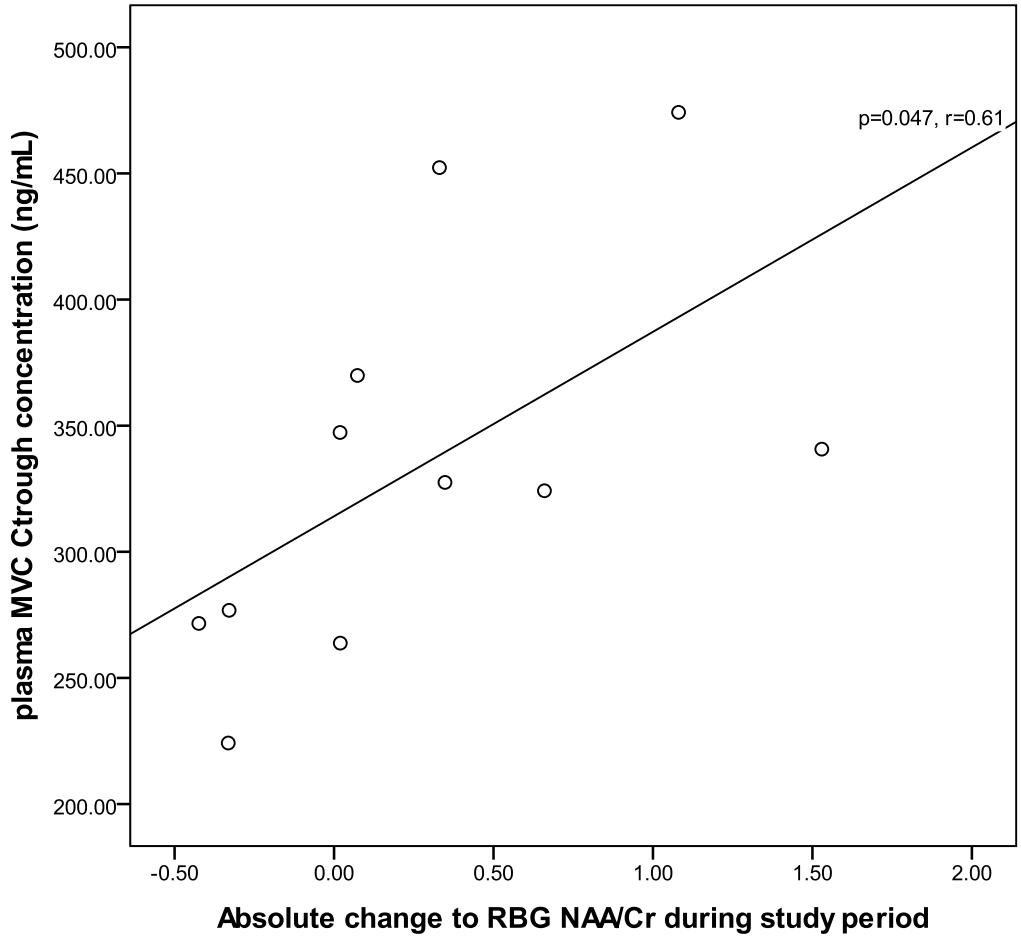
[Legend Table 5.5: RBG=right basal ganglia; NAA=N-acetyl aspartate; Cr=creatine; Cho =choline; mI=myo-inositol; Ctrough=trough concentration immediately pre-dose; C4 or 6h = concentration 4 or 6 hours post-dosing]

Figure 5.1: Correlation between maraviroc CSF C4-6h exposure and both plasma Ctrough(●) and plasma C4-6h (◇) exposure



[Legend Figure 5.1 MVC=Maraviroc; CSF=cerebrospinal fluid; Ctrough=trough concentration immediately pre-dose; C4 or 6h = concentration 4 or 6 hours post-dosing]

Figure 5.2: Correlation between absolute change to RBG NAA/Cr and maraviroc plasma Ctrough concentration



[Legend Figure 5.2 MVC=Maraviroc; Ctrough=trough concentration immediately pre-dose; RBG=right basal ganglia; NAA/Cr=N-acetyl aspartate/creatine ratio]

CHAPTER 6

A Cross-sectional Study to Compare Cognitive Performance in Subjects with Chronic HIV-1 and Acute HCV Coinfection and Subjects with Chronic HIV-1 Infection

Chapter 6: A Cross-Sectional Study to Compare Cognitive Performance in Subjects with Chronic HIV-1 and Acute HCV Coinfection and Subjects with Chronic HIV-1 Infection

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6.3 Results

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6.3.3 Association of assessment scores and study group

6.4 Discussion

6.1 Introduction

HIV-1 and HCV coinfection is an important cause of morbidity and mortality in the current day and approximately 10-20% of subjects in Europe living with chronic HIV-1 infection are co-infected with chronic HCV [1]. In addition, in the past 7 years, an epidemic of sexually-transmitted acute HCV infection has been identified in HIV-1 infected homosexual men. Individuals with HIV-1 and chronic HCV coinfection remain at risk of accelerated liver disease from HCV and higher rates of cART-associated hepatotoxicity[2].

Neurocognitive disturbance is widely reported in chronic HIV-1 infection with rates of HAND approaching 50% in some cohorts despite the availability of effective cART (Dore, et al. 1999; Larussa, et al. 2006; Tozzi, et al. 2007). Features of this condition can include slowed motor function and concentration difficulties (Tozzi, et al. 2007). A similar cognitive disturbance in chronic HCV infection has also been described in subjects without evidence of significant liver injury and characteristics include depression, anxiety, fatigue and apathy (Forton, et al. 2001). Individuals with impaired immune function secondary to chronic HIV infection may be more susceptible to the cerebral effects of HCV infection. Whether CNS disturbance is associated with the acute phase of HCV acquisition in subjects with chronic HIV-1 infection is not yet known.

In order to address the second hypothesis of thesis, the aim of this study was to compare the rates and features of neurocognitive performance in individuals with HIV-1 monoinfection, individuals with HIV-1 and *chronic* HCV coinfection or HIV-1 and *acute* HCV coinfection.

6.2 Methods

This study took place at the HIV Outpatient department, St Mary's Hospital, Imperial College Healthcare NHS Trust, London between 2008 and 2009. Ethical approval was attained as described in Section 2.1.

6.2.1 Subject Selection

Subjects were recruited during routine HIV Outpatient attendances. They were eligible to participate if aged over 18 years, had chronic HIV-1 infection and were proficient in English. Exclusion criteria included current AIDS defining illness, any active neurological disease, dementia, untreated syphilis, chronic hepatitis B infection, current receipt of interferon and/or ribavarin treatment, hepatic synthetic functional impairment (a serum albumin below 30 g/dL), use of recreational drugs within

the past month and alcohol abuse. Subjects were also required to meet the specific eligibility criteria for one of the 3 study groups (see below).

Group 1: HIV-1 monoinfection [control subjects]

Subjects were required to be receiving stable cART (containing a minimum of 3 drugs) with an undetectable plasma HIV RNA level for a minimum of 3 months. Additional exclusion criteria included HCV infection.

Group 2: HIV-1 and chronic HCV coinfection

Subjects were required to have evidence of chronic HCV as defined by HCV antibody positivity for a minimum of 12 months and detectable plasma HCV RNA on most recent testing.

Group 3: HIV-1 and acute HCV coinfection

Subjects were required to have acute HCV as defined by a new positive HCV RNA test within 12 months of a negative HCV RNA test.

6.2.2 Study procedures

All participants provided informed consent. Demographic information was collected from medical records including age, sex, nadir CD4 count, time-elapsed since HIV-1 diagnosis (years), current plasma CD4 count and HIV RNA level and current cART. For subjects with HCV, additional information regarding time-elapsed since HCV diagnosis, current and peak ALT level, current HCV RNA level and HCV genotype was also collected. Current cART was scored for CNS penetration, using the CPE Score (Letendre, et al. 2008).

6.2.3. Computerised Cognitive Assessment

Subjects then completed the Cogstate^{TM} assessment as described in Section 2.4.3 on a single occasion during an attendance at the HIV Outpatient department.

6.2.4 Definition of Neurocognitive Impairment (NCI)

In this study 6 cognitive assessment scores were compared to age-stratified population data provided by the manufacturer (n=879 healthy adults). Participants' scores were then ranked according to the distance (in SD) from the mean of the general population. NCI was diagnosed when scores in at least 2 cognitive tasks fell more than 1SD below the normal population mean.

6.2.5 Statistical analysis

The mean (SD) scores for each computerised task and the rates of NCI were calculated for each study group. Group 1 [control subjects] results were further examined for association between cognitive task scores, diagnosis of NCI and clinical parameters or patient demographics using linear regression analysis. Association between cognitive assessment scores and antiretroviral CPE score was also evaluated.

Cognitive assessment scores in Groups 2 and 3 were then compared individually to Group 1 [control subjects]. Where significant differences in neurocognitive task scores were found, linear regression analysis was performed to investigate the presence of association between task score and clinical or demographic parameters. SPSS version 18.0 was used for analysis. Any association with a significance of $p<0.1$ was taken forward to multivariate analysis. *p*-values of <0.05 were considered statistically significant.

6.3 Results

96 subjects participated in the study. Forty-five (47%) in Group 1, 27 (28%) in Group 2 and 24 (25%) in Group 3. Patient demographics are shown in Table 6.1. Overall, subjects with acute HCV coinfection (Group 3) were younger, exclusively male, had higher current and nadir plasma CD4+ cell counts and had a shorter time-elapsed since HIV-1 diagnosis than subjects from Groups 1 or 2.

6.3.1 Cognitive assessment scores and rates of neurocognitive impairment

Mean cognitive task scores are shown in Table 6.2. When compared to age-matched population data, the proportion of subjects meeting the criteria for NCI was 24% in Group 1, 11% in Group 2 and 25% in Group 3 (Figure 6.1). When compared individually to Group 1, no significant differences between the frequency of NCI in either Group 2 ($p=0.26$, 95% CI -0.31, 0.08) or Group 3 ($p=0.79$, 95% CI -0.17, 0.23) when compared to Group 1.

When individual cognitive task scores were examined by study group however, subjects from Group 3 (HIV-1 and acute HCV coinfection) had significantly worse executive function performance than control subjects in Group 1 ($p=0.02$, 95% CI 1.43, 13.40, see Table 6.3). They also had significantly faster composite speed scores ($p=0.04$, 95% CI -0.31, 0.01) than subjects from Group 1. No differences in individual task performance were observed between subjects from Group 2 and Group 1 (*p*-value>0.21 all observations).

In the multivariate analysis, worse executive function performance was significantly associated with lower nadir CD4+ cell count (*p*=0.001 95% CI-5, -1 per 100 cell/uL increase) and acute HCV study group (*p*<0.001 95% CI 7,20 see Table 6.4).

6.3.2 Association of assessment scores and clinical parameters in Group 1 (HIV-1 infected control subjects)

Eleven (24%) participants in Group 1 fulfilled the definition of NCI when results were compared to population data. The presence of NCI was significantly associated with younger age of participant (p=0.04, 95%CI -0.19, -0.01, per 10-year increase) in this control group. NCI was present in 54, 27, 36 and 9% of subjects in ascending inter-quartile age groups (p-value for trend=0.22 see Figure 6.2). No significant associations were observed between the presence of NCI in this group and current or nadir CD4 count, time-elapsed since HIV-1 diagnosis, CPE score or type of cART (*p*-value>0.22 for all observations, see Table 6.5).

These analyses also revealed that in the control group (HIV-1 infected subjects with undetectable plasma HIV RNA), a lower nadir CD4+cell count was significantly associated with worse executive function performance (*p*=0.01, 95% CI -4.33, -0.57, per 100 cell/uL increase). Individuals receiving PI-based cART were also noted to have a significantly worse overall speed (p=0.01, 95% CI -0.37, -0.07) and overall accuracy (p=0.03, 95%CI 0.14, 0.55) of performance. No significant association between cognitive scores and CPE score, current CD4+ cell count or gender was observed (p>0.05 all values, see Table 6.6).

6.4 Discussion

This study attempted to investigate the frequency and nature of cognitive deficits in three HIV-1 infected clinic populations in order to examine the second hypothesis of this thesis. For this reason, HIV-1 infected subjects, who were neurologically asymptomatic and without other reasons to have cognitive problems were selected as a control group. In addition, all control subjects (Group1) were required to be virologically suppressed in plasma and receiving stable cART in order to minimise any negative effect of HIV-1 replication on cognitive function. A second group was then examined in order to distinguish the cerebral effects of chronic HCV from those of acute HCV in HIV-1 infected subjects. The results for each coinfected group were compared to the monoinfected asymptomatic control group separately to assess effect. Examination of the HIV-1 monoinfected control group importantly also gave a secondary opportunity to address the hypothesis that the use of antiretrovirals with greater CNS penetration results in improved cerebral functioning.

Several interesting and novel observations were made. Firstly, a high proportion (24%) of HIV-1 infected adults (without HCV), with undetectable plasma HIV RNA on stable cART, met the definition for NCI when examined using this detailed, computerised assessment. This study was, to our knowledge, the first to establish the frequency of this neurological deficit in a UK cohort. Our findings are consistent with the recently reported rates of asymptomatic NCI within both Swiss and Thai HIV-infected cohorts (Pumpradit, et al. 2010; Simioni, et al. 2010).

The clinical significance of this asymptomatic neurological deficit has not yet been fully elucidated. Previous studies suggest the clinical progress will be variable, with some individuals exhibiting progressive cognitive decline, while others remain stable or have improved scores on longitudinal cognitive assessments (Antinori, et al. 2007; Tozzi, et al. 2007). In the absence of established co-pathologies, such as concurrent neurological disease or recreational drug misuse, the factors which cause ongoing neurocognitive decline in such individuals, despite adherence to cART remain unclear. Although no association was observed between NCI and type of cART or CPE score in this study, an association *was* observed between PI-based cART and worse simple reaction speed, visual learning and memory, overall speed and overall accuracy. This suggests that an association may exist between antiretroviral therapy and neurocognitive performance, but which is more complex than simply assigning and summing drug scores using the CPE score. No association between type of cART and patient demographics or clinical parameters was observed in this cohort and therefore we are unable to explain any obvious prescribing bias causing a cART-drug class effect. It is possible, however, that other biological or treatment factors, not assessed within this study, will explain the neurocognitive dysfunction we observed in subjects receiving PI-based cART. An alternative explanation for progressive cognitive decline, despite virological suppression in plasma, may be that antiretrovirals themselves can cause direct neurotoxic damage to cerebrovascular endothelium and disruption of the blood-brain-barrier permeability in some individuals. Associations between neurotoxicity and drugs with potential to cause mitochondrial damage have previously been reported. As the entire control group were receiving cART at the time of assessment, this factor was unable to be addressed within this study.

Interestingly, NCI was associated with younger age in Group 1. All subjects in this group had acquired HIV-1 through horizontal transmission. This finding contrast with the general population, where cognitive function generally declines with increasing age (Drag and Bieliauskas 2010). Possible reasons for the differences observed in this cohort may include that the younger, less mature brain is more susceptible to the direct neurotoxic effects of HIV-1 than in older patients, a theory supported by the observation of more frequent and fulminant CNS inflammation and disease in HIV-

infected children than adults (Sharer and Cho 1989). Alternatively these age-related differences observed may reflect differing socio-economic and education levels, or historical recreational drug misuse between our cohort and normative controls (for whom detailed demographic data is not available).

A low nadir CD4 count was associated with worse divided attention and executive function in Group 1. An association between nadir CD4+ cell count and symptomatic HIV-1 associated NCI which can persist (despite immune restoration with cART) has previously been reported (Munoz-Moreno, et al. 2008) and suggests the condition is, at least partly, irreversible. This association is of clinical relevance in our cohort, as late presentation of advanced HIV-1 disease remains common, even in resource-rich settings. Clinicians must be aware that such individuals with very low nadir CD4+ cell counts, maybe at increased risk of developing cognitive disturbance and monitor for symptoms closely, irrespective of current CD4+ cell count.

Acute HCV coinfection was significantly associated with worse executive functioning in this study, even after adjustment for other clinical parameters. While this form of cognitive deficit has previously been reported in individuals with chronic HCV and HIV-1 coinfection (Morgello, et al. 2005; Ryan, et al. 2004) and individuals with HIV-1 associated NCI (Heaton, et al. 2004; Morgello, et al. 2005; Ryan, et al. 2004), to my knowledge this has not previously been associated with the acute phase of HCV. The acute phase of HCV has rarely been identified prior to this current epidemic and the clinical significance of this finding requires further elucidation. It suggests that in some individuals, HCV may enter the CNS during the acute phase, causing a degree of neurological disturbance involving the prefrontal cortex and fronto-striatal regions in a similar manner to HIV-1 infection (Melrose, et al. 2008; Ridderinkhof, et al. 2004). This disturbance may result in a subject being less able to plan, sequence, initiate, and sustain behaviour, incorporating feedback and making appropriate adjustments.

If HCV does migrate to the CNS during the acute phase, there may be important clinical consequences. Currently, treatment for HCV consists of pegylated-interferon and ribavirin. It has been suggested that the very low efficacy of using interferon as monotherapy, may be due to its inability to cross the blood brain barrier – a limitation at least partly overcome by combination therapy with the non-protein bound nucleoside analogue, ribavirin (Thomas, et al. 1999). Furthermore, treatment success rates appear greater when therapy is commenced soon after acquiring HCV, rather than when the infection has become established (Brook, et al. 2010; Dominguez, et al. 2006). The reasons for such differences in treatment outcomes are not yet known,

but it may be hypothesised that a shorter duration of time for HCV to migrate and establish extrahepatic (including CNS) sanctuary sites with potential for treatment escape, may be a relevant factor. Further work in this area is required.

This study has several limitations. Firstly, a computerised neurocognitive test was used to assess subjects and define NCI. Computerised assessments have inferior sensitivity for detecting HIV-associated NCI when compared to formal neuropsychometric testing, raising the possibility for misclassification of subjects (Maruff, et al. 2009). Of relevance, the population examined in this study had higher mean CD4+ lymphocyte counts than subjects participating in the validation study of this tool for the diagnosis of HIV-associated NCI. A second limitation is that the HIV-1 disease stage varied between Groups 1 to 3, with individuals from Group 3 having higher mean CD4+ cell counts, a shorter time-elapsed since HIV-1 diagnosis and a lower frequency receiving cART. It is also acknowledged that the presence of multiple associations were investigated in this work without adjusting for multiple comparisons via the use of the Bonferroni correction (Perneger 1998). In this way, there remains the possibility of Type 1 error within our findings, however use of this tool, particularly in a small cross-sectional study, may make the significance of results difficult to interpret (as each result would vary according to the number of tests performed) and may increase the risk of Type 2 error throughout.

Finally, an association between recreational drug use and acquisition of acute HCV has been described and also that drug use (parenteral or non-parenteral) may result in cerebral function disturbance (Christensen, et al. 1996). While this study attempted to address this potential confounding factor by excluding subjects with any recent (within 1 month) drug misuse, it is possible that higher rates of historical drug misuse with resultant cerebral damage were observed in group 3 and may account for our observations. Without more detailed recreational drug histories from subjects however, this remains a potential confounder. Reassuringly, however, 16/27 (59%) of subjects in Group 2 had a history of injecting drug use and no observed differences in executive function or other cognitive tasks were observed in this group. In summary therefore this work provides some supportive evidence for the hypothesis that acquisition of acute HCV in HIV-1 infected subjects is associated with a deterioration of cerebral function parameters (executive functioning). In addition, deterioration of cerebral function parameters was observed in individuals receiving PI-based cART which may be due to worse CNS penetration of this drug class.

Table 6.1: Demographic factors and clinical parameters of subjects

	Group 1	**Group 2**	***Group 3***
Number, n	45	27	24
Male gender, n(%)	38 (84)	22 (88)	24 (100%)
Current CD4+, cells/µL	546 (271)	562 (290)	613 (189)
Current plasma HIV RNA below 50 copies/mL, n (%)	45 (100)	25 (93)	16 (67)
Mean current plasma HIV RNA level of remaining subjects	-	3211	17099
Nadir CD4+, cells/µL	180 (129)	214 (166)	315 (181)
Time-elapsed since HIV diagnosis (years)	12 (6.0)	14 (5.1)	7.9 (5.8)
Age (years)	48 (11)	46 (8)	40 (7.6)
Receiving antiretroviral therapy, n (%)	45 (100)	25 (93)	17 (71%)
NNRTI	20 (44)	11 (44)	11 (65)
PI	25 (56)	14 (56)	5 (29)
Quadruple nucleoside analogue	0	-	1 (6)
CPE Score, 2008 version	1.76 (0.68)	1.52 (0.47)	1.58 (0.59)
Time elapsed since negative HCV RNA (weeks)	-	-	26.42 (10.47)
Current ALT, IU		109 (66)	213 (210)
Peak ALT, IU	-	-	574 (581)
HCV genotype, n (%)			
1	-	9 (33)	19 (79)
2	-	1 (4)	0
3	-	2 (7)	1 (4)
4	-	15 (56)	2 (8)
Not known	-	0	2 (8)
Most recent HCV PCR, copies/mL	-	2 926 102	3 849 936

[Legend Table 6.1: NNRTI = non-nucleoside reverse transcriptase inhibitor; PI=HIV-1 protease inhibitor; CPE score = Cerebrospinal fluid Penetration Effectiveness Rank Score; ALT =alanine aminotransferase; HCV=hepatitis C virus]

Table 6.2: Results of computerised cognitive assessment by study group

Cognitive task	**Simple reaction speed**	**Identification speed**	**Divided attention speed**	**Complex reaction speed**	**Associate, non visual learning**	**Visual learning and memory**	**Working memory**	**Executive function**	**Composite speed score**	**Composite accuracy score**
Unit of measure	*log_{10}ms*	*log_{10}ms*	*log_{10}ms*	*log_{10}ms*	*arcsine proportion correct*	*arcsine proportion correct*	*arcsine proportion correct*	*error rate*	*log_{10}ms*	*arcsine proportion correct*
Group 1	2.53 (0.12)	2.71 (0.08)	2.64 (0.12)	2.85 (0.87)	0.84 (0.17)	0.77 (0.16)	1.27 (0.23)	19.09 (8.12)	10.73 (0.33)	2.88 (0.42)
Group 2	2.52 (0.11)	2.71 (0.08)	2.60 (0.92)	2.85 (0.85)	0.80 (0.16)	0.79 (0.10)	1.32 (0.22)	21.63 (10.70)	10.69 (0.26)	2.91 (0.31)
Group 3	2.49 (0.11)	2.69 (0.05)	2.55 (0.14)	2.83 (0.86)	0.89 (0.16)	0.78 (0.14)	1.32 (0.16)	26.50 (17.87)	10.57(0.28)	3.00 (0.26)

Table 6.3: Linear egression analysis to compare differences in the frequency of neurocognitive impairment and individual cognitive task scores between Groups 2 and 3 (when compared to Group 1)

	Group 2		**Group 3**	
	HIV-1 and chronic HCV coinfection		**HIV-1 and acute HCV coinfection**	
	***p*-value**	**95% CI**	***p*-value**	**95% CI**
Frequency of NCI	0.26	[-0.31, 0.08]	0.79	[-0.17, 0.23]
Detection speed	0.67	[-0.07, 0.04]	0.13	[-0.10, 0.01]
Identification speed	0.81	[-0.03, 0.04]	0.38	[-0.05, 0.02]
Divided attention speed	0.21	[-0.10, 0.02]	0.06	[-0.15, 0.03]
Complex reaction speed	0.78	[-0.03, 0.04]	0.47	[-0.06, 0.03]
Associate non-visual learning	0.34	[-0.12, 0.04]	0.17	[-0.03, 0.14]
Visual learning and memory	0.53	[-0.05, 0.09]	0.83	[-0.06, 0.08]
Working memory	0.30	[-0.05, 0.16]	0.30	[-0.05, 0.16]
Executive function	0.38	[-3.22, 8.31]	**0.02**	**[1.43, 13.40]**
Composite speed score	0.60	[-0.19, 0.11]	**0.04**	**[-0.31, -0.01]**
Composite accuracy score	0.66	[-0.13, 0.21]	0.18	[-0.06, 0.30]

[Legend Table 6.3: 95% CI= 95% confidence interval; HCV= hepatitis C virus; NCI=neurocognitive impairment]

Table 6.4: Association between worse executive function performance, clinical parameters and study group in Groups 3 and 1

Parameter	Univariate analysis		Multivariate analysis	
	***p*-value**	**95% CI**	***p*-value**	**95% CI**
Acute HCV study group	**0.02**	**[1.43, 13.40]**	**0.001**	**[7, 20]**
Age, per 10 year increase	0.68	[-4, 2]	-	
Current CD4+ count, per 100 cell/uL increase	0.70	[-2, 1]	-	
Nadir CD4+ count , per 100 cell/uL increase	0.09	[-4, 0]	**0.001**	**[-5, -1]**
Receiving cART	0.60	[-13, 21]	-	
Receiving PI-based cART	0.67	[-20, 13]	-	
Time since HIV diagnosis, per 10 year increase	0.41	[-2, 1]	-	
CPE 2008	0.95	[-5, 5]	-	

[Legend Table 6.4: 95% CI=95% confidence interval; cART = combination antiretroviral therapy; CPE= Cerebrospinal Fluid Penetration Effectiveness Rank; PI= protease inhibitor; HCV=hepatitis C virus]

Table 6.5: Results of univariate linear regression to investigate presence of association between neurocognitive impairment and clinical parameters in Group 1 (HIV-1 monoinfected control subjects)

Clinical parameter / demographic	*p*-value	95% confidence interval
Age, per 10 year increase	**0.04**	**[-0.19, -0.01]**
Male gender	0.70	[-0.43, 0.28]
Current CD4+ count, per 100 cell/uL increase	0.87	[-0.05, 0.04]
Nadir CD4+ count, per 100 cell/uL increase	0.91	[-0.10,0.11]
PI based cART	0.22	[-0.37, 0.09]
Years elapsed since HIV diagnosis, per 10 year increase	0.35	[-0.31, 0.11]
CPE score, 2008 version	0.23	[-0.08, 0.33]
CPE 2010, 2010 version	0.87	[-0.08, 0.09]

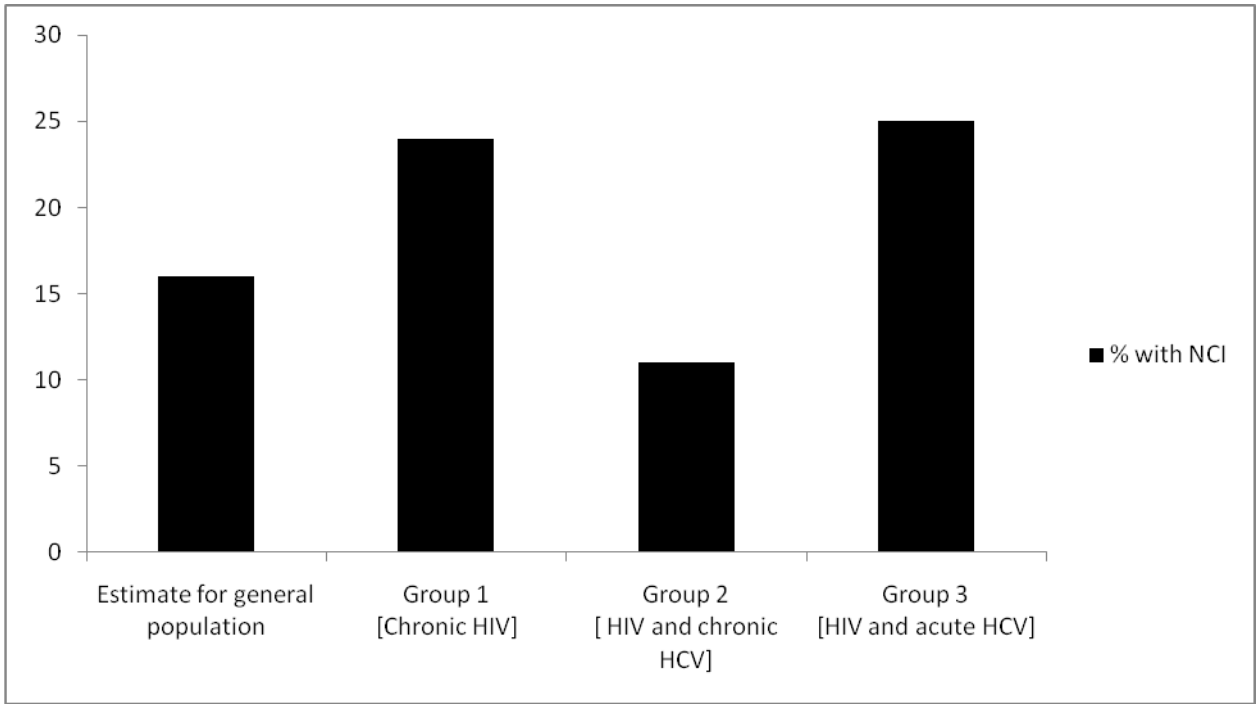
[Legend Table 6.5: cART = combination antiretroviral therapy; CPE= Cerebrospinal Fluid Penetration Effectiveness Score; PI= protease inhibitor]

Table 6.6: Results of linear regression analysis to investigate the presence of association between individual neurocognitive task scores and clinical or demographic parameters in Group 1

Cognitive task ***p*-value [95% CI]**	**Executive function**	**Composite speed score**	**Composite accuracy score**
Age, per 10 year increase	0.37 [-1.25, 3.25]	0.33 [-0.05, 0.14]	0.48 [-0.16, 0.08]
Male Gender	0.67 [-5.34, 8.30]	0.41 [-0.39, 0.16]	0.95 [-0.36, 0.34]
Current CD4+ count, per 100 cell/uL increase	0.27 [-1.44, 0.41]	**0.05 [-0.07, 0.00]**	0.29 [-0.02, 0.07]
Nadir CD4+ count, per 100 cell/uL increase	**0.01 [-4.33, -0.57]**	0.09 [-0.14, 0.01]	0.51 [-0.07, 0.14]
Receiving PI-based cART	0.43 [-6.90, 3.01]	**0.03 [-0.37, -0.02]**	**0.01 [0.14, 0.55]**
Years elapsed since HIV diagnosis, per 10 year increase	0.43 [-2.50, 5.72]	0.97 [-0.17, 0.17]	0.15 [-0.04, 0.01]
CPE, version 2008	0.49 [-2.80 5.70]	0.38 [-0.10, 0.25]	0.87 [-0.20, 0.24]
CPE, version 2010	0.57 [-1.23, 2.22]	0.96 [-0.07, 0.07]	0.47 [-0.05, 0.12]

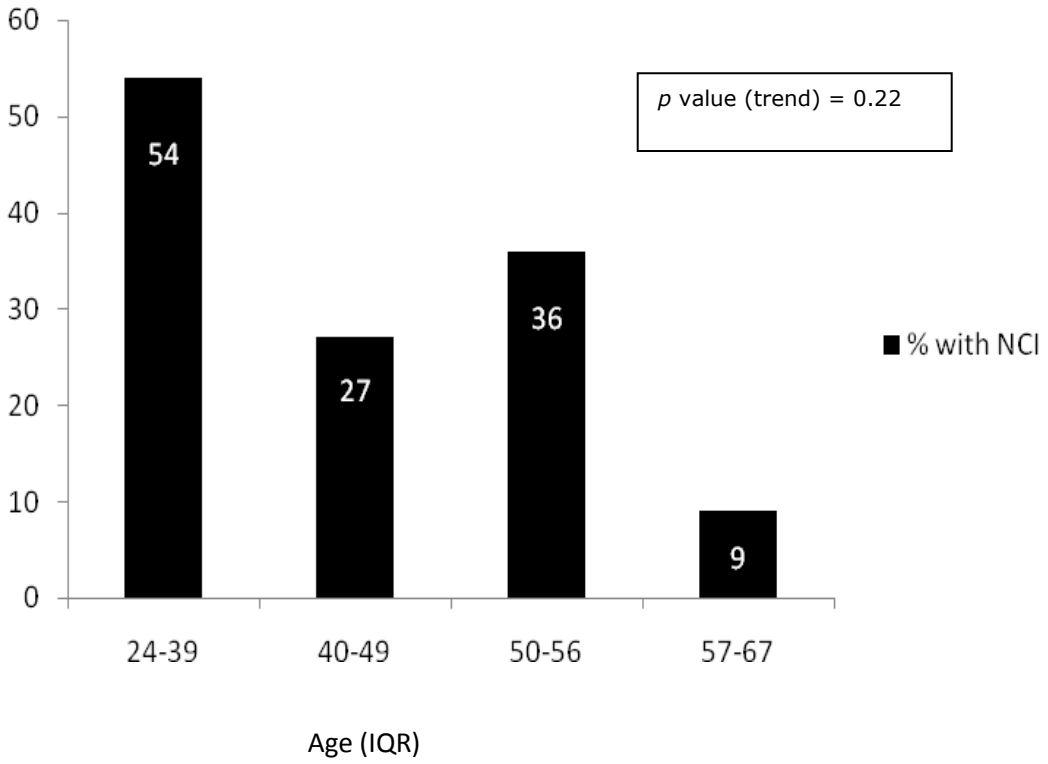
[Legend Table 6.6: cART = combination antiretroviral therapy; CPE= Cerebrospinal Fluid Penetration Effectiveness Rank; PI= protease inhibitor; 95% CI= 95% confidence interval]

Figure 6.1: Bar graph showing the proportion of subjects with neurocognitive impairment in each study group



[Legend Figure 6.1: NCI=neurocognitive impairment; Y-axis: percentage of subjects in each study group with NCI; X-axis= study group]

Figure 6.2: Proportion of HIV-1 infected subjects on stable antiretroviral therapy with neurocognitive impairment by age quartile (n=45)



[Legend Figure 6.2: NCI = neurocognitive impairment, IQR = interquartile range. [Y-axis = proportion of subjects with neurocognitive impairment, X axis = age interquartile range (years)].

Chapter 7

A Case-control Study to Compare Cerebral Metabolite Ratios in Subjects with Chronic HIV-1 and Acute HCV Coinfection and Subjects with Chronic HIV-1 Infection

Chapter 7: A Case-Control Study to Compare Cerebral Metabolite Ratios in Subjects with Chronic HIV-1 and Acute HCV Coinfection and Subjects with Chronic HIV-1 infection

7.1 Introduction

As described in Chapter 6, extra-hepatic manifestations of chronic HCV infection, including neurological disturbance, have been reported in recent years. Reported features of this include concentration, memory (Forton, et al. 2002), attention and executive function impairments (Weissenborn, et al. 2004). Individuals with HIV-1 and chronic HCV coinfection have, in some studies, shown greater neurocognitive deficits (frequently affecting executive functioning) than in subjects with HIV-1 alone (Letendre, et al. 2005; Murray, et al. 2008; Richardson, et al. 2005; Ryan, et al. 2004).

A biological mechanism for the neurological pathology caused by chronic HIV-1 and HCV has been explored. Replication of both viruses can be identified in the cerebrospinal fluid and brain tissue at autopsy (Laskus, et al. 2002a; Murray, et al. 2008) and changes in cerebral metabolites measured *in vivo* using ^{1}H MRS have also been reported (Barker, et al. 1995; Chang, et al. 2002; Chong, et al. 1993; Forton, et al. 2002; Weissenborn, et al. 2004). Such changes described include elevated mI and Cho in FWM and basal ganglia, respectively in chronic HCV (Forton, et al. 2001; Forton, et al. 2008a) and reduced levels of NAA and increased levels of mI and Cho in HIV-1 infection (Barker, et al. 1995; Chong, et al. 1993).

At present, no data exist regarding whether acute HCV infection is associated with deterioration of cerebral function parameters. Until recently, HCV infection was not generally identified by healthcare providers during the acute phase. However, due to the current epidemic of acute HCV in HIV-1 infected men, this situation has now changed (Browne, et al. 2004; Ghosn, et al. 2004) and the infection is increasingly being identified on routine laboratory tests by HIV-1 healthcare providers. In order to investigate the second hypothesis of thesis therefore, the aim of this study, was to identify whether acquisition of acute HCV infection is associated with changes in cerebral metabolites in a cohort of HIV-1 infected men.

7.2 Methods

This study took place at the HIV Outpatient department, St Mary's Hospital, London and the Robert Steiner Magnetic Resonance Unit, Hammersmith Hospital, Imperial College London between 2008 and 2010. Ethical approval was attained as described in Section 2.1.

7.2.1 Subject Selection

Male patients attending St. Mary's Hospital, London for HIV care were eligible. Subjects were assigned as cases (chronic HIV-1 and acute HCV, *Group 1*) or HIV-1 infected controls (chronic HIV-1 infection with no evidence of hepatitis C infection, *Group 2*). Chronic HIV-1 infection was defined as being HIV-1 antibody positive for at least 6 months. Exclusion criteria included commencing or undergoing any changes to antiretroviral therapy in the past 3 months, current or recent use of anti-depressant or anti-psychotic therapies (past 3 months), current opportunistic infection, active neurological disease, dementia, untreated syphilis, chronic hepatitis B infection, current receipt of interferon and/or ribavirin treatment, hepatic synthetic functional impairment (a serum albumin below 30 g/dL), use of recreational drugs within the past month and alcohol abuse.

Group 1: Chronic HIV-1 and acute HCV coinfection [cases]

Subjects were HIV-1 antibody positive and required to have acute HCV defined by a positive HCV RNA test within 12 months of a negative HCV RNA test.

Group 2: HIV-1 monoinfection [control subjects]

Matched HIV-1 antibody positive subjects were selected according to age, time-elapsed since HIV-1 diagnosis, antiretroviral therapy and current plasma CD4+cell count. All were required to be HCV antibody negative within the past year and with normal liver function tests thereafter.

7.2.2 Study procedures

All participants provided informed consent. Demographic information was collected from medical records including age, sex, nadir plasma CD4+ cell count, time-elapsed since HIV-1 diagnosis (years), current CD4+ cell count and HIV RNA level and current antiretroviral therapy. For subjects with acute HCV, additional information regarding time-elapsed since HCV diagnosis, current and peak ALT level, current HCV RNA level and HCV genotype was also collected. Current cART was scored for central nervous system penetration, using the CPE score (Letendre, et al. 2008).

7.2.3 Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

Cerebral ^{1}H-MRS was performed on all subjects once, using the same scanner as described in Section 2.4.1.

7.2.4 Statistical Methods

All statistical calculations were performed using SPSS (version 18.0). Between group differences in cerebral metabolite ratios were evaluated in univariate model by linear regression. Association between cerebral metabolite ratios and clinical parameters or demographic factors were investigated using univariate linear regression and those with a *p*-value ≤0.1 were entered into multivariate models. Variables with a skewed distribution were logarithm transformed and parameters with a *p*-value below 0.05 were considered significant.

7.3 Results

Patient characteristics

Twenty-four subjects with acute HCV (Group 1) and 12-matched subjects with HIV-1 monoinfection (Group 2) were recruited. Patient demographics and clinical parameters are shown in Table 7.1. All subjects from Group 1 had detectable HCV viraemia and elevated serum ALT at the time of study entry and had a previous negative HCV RNA test within a mean of 26.4 weeks (range 4-48). Groups were well matched for age, gender, whether currently receiving antiretrovirals, type of antiretroviral drug regimen and CPE scores. Subjects with acute HCV had a slightly higher mean current CD4 cell count (613 versus 525 cells/uL) and nadir CD4 count (315 versus 270 cells/uL respectively).

7.3.1 Results of cerebral metabolite ratios

The cerebral metabolite ratios for each group are shown in Table 7.2. Overall there were no significant differences in cerebral metabolite ratios between Groups 1 and 2, however a trend towards higher mI1/Cr was observed in the RBG of Group 1 (*p*=0.06, 95% CI -0.03, 1.32).

7.3.2 Association of RBG mI1/Cr and clinical parameters

In the multivariate analysis, nadir CD4 count and acute HCV study group were both independently associated with higher mI/Cr (p=0.05 and p=0.03 respectively, see Table 7.3). No significant associations were observed between RBG mI1/Cr and clinical parameters including the time elapsed since HCV RNA negative test, age or current CD4+ cell count (p-value>0.28 all values).

7.4 Discussion

This study attempted to investigate an association between acute HCV acquisition and cerebral function parameters using cerebral metabolite ratios as an objective measure. Differences between subjects with acute HCV and their matched controls were observed in RBG mI/Cr ratio, suggesting

changes to cerebral metabolite patterns are associated with the acute phase of infection when high levels of plasma HCV viraemia are observed. This finding became more signficant after adjustment for the weak association between higher mI/Cr and lower nadir CD4+ cell counts observed.

Forton *et al* (Forton, et al. 2008a) also observed changes in mI/Cr ratio in patients with chronic HCV, reporting an increase in this ratio in the FWM, rather than the RBG where I observed changes. The basal ganglia has a higher blood flow per unit volume (Kim, et al. 2008), compared to other cerebral locations, suggesting greater and earlier exposure of this part of the brain, which may explain why changes were observed here in acute infection, but had not yet evolved in other cerebral locations. Furthermore, basal ganglia dysfunction is recognised to be associated with symptoms of fatigue and inertia in other neurological disorders, therefore if cerebral inflammation occurs here in acute HCV, it may explain some of the neuropsychological disturbance we report in Chapter 6 (Chaudhuri and Behan 2004).

mI is an osmosensitive glial marker and plays a crucial role in cell volume regulation (Haussinger, et al. 2000). Organic osmolytes, such as mI accumulate inside the cell in response to cell shrinkage, and are rapidly released in response to cell swelling via osmoregulated membrane channels (Burg 1995; Lang, et al. 1998). The changes to mI/Cr ratio that were observed may therefore represent part of an acute response to the presence of the HCV virus, indicating increased neuroinflammation and glial proliferation during the early phase of HCV. It has been postulated that the effects of HCV on the CNS may be due to cerebral immune activation (Huang, et al. 1999) or direct viral replication (Forton, et al. 2004). In the context of HIV disease, both of these processes may occur at increased rates.

Abnormal cerebral metabolism has been documented in recreational drug-dependent subjects (Christensen, et al. 1996) and may confound results in studies assessing CNS function in HCV. All subjects in our study acquired HIV and HCV infections through sexual transmission. We also excluded subjects currently using recreational-drugs, attempting to limit this potential bias, however it is acknowledged that data on historical drug use was not collected. In addition, subjects with other sexually transmitted infections which are known to cause CNS disease, such as early syphilis, were excluded.

In summary, this study supports my second hypothesis that acquisition of acute HCV is associated with changes to cerebral function parameters, as statistically significant differences in RBG mI/Cr were observed between the 2 study groups. These alterations in mI metabolism during the early

stage of infection require further investigation as their longer-term clinical significance and reversibility remain uncertain. Further, larger-scale studies are required.

Table 7.1: Patient demographics and clinical parameters

Parameter, mean (SD) unless otherwise stated	Group 1 (HIV and acute HCV)	Group 2 (HIV)
Number of subjects, n	24	12
Male gender, n(%)	24 (100)	12(100)
Age (years)	40 (8)	44 (12)
HIV-acquisition risk group – men-having-sex-with-men, n (%)	24 (100)	12 (100)
Current CD4+ cell count (cells/µL)	613 (189)	525 (171)
Current plasma HIV RNA below 50 copies/mL, n (%)	17 (71)	9 (75)
Current plasma HIV RNA level of remaining subjects	17099	10410
Nadir CD4+ cell count (cells/µL)	315 (181)	270 (137)
Time-elapsed since HIV diagnosis (years)	7.9 (5.8)	7.08 (6.5)
Receiving antiretroviral therapy, n (%)	17 (71)	9 (75)
NNRTI –containing regimen	11 (65)	6 (67)
PI –containing regimen	5 (29)	2 (22)
Quadruple nucleoside analogue regimen	1 (6)	1 (11)
CPE Score (2008)	1.58 (0.59)	1.56 (0.58)
Time elapsed since negative HCV RNA (weeks)	26.42 (10.47)	-
Current ALT, IU	213 (210)	-
Peak ALT, IU	574 (581)	-
HCV genotype, n (%)		-
1	19 (79)	
2	0	
3	1 (4)	
4	2 (8)	
Not known	2 (8)	
Most recent HCV PCR (copies/mL)	3 849 936	-

[Legend Table 7.1: NNRTI = non-nucleoside reverse transcriptase inhibitor; PI=HIV-1 protease inhibitor; CPE score = Cerebrospinal fluid Penetration Effectiveness Rank Score; ALT =alanine aminotransferase, HCV= hepatitis C virus]

Table 7.2: Results of cerebral metabolite ratios in Groups 1 and 2

	Group 1	Group 2	Difference between Group 1 and 2	
Cerebral metabolite ratio	**Acute HCV + HIV**	**HIV**	***p*-value**	**95% CI**
FGM				
NAA/Cr	1.42 (0.25)	1.35 (0.10)	0.32	[-0.41, 1.21]
Cho/Cr	0.59 (0.12)	0.63 (0.17)	0.31	[-2.38, 0.77]
mI1/Cr	0.70 (0.28)	0.62 (0.13)	0.39	[-0.10, 0.25]
Total mI/Cr	2.71 (0.74)	2.55 (0.46)	0.53	[-0.19, 0.37]
FWM				
NAA/Cr	1.53 (0.32)	1.48 (0.26)	0.63	[-0.42, 0.68]
Cho/Cr	1.04 (0.20)	1.02 (0.19)	0.73	[-0.69, 0.98]
mI1/Cr	0.96 (0.48)	0.83 (0.54)	0.47	[-0.23, 0.49]
Total mI/Cr	3.06 (0.71)	3.03 (0.96)	0.90	[-0.22, 0.25]
RBG				
NAA/Cr	1.71 (0.25)	1.65 (0.29)	0.54	[-0.45, 0.84]
Cho/Cr	0.77 (0.12)	0.80 (0.19)	0.36	[-2.27, 0.85]
mI1/Cr	0.71 (0.22)	0.55 (0.23)	0.06	[-0.03, 1.32]
Total mI/Cr	2.50 (0.29)	2.70 (0.88)	0.34	[-0.49, 0.17]

[Legend Table 7.2: FGM= frontal grey matter; FWM=frontal white matter; RBG=right basal ganglia; NAA=N-acetyl aspartate; Cr= creatine; Cho = choline; mi1=first myo-inositol peak; Total mI= total of myo-inositol peaks; 95% CI= 95% confidence interval]

Table 7.3: Results of linear regression analysis to investigate associations between mI1/Cr ratio, study group and clinical parameters

Clinical Parameter	Right basal ganglia mI1/Cr *p*-value [95%CI]	
	Univariate analysis	Multivariate analysis
Study Group 1 (Acute HCV + HIV)	0.06 [-0.01, 0.32]	**0.03 [0.02, 0.35]**
Age, per 10 year increase	0.45 [-0.12, 0.06]	-
Current CD4+cell count, per 100 cell/uL increase	0.83 [-0.05, 0.04]	-
Nadir CD4+ cell count, per 100 cell/uL increase	0.09 [-0.10, 0.01]	**0.05 [-0.11, 0.00]**
Weeks elapsed since HCV RNA negative, per week increase	0.28 [-0.01, 0.02]	-
Current undetectable plasma HIV RNA level	0.64 [-0.23, 0.14]	-
Years since HIV diagnosis	0.81 [-1.58, 1.25]	-
CPE score 2008	0.21 [-0.27, 0.06]	-

[Legend Table 7.3: mi1=first myo-inositol peak; Cr= creatine; 95% CI= 95% confidence interval; CPE=CSF Penetration Effectiveness Rank]

CHAPTER 8

A Case-control Study Using PK-11195 Radio-labelled Cerebral Positron Emission (PET) Scanning to Compare Microglial Cell Activity in Subjects with Chronic HIV-1 and Acute HCV Coinfection and Subjects with Chronic HIV-1 Infection

Chapter 8: A Case-Control Study using PK-11195 Radio-labelled Cerebral Positron Emission (PET) Scanning To Compare Microglial Cell Activation in Subjects with Chronic HIV-1 and Acute HCV Coinfection and Subjects with Chronic HIV-1 Infection

8.1 Introduction

8.2 Methods

8.2.1 Subject selection

8.2.2 Study procedures

8.2.3 Cerebral PET scanning with ^{11}C PK11195 ligand

8.2.4 Statistical analysis

8.3 Results

8.3.1 ^{11}C PK11195 Binding Potentials

8.3.2 Association between ^{11}C PK11195 Binding Potentials and clinical parameters

8.3.2 Association between ^{11}C PK11195 Binding Potentials and cerebral metabolite ratios

8.4 Discussion

8.1 Introduction

HCV core proteins have recently been found to have the ability to infect cultured human microglial cells with induced expression of pro-inflammatory cytokines including IL-1α, IL-1β, IL-6, IL-12, IL-18 and tumor necrosis factor-α (Vivithanaporn, et al.; Wilkinson, et al. 2010). Pathological studies of post-mortem brain tissue from 12 HCV infected subjects (6 with HIV-1 coinfection), using monoclonal antibody staining have also detected HCV RNA in CD68+ positive cells (specific for macrophages/microglial cells) and also in GFAP mRNA+ cells (specific for astrocytes). This evidence suggests these cell types may be neurological targets for HCV and potentially responsible for the cerebral disturbance reported in some subjects (Wilkinson, et al. 2009).

Microglia are the major intrinsic immunocompetent phagocytic cells in the CNS, comprising 20% of all glial cells and are developmentally derived from bone marrow precursors of monocytic lineage. It is believed that resident microglia turn over slowly and are replaced by circulating monocytes. They are activated in response to most cerebral insults including trauma, infection and ischaemia (Tai, et al. 2007). Chemokines from activated microglia may further attract infected circulating cells of monocytic lineage to the brain and/or peripherally-derived cytokines may induce proinflammatory cytokine release from perivascular macrophage-like cells in the brain. Microglial cells have been implicated in promoting neurodegeneration in several disorders including HIV-E (Gonzalez-Scarano and Baltuch 1999) and chronic HCV (Forton, et al. 2008a).

The isoquinoline PK11195 is a highly specific, high-affinity ligand for the peripheral benzodiazepine binding site (PBBS) on microglia cells. Upregulation of the PBBS (which is found exclusively in non-neuronal cells), occurs in activated microglial cells and in infiltrating macrophages if the BBB is damaged. In the normal brain there is minimal binding of PK11195, but a significant increase of PBBS expression is seen in activated microglia after neuronal injury (Banati 2002; Banati, et al. 1997). PK11195 can be carbon 11-radio-labelled (^{11}C) and used as a non-invasive radiological marker of microglial activation with PET scanning technology. This allows for the extraction of quantitative data with respect to the physiological process of microglial cell activation, occuring at the cellular level.

Although *in vitro*, there are reports of astrocytes expressing upregulated binding of PK11195 (Itzhak and Norenberg 1994), it is regarded as a selective marker of activated microglia *in vivo* in the context of an intact blood brain barrier (Banati 2002). Thus, PK11195 has been used as a generic marker of neuroinflammation in studies of various neurological disorders including Huntingdon's disease (Tai, et al. 2007), Parkinson's disease (Gerhard, et al. 2006) and cerebral HIV infection (Hammoud, et al.

2005; Wiley, et al. 2006). ^{11}C PK11195 has shown increased uptake (or binding potential) in human subjects with HIV-E (Hammoud, et al. 2005). Interestingly, no increase was reported in subjects with HIV-1 and mild neurocognitive deficits when compared to matched asymptomatic HIV-1 infected subjects, suggesting that microglial cell activation occurs only in the advanced stages of HAND (HIV-E) in HIV-1 infected subjects (Wiley, et al. 2006).

During the current epidemic of sexually-transmitted acute HCV in MSM with chronic HIV-1 infection, there is a unique opportunity to further explore the neuropathological mechanism of reported HCV-associated cerebral disturbance *in vivo* and to establish whether microglial cell activation is associated with the acute infective process of HCV. This chapter will therefore address the second hypothesis of this thesis, that individuals with chronic HIV-1 infection will experience a change in cerebral function parameters (via microglial cell activation) following acquisition of acute HCV.

8.2 Methods

This study took place at the HIV Outpatient department, St Mary's Hospital, London and the Robert Steiner Magnetic Resonance Unit and Cyclotron Building, Hammersmith Hospital, Imperial College, London between 2009 and 2010. Ethical and ARSAC approval was obtained as described in Chapter 2.1.

8.2.1 Subject selection

Male patients attending St. Mary's Hospital, London for HIV care were eligible. Subjects were assigned as cases (chronic HIV-1 and acute HCV, *Group 1*) or HIV-1 infected controls (chronic HIV-1 infection with no evidence of hepatitis C infection, *Group 2*). Chronic HIV-1 infection was defined as being HIV-1 antibody positive for at least 6 months. Exclusion criteria included commencing or undergoing any changes to antiretroviral therapy in the past 3 months, current or recent use of benzodiazepines (due to known effect upon PBBS expression), anti-depressant or anti-psychotic therapies (past 3 months), current opportunistic infection, active neurological disease, dementia, untreated syphilis, chronic hepatitis B infection, current receipt of interferon and/or ribavirin treatment, hepatic synthetic functional impairment (a serum albumin below 30 g/dL), use of recreational drugs within the past month and alcohol abuse.

Group 1: Chronic HIV-1 and acute HCV coinfection [cases]

Subjects were HIV-1 antibody positive and required to have acute HCV defined by a positive HCV RNA test within 8 months of a negative HCV RNA test.

Group 2: HIV-1 monoinfection [control subjects]

Matched HIV-1 antibody positive subjects were selected according to age, time-elapsed since HIV-1 diagnosis, antiretroviral therapy and current CD4+cell count. All were required to be HCV antibody negative within the past year with normal liver function tests thereafter.

8.2.2 Study procedures

All participants provided informed consent. Demographic information was collected from medical records including age, sex, nadir CD4+ cell count, time-elapsed since HIV diagnosis (years), current CD4+ cell count and HIV RNA level and current antiretroviral therapy. For subjects with acute HCV, additional information regarding time-elapsed since most recent negative HCV RNA test, current and peak ALT level, current HCV RNA level and HCV genotype was also collected.

8.2.3 Cerebral PET scanning with ^{11}C PK11195

PET-CT scanning and T1-weighted cerebral MR imaging with ^{1}H-MRS were performed as described in Chapters 2.4.1 and 2.4.2. All subjects had both imaging tests performed either on the same day, or where ^{11}C PK11195 radioisotope production did not exceed the minimum quantity of 325 MBq (=8.78mCi) or other quality control test (n=2 cases), the PET-CT was performed within one week of MR. All subjects completed one PET-CT, MR and ^{1}H-MRS assessment.

8.2.4 Statistical analysis

^{11}C PK11195 binding potential (BP) analysis was completed as described in Chapter 2.4.2. Individual and study group mean (SD) ^{11}C PK11195 BP of left, right and total ventral striatum, caudate, putamen and thalamic regions were established. Any association between ^{11}C PK11195 BP and study group was investigated using the non-parametric Mann-Whitney Statistic. Association between clinical parameters (including age, CD4+ cell count, plasma HIV RNA level and for Group 1, time elapsed since HCV acquisition, plasma ALT, HCV RNA level and HCV genotype) were then investigated using linear regression. SPSS software (v18.0) was used for analysis. *P*-values below 0.05 were considered statistically significant.

8.3 Results

16 subjects (8 in each study group) were recruited and scans from 15 subjects were included in the analysis (1 subject had concurrent pneumonia diagnosed after completing the research and was subsequently excluded as levels of immune activation maybe influenced by the systemic inflammatory process). Patient demographics and clinical parameters are displayed in Table 8.1. Subjects from Group 1 had slightly higher current and nadir CD4+ lymphocyte counts than those from Group 2. The proportion of subjects receiving no cART, NNRTI-based and boosted PI-based cART was the same for each group. The mean number of weeks elapsed since most recent negative plasma HCV RNA test in Group 1 was 20.5 (range 16-28).

8.3.1 ^{11}C PK11195 Binding Potentials

Each subject's ^{11}C PK11195 BP in each cerebral region of interest is shown in Table 8.2. The mean (SD) BP of the total ventral striatum, caudate, putamen and thalamus were 0.17 (0.12) vs. 0.21 (0.15), 0.06 (0.05) vs. 0.06 (0.09), 0.21 (0.11) vs. 0.29 (0.14), 0.53 (0.31) vs. 0.55 (0.17) for Groups 1 and 2 respectively (see Table 8.3).

8.3.2 Association of ^{11}C PK11195 Binding Potentials and clinical parameters

No significant difference between study groups was observed in any cerebral region ($p>0.30$ all values, see Figure 8.1). Furthermore no association between ^{11}C PK11195 BP and any HIV-1 related clinical parameter was observed (*p*-value>0.20 all values, see Table 8.4). The ^{11}C PK11195 BPs were not significantly associated with basal ganglia cerebral metabolite ratios (see Table 8.5).

8.4 Discussion

In this study using ^{11}C PK11195 as an *in vivo* tracer of PBBS expression, I investigated the presence of microglial cell activation in individuals with chronic HIV-1 infection and acute HCV coinfection as a possible mechanism for causing cerebral neurocognitive deficits. When compared to control subjects matched for age, CD4+ cell count, nadir and current CD4+ cell count, cART and plasma HIV RNA level, I found no evidence of increased microglial cell activation in subjects with acute HCV when compared to HIV-monoinfected individuals, despite acute HCV cases being in the very early months of coinfection, with high levels of plasma HCV viraemia and deranged liver function tests. To my knowledge, this is the first time this acute phase of HCV has been investigated for the presence of such neuroimmune activation.

These novel results are of clinical importance as they provide evidence that microglial cell activation does not occur during the early months of HCV plasma viraemia, despite previous evidence that HCV proteins have been identified in microglial cells and can induce their production of inflammatory chemokines *in vitro* (Vivithanaporn, et al.). My work therefore suggests that either microglial cells are not activated by HCV *in vivo*, or that microglial cell recruitment takes place during later stages of chronic HCV infection. The former theory is disputed by the findings of Forton *et al*, who report high levels of microglial cell activation in a small number of subjects with advanced, chronic HCV infection using a similar radiological technique (Forton, et al. 2008b).

It is possible that the very acute nature of infection in the subjects I studied is responsible for the absence of microglial cell activation. It is known that microglia are recruited from circulating macrophage/monocytes and animal model data has demonstrated that this process may take several months following cerebral insult (Malm, et al. 2005). The mean time elapsed since negative HCV RNA test was 21 weeks in this cohort, which is obviously an over-estimation of the *actual* time elapsed since transmission of the virus. It is therefore possible that if the study was repeated several months later, different results would be observed.

Microglia activation may also be a proportional to the intensity of the insult and in acute HCV the CNS viral load will be low, even if the plasma viraemia is significant. The postulated mechanism for HCV to enter the brain is via transportation with recruited microglial cells from circulating macrophages and there simply may not have been enough time elapsed for thisprocess to yet be effected.

If microglial activation is a late feature of chronic HCV infection, then there may be an additional clinical reason to treat and eliminate HCV in the acute phase where possible, as therapy-response rates are higher and recommended treatment courses shorter (Vogel, et al. 2010), potentially preventing later neuroinvasion and immune activation occurring with chronic disease. If a HCV CNS viral reservoir does occur in the later stages of disease in some individuals, this may cause inferior anti-HCV therapy response outcomes, as pegylated interferon does not cross the BBB (Thomas, et al. 1999).

The very low levels of ^{11}C PK11195 BP in all selected cerebral locations and in both study groups, are similar to levels of ^{11}C PK11195 BP reported in previous healthy volunteer studies (Pavese, et al. 2006). Microglia are recognised as cells which respond rapidly to traumatic, inflammatory and degenerative cerebral changes and the low ^{11}C PK11195 BPs therefore provide further reassuring

evidence that in asymptomatic HIV-1 infected individuals without advanced disease or immune suppression, significant microglial activation indicating cerebral disturbance does not occur.

Limitations of this chapter include the small number of subjects recruited to each study group, restricting the power to detect subtle differences and associations. PET scans with PK11195 cost over £10,000 per scan and therefore are too expensive to perform on a larger scale, however this work is of similar size to previous physiological PET studies. Further limitations include the imperfect matching of cases with controls (for age, current and nadir CD4+ cell count) in a real-life clinical setting, the absence of longitudinal follow-up studies to identify if any increase of ^{11}C PK11195 BP evolves with duration of HCV coinfection and if so, identification of the time this occurs and whether any correlation with treatment outcome or neurocognitive dysfunction is observed.

Nevertheless this study addresses the second hypothesis of this thesis and provides evidence that acquisition of acute HCV coinfection in individuals with chronic HIV-1 infection, is not associated with a deterioration of cerebral function parameters (in the form of microglial cell activation).

Table 8.1: Subject demographics and clinical parameters

Parameter, mean (SD) unless otherwise stated)	Group 1 (HIV and acute HCV)	Group 2 (HIV)
Number, n	8	8
Male gender, n(%)	8 (100)	8 (100)
Age (years)	41 (9)	48 (11)
Current CD4+, cells/µL	617 (229)	490 (141)
Nadir CD4+, cells/µL	363 (230)	275 (168)
Time-elapsed since HIV diagnosis (years)	9 (8)	8 (7)
Receiving cART, n (%)	6 (75)	6 (75)
TDF FTC NVP	1	1
ABC 3TC NVP	1	1
TDF FTC EFV	2	2
TDF FTC DRV/r	1	2
ABC 3TC DRV/r	1	0
Current plasma HIV RNA below 50 copies/mL, n (%)	6 (75)	6 (75)
Mean current plasma HIV RNA level of subjects not on cART (copies/mL)	5755	8523
Weeks since negative plasma HCV RNA test	21 (4)	
Peak ALT, IU	847 (689)	-
Current ALT, IU	245 (162)	-
HCV genotype, n (%)		
1	6 (75)	-
4	2 (25)	-
Most recent HCV RNA, copies/mL	6 596 560	-

[Legend Table 8.1: TDF=tenofovir; FTC = emtricitabine; 3TC=lamivudine; NVP=nevirapine; EFV=efavirenz; DRV/r=darunavir/ritonavir; ALT =alanine aminotransferase]

Table 8.2: Results for ^{11}C PK11195 Binding Potentials of individual subjects in selected cerebral regions of interest

		BP in ventral striatum			BP in caudate			BP in putamen			BP in thalamus			Total BP all selected areas
	Subject	**Left**	**Right**	**Total**	**Left**	**Right**	**Total**	**Left**	**Right**	**Total**	**Left**	**Right**	**Total**	
	1	0.03	0.08	0.11	0.00	0.00	0.00	0.18	0.13	0.32	0.36	0.39	0.74	1.17
	2	0.08	0.15	0.23	0.00	0.05	0.05	0.16	0.16	0.31	0.41	0.40	0.81	1.41
	3	0.16	0.16	0.32	0.02	0.04	0.06	0.06	0.08	0.14	0.17	0.13	0.30	0.82
Group 1	4	0.00	0.03	0.03	0.00	0.00	0.00	0.04	0.07	0.11	0.17	0.13	0.30	0.44
(Acute HCV and HIV-1 coinfection)	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	0.00	0.00	0.00	0.00	0.08	0.08	0.02	0.02	0.05	0.02	0.02	0.03	0.16
	7	0.12	0.13	0.26	0.03	0.09	0.13	0.13	0.13	0.26	0.36	0.33	0.69	1.33
	8	0.10	0.10	0.20	0.02	0.10	0.12	0.12	0.17	0.29	0.41	0.38	0.79	1.4
	9	0.20	0.24	0.44	0.13	0.12	0.24	0.19	0.24	0.43	0.29	0.22	0.50	1.62
	10	0.12	0.10	0.23	0.07	0.05	0.12	0.08	0.09	0.16	0.19	0.15	0.34	0.85
	11	0.04	0.06	0.11	0.00	0.00	0.00	0.03	0.05	0.08	0.17	0.12	0.29	0.48
Group 2	12	0.17	0.12	0.28	0.00	0.01	0.01	0.14	0.10	0.24	0.34	0.36	0.70	1.25
(HIV-1 infection)	13	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.21	0.36	0.29	0.25	0.54	0.91
	14	0.09	0.15	0.24	0.03	0.07	0.09	0.23	0.27	0.50	0.39	0.41	0.79	1.62
	15	0.04	0.00	0.04	0.00	0.00	0.00	0.10	0.12	0.21	0.31	0.30	0.61	0.87
	16	0.15	0.19	0.34	0.00	0.00	0.00	0.13	0.16	0.29	0.29	0.32	0.62	1.25

[Legend Table 8.2: BP= ^{11}C PK11195 Binding Potential]

Table 8.3: Difference in mean (SD) of ^{11}C PK11195 Binding Potential in cerebral regions between Group 1 and 2

Cerebral region		Group 1 (acute HCV) N=7	Group 2 (control subjects) N=8	*p*-value for difference between groups	Mann-Whitney Statistic
Ventral striatum	*Left*	0.07 (0.06)	0.10 (0.07)	0.35	20
	Right	0.09 (0.06)	0.11 (0.08)	0.86	27
	Total	0.17 (0.12)	0.21 (0.15)	0.52	23
Caudate	*Left*	0.01 (0.01)	0.03 (0.05)	0.62	24
	Right	0.05 (0.04)	0.06 (0.05)	0.34	36
	Total	0.06 (0.05)	0.06 (0.09)	0.72	31
Putamen	*Left*	0.10 (0.06)	0.13 (0.06)	0.42	21
	Right	0.11 (0.05)	0.15 (0.08)	0.30	19
	Total	0.21 (0.11)	0.29 (0.14)	0.36	20
Thalamus	*Left*	0.27 (0.15)	0.28 (0.07)	0.73	31
	Right	0.25 (0.16)	0.27 (0.10)	0.91	29
	Total	0.53 (0.31)	0.55 (0.17)	0.82	30
Total all regions		0.96 (0.50)	1.10 (0.40)	0.56	23

Table 8.4: Association between ^{11}C PK11195 Binding Potentials and clinical parameters using linear regression

	Ventral striatum BP p-value [95% CI]	Caudate BP p-value [95% CI]	Putamen BP p-value [95% CI]	Thalamus BP p-value [95% CI]	Total BP p-value [95% CI]
Age, per 10 year increase	0.42 [-0.10, 0.04]	0.53 [-0.05, 0.03]	0.27 [-0.03, 0.10]	0.79 [-0.11, 0.14]	0.91 [-0.22, 0.25]
CD4+ count, per 100 cell/uL increase	0.91 [-0.04, 0.04]	0.87 [-0.02, 0.02]	0.67 [-0.05, 0.03]	0.89 [-0.07, 0.08]	0.92 [-0.14, 0.13]
Nadir CD4+ count, per 100 cell/uL increase	0.39 [-0.02, 0.06]	0.21 [-0.01, 0.04]	0.88 [-0.04, 0.04]	0.33 [-0.03, 0.09]	0.28 [-0.06, 0.19]
Time elapsed since HIV diagnosis, per 10 year increase	0.91 [-0.11, 0.12]	0.52 [-0.08, 0.04]	0.45 [-0.14, 0.07]	0.94 [-0.20, 0.19]	0.74 [-0.42, 0.31]
Receiving cART	0.90 [-0.18, 0.21]	0.75 [-0.12, 0.09]	0.28 [-0.27, 0.09]	0.20 [-0.52, 0.12]	0.32 [-0.91, 0.32]

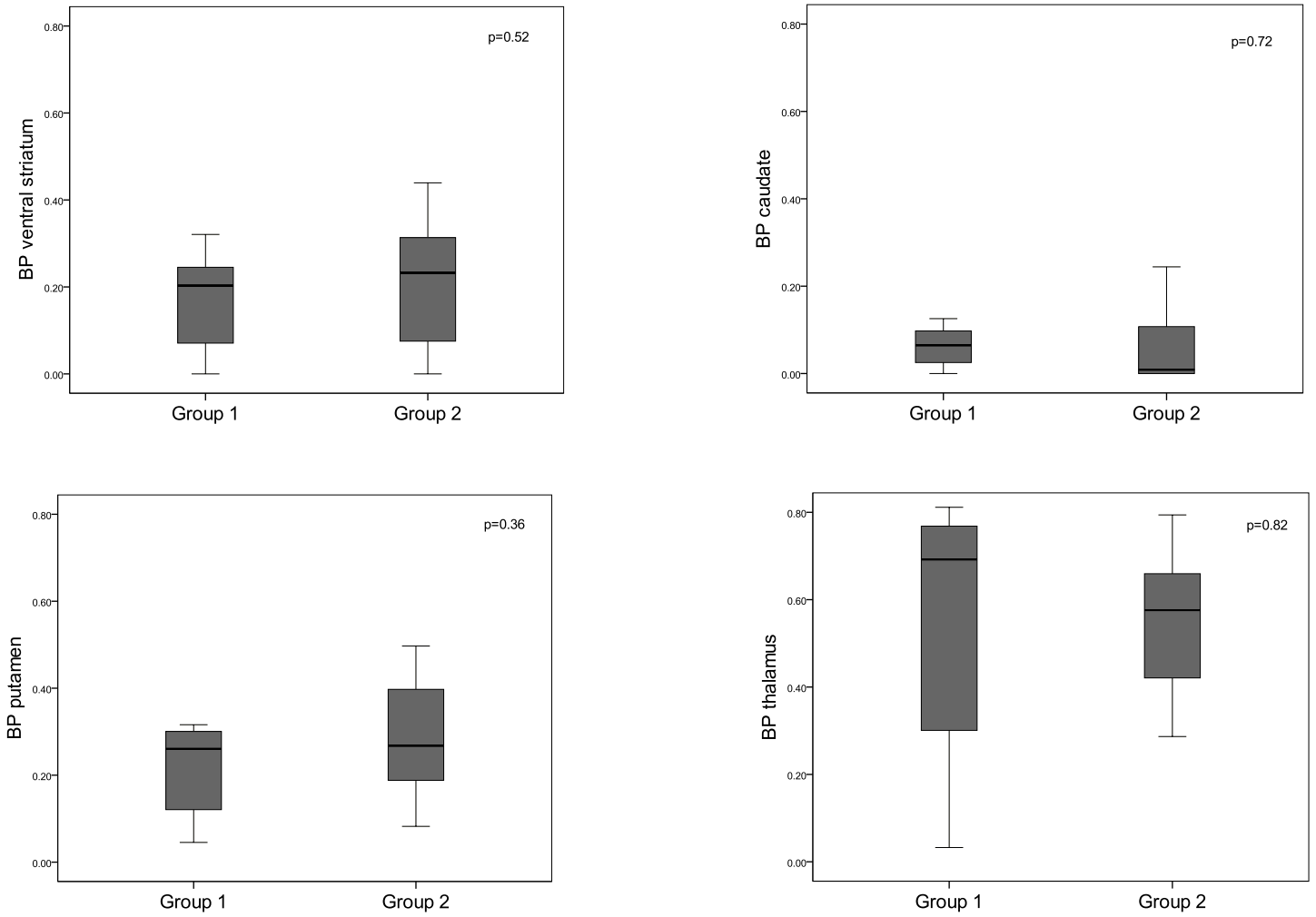
[Table 8. 4 legend: BP=^{11}C PK11195 Binding Potential; cART=combined antiretroviral therapy; 95%CI = 95% confidence interval]

Table 8.5: Association between ^{11}C PK11195 Binding Potentials and right basal ganglia cerebral metabolite ratios using linear regression

Cerebral metabolite ratio	NAA/Cr *p*-value [95%CI]	Cho/Cr *p*-value [95%CI]	mI/Cr *p*-value [95%CI]
Total BP in thalamus	0.71 [-0.56, 0.78]	0.83 [-0.20, 0.25]	0.97 [-0.53, 0.55]
Total BP in ventral striatum, caudate, putamen and thalamus	0.82 [-0.40, 0.32]	0.73 [-0.10, 0.14]	0.85 [-0.32, 0.26]

[Table 8.5 legend: NAA=N-acetyl aspartate, Cr=creatine; Cho = choline; mI=myoinositol; BP=^{11}C PK11195 Binding Potential]

Figure 8.1: Boxplots to represent^{11}C PK11195 Binding Potentials in cerebral locations by study group



[Figure 8.1 legend: BP=^{11}C PK11195 Binding Potential;Group 1: Subjects with chronic HIV-1 and acute HCV coinfection; Group 2: Subjects with chronic HIV-1 only]

CHAPTER 9

Overall Conclusions

Chapter 9: Overall Conclusions

9.1 Conclusions

It has recently been proposed that HCV co-infection and poor CNS penetration of antiretroviral drugs may be risk factors for the development of HAND. This thesis sought to examine the following two hypotheses:

- **Use of antiretroviral drugs with greater CNS penetration is associated with greater improvements in cerebral function parameters in HIV-1 infected subjects**
- **Acquisition of acute HCV coinfection is associated with a deterioration of cerebral function parameters in HIV-1 infected subjects**

9.2 First hypothesis

Use of antiretroviral drugs with greater CNS penetration is associated with greater improvements in cerebral function parameters in HIV-1 infected subjects

This hypothesis was tested in 3 clinical studies. Firstly in Chapter 3, data from the largest UK Cohort Study of HIV-1 infected adults were examined to establish the impact of antiretroviral therapy CNS penetration upon the incidence of HIV-associated brain diseases (including HIV-E, PML, TOXO and CRYPTO) and overall survival between 1996 and 2008. CNS penetration was assessed using the CPE Ranking system (Letendre S 2010b) as this is currently the most widely used tool in HIV clinical research. Previous European data reported improved survival following such CNS diagnoses in individuals prescribed higher CPE scoring antiretroviral therapy (Gasnault J 2008; Lanoy E 2007). This was the first UK study to assess impact of CPE score upon such clinical outcomes.

This initial study found no evidence to support the first hypothesis of this thesis. Lower CPE scoring therapy (with presumed lower CNS penetration) was found to be associated with a higher risk of developing a CNS disease in univariate analysis, but after adjustment for relevant clinical parameters, the factors independently associated with increased risk of CNS disease did not include CPE score, but rather lower CD4+ cell count, higher plasma HIV RNA level, heterosexual-HIV-1 risk group, older age and commencing cART within the past 6 months. In fact, rates of new CNS disease were extremely low in individuals with undetectable plasma HIV RNA levels, irrespective of CPE score, inferring that suppression of viral replication and the associated immune recovery are more important protective factors against the development of CNS diseases than the utilisation of higher CPE ranked regimens.

Interestingly, this study also revealed a novel finding regarding the use of the CPE Score as a method of estimating antiretroviral CNS penetration. I demonstrated that in this large cohort, the CPE score of a subject's prescribed regimen is significantly associated with clinical parameters at baseline, including CD4+ cell count and HIV RNA level, age and calendar year. This phenomenon has not previously been described and is extremely important to consider whenever the CPE score is applied to assess endpoints without therapy randomisation, particularly in retrospective analyses as inherent bias is inevitable. This finding may, at least partly, explain the currently unpublished findings of Gasnault *et al.,* who reported improved survival following PML when higher CPE ranked regimens were used, as prescribing bias for the most unwell and advanced patients may have influenced data outcomes.

Importantly, the results presented in Chapter 3 are dependent upon accurate clinical reporting of CNS disease and due to the large numbers of subjects in the cohort and de-linkage of data, individual reports were not independently verified or confirmed with case note review. This limitation must be considered when interpreting our data. Secondly we were unable to assess the impact of CPE score upon the milder forms of HAND (including MND and ANI) as currently UKCHIC do not collect reports of such diagnoses and no uniform method for diagnosing these conditions are being used routinely in UK centres at the present time.

Chapters 4 and 5 sought to further examine the first hypothesis assessing prospective changes to cerebral function parameters in stable HIV-1 infected subjects switching to novel antiretroviral regimens (with increased or decreased CNS penetration) via the use of longitudinal cerebral proton spectroscopy and computerised neurocognitive assessment. In Chapter 4, subjects switched therapy from a boosted-protease inhibitor containing triple-therapy regimen to eitherDRV/RTV monotherapy or DRV/RTV plus 2 nucleosides. Interestingly, the results from this study did provide evidence in support of the first hypothesis as overall, subjects switching to DRV/RTV demonstrated improvements in both types of cerebral function parameter assessments over a 48-week period. DRV/RTV, despite being highly bound to plasma proteins, has previously been demonstrated to cross the BBB with detectable CSF concentrations in the range of or which exceed the protein adjusted IC_{50} (Yilmaz A 2009). However, the effect of nucleoside discontinuation (thereby reducing CPE score) in one arm, did not appear to influence results as no significant differences were found between study treatment arms (DRV*mono* versus DRV*triple*). While the very small sample size is highly likely to have limited our power to detect such differences, other possible explanations may include the limited contribution of nucleoside analogues such as TDF and ddI and lower CNS penetration of ATV/RTV and SQV/RTV (when compared to DRV/RTV) which some subjects were receiving until

randomisation and therefore the switch to either treatment arm was not associated with a deterioration of cerebral function parameters. In order to better estimate the impact of DRV/RTV monotherapy upon cerebral function parameters, future work should investigate larger numbers of subjects using similar objective, longitudinal repeat assessments, all receiving DRV/RTV plus 2 identical nucleoside analogues before randomisation. A neurological sub-study of the currently ongoing Medical Research Council PIVOT study also seeks to further evaluate the question of any potential CNS sequelae of using PI-monotherapy as a maintenance strategy in virologically-stable patients.

In Chapter 5, changes to cerebral function parameters in stable patients undergoing therapy intensification with MVC were assessed in order to address the first hypothesis. MVC is estimated to have good CNS penetration due to its level of plasma protein binding, the described exposure when sampled in the female genital tract and CSF and the predominance of CCR5 tropic viruses in the CSF. This short study therefore studied stable patients (all receiving identical cART) at baseline and following intensification of MVC to increase potential CNS penetration and CPE score. This study firstly described the steady state CSF exposure of MVC and LPV in neuroasymptomatic individuals in a controlled setting, however also strongly supports the first hypothesis of this thesis as therapy-intensification with MVC, an antiretroviral with good CNS penetration, was associated with improvements in cerebral function parameters (namely RBG cerebral metabolite ratios) and in addition, such improvements were directly associated with plasma trough exposure, supporting causality.

This is the first work to describe a positive cerebral effect of MVC therapy *in vivo,* observed by measuring cerebral metabolite ratios, and a direct relationship of this effect to MVC exposure. These findings may have implications for clinical practice. For example, it would be interesting to see if such changes in cerebral metabolites could be associated with changes in neurocognitive function or other functional assessments. Future work to assess these effects over longer treatment periods, in both neurosymptomatic and asymptomatic HIV-1 infected subjects is justified and studies to assess the clinical implications of these findings are needed.

9.3 Second hypothesis

The acquisition of acute HCV coinfection is associated with a deterioration of cerebral function parameters in HIV-1 infected subjects

In order to examine the second hypothesis of this thesis, a series of clinical case-control studies were performed to evaluate any potential impact of acute HCV acquisition upon cerebral function

parameters including cognitive performance, cerebral metabolite ratios and microglial cell activation. Whether deterioration of cerebral function occurs during this acute phase of HCV has never previously been reported and due to the current epidemic of acute HCV in HIV-1 infected MSM, I had a unique opportunity to study this novel hypothesis.

In Chapter 6, subjects with acute HCV and HIV-1 coinfection were compared using a detailed, computerised cognitive assessment in 2 groups of control subjects: first, stable subjects with HIV-1 monoinfection and second, subjects with chronic HCV and HIV-1 coinfection. The reason for selection of such control groups was to minimise the impact of HIV-1 associated factors upon study outcomes and also to ensure any no factors related to chronic HCV coinfection were responsible for results. Interestingly, although no differences in the frequency of overt NCI were observed in acute HCV, significant impairment of executive functioning was observed in this subject group, despite their relatively well preserved CD4+ cell counts, younger age and shorter duration of HIV-1 infection. This evidence supports the hypothesis that deterioration of cerebral function parameters is observed in acute HCV coinfection which has not previously been described. No association with executive functioning and other clinical parameters was found as an alternative explanation, supporting acute HCV viraemia as a potential independent cause of this deficit. Unfortunately, without retrospective cognitive assessments for direct comparison in those subjects with acute HCV and therefore it is not possible to confirm an acute deterioration in cognitive performance.

In Chapter 7 an alternative tool, ^{1}H-MRS, was used to assess cerebral function in individuals with acute HCV. This technique can provide objective comparisons between cases and controls. Here, we observed a significant increase in RBG mI/Cr in subjects with acute HCV in the multivariate model, also supporting my second hypothesis that changes occur during the acute phase of HCV viraemia. An alteration of mI metabolism suggests cerebral inflammation in the RBG is occurring in acute HCV and this may ultimately be responsible for the deterioration of executive function performance previously described.

Finally, in Chapter 8, a potential mechanism of cerebral function deterioration was investigated using ^{11}C-PK11195 PET scanning as an *in vivo* marker of microglial cell activation. Researchers have recently identified the ability of HCV proteins to infect microglial cells *ex vivo* and using similar techniques have shown microglial cell activation in individuals with advanced HIV-E (Gonzalez-Scarano and Baltuch 1999) and chronic HCV with encephalopathy (Forton, et al. 2008a). It is reasonable, therefore to investigate whether a similar process of neuroimmune activation occurs during acute HCV. Of note, there was no increase in microglial activation observed and therefore this

cell-type does not appear to be involved in the mechanism of cognitive deficits that I reported during acute HCV coinfection. This may be due to the very acute nature of the condition studied (mean 21 weeks elapsed), since there may not have been enough time for microglial recruitment to have occured in the brain. Thus, it is likely that the effects of circulating cytokines, rather than microglial cell activation, are responsible for the clinical syndrome of neuropsychological disturbance I have observed in acute HCV infection. The neuropsychological effects of endogenous circulating cytokines and chemokines are observed in the presence of chronic infection, which are very similar to the CNS effects of therapeutically-administered interferon.

Further serial studies investigating the cerebral effects following acute HCV coinfection are warranted, including antiviral post-treatment effects, the long-term effects of viral eradication and genotype-specific changes.

Summary:

Mild forms of cerebral impairment affecting individuals with chronic HIV-1 infection have become increasingly apparent in recent years and in many cases, the reasons for its development are unclear. This is an area of great clinical importance for HIV-1 infected subjects, who now have dramatically improved life-expectancy with access to cART, making prevention and treatment for this co-morbidity a priority. In this thesis, evidence was presented to show that in some controlled settings, antiretroviral drugs with greater CNS penetration (including novel switching-strategies to darunavir-containing triple or monotherapy and intensification with maraviroc) can improve cerebral function parameters but found no evidence that the widespread use of regimens with higher CPE scores are associated with reduced incidence of the HIV-1 associated CNS diseases PML, HIV-E, TOXO and CRYPTO.

This thesis also presented evidence to demonstrate that cerebral disturbance and a deterioration of cerebral function parameters is observed during acute HCV coinfection. This does not appear to result in overt cognitive impairment, but rather of subtly impaired decision-processing skills. A dynamic metabolic disturbance was identified within the basal ganglia using ^{1}H-MRS, however the mechanism for these deficits was not demonstrated in this work as microglial cell activation does not appear to take place during this acute phase of HCV disease.

List of communications

Data contained in this thesis published in scientific journals or presented at scientific conferences are listed below:

Publications:

Antiretroviral therapy CNS penetration and HIV-1 associated CNS disease
Neurology, 2011; 76(8):693-700
Garvey L, Winston A, Walsh J, Post F, Porter K, Gazzard B, Fisher M, Leen C, Pillay D, Hill T, Johnson M, Gilson R, Anderson J, Easterbrook P, Bansi L, Orkin C, Ainsworth J, Palfreeman A, Gompels M, Phillips A, Sabin CA for the UK Collaborative HIV Cohort (CHIC) Study.

HIV-associated central nervous system diseases in the recent combination antiretroviral therapy era
Eur J Neurol, 2011: 18(3)527-534
Garvey L, Winston A, Walsh J, Post F, Porter K, Gazzard B, Fisher M, Leen C, Pillay D, Hill T, Johnson M, Gilson R, Anderson J, Easterbrook P, Bansi L, Orkin C, Ainsworth J, Palfreeman A, Gompels M, Phillips A, Sabin CA for the UK Collaborative HIV Cohort (CHIC) Study.

Changes in cerebral function parameters in HIV-1 infected subjects switching to darunavir/ritonavir, either as monotherapy or with nucleoside analogues.
Garvey L, Higgs C, Mohammed P, Hill A, Allsop JM, Taylor-Robinson SD, Nelson M, Winston A.
AIDS Res Hum Retroviruses. 2011; Jan 5 *(epub ahead of print)*

Correlations between Computerised Battery Testing and a Memory Questionnaire for Identification of Neurocognitive Impairment in HIV-1 Infected Subjects on Stable Antiretroviral Therapy.
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Garvey L, Yerrakalva D, Winston A.

Does acute hepatitis C infection affect the central nervous system in HIV-1 infected individuals?
J Viral Hepat. 2009 Sep 25.
Winston A, Garvey L, Scotney E, Yerrakalva D, Allsop JM, Thomson EC, Grover VP, Main J, Cox IJ Wylezinska M, Taylor-Robinson SD.

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Scientific conferences:

Oral presentations

Features of Neurocognitive Performance in over 100 Neurologically-Asymptomatic HIV-Infected Adults Receiving cART
British HIV Association Spring Conference, Bournemouth, UK, April 2011
Garvey L, Surendrakumar V, Winston A

Acquisition of acute HCV in HIV-infected subjects is associated with cerebral disturbances but not increased microglial cell activation: a PET study
British HIV Association Spring Conference, Bournemouth, UK, April 2011
Garvey L, Ramlackhansingh A, Allsop JM, Thomson E, Main J, Kulasegaram R, Brooks D, Taylor-Robinson SD, Pavese N, Winston A

Factors associated with central nervous system penetration effectiveness (CPE) score of antiretroviral regimens within a large UK cohort
British HIV Association Spring Conference, Manchester UK, April 2010
Garvey L, Winston A, Sabin C on behalf of UK CHIC

Continuing decline in incidence of central nervous system (CNS) events in the combination antiretroviral therapy (cART) era
12th European AIDS Conference, Cologne, 2009
Garvey L, Winston A, Sabin C on behalf of UK CHIC

Poster presentations

A Prospective Study to Assess the Central Nervous System Effects of a CCR5-Inhibitor in HIV-1 Infected Subjects on Stable Antiretroviral Therapy; A Pharmacokinetic and Cerebral Metabolite Study
*18th Conference on Retroviruses and Opportunistic Infections (CROI), Boston, USA, February 2011*Garvey L, Nelson N, Latch N, Erlwein OW, Allsop JM, Mitchell A, Kaye S, Watson V, Back D, Taylor-Robinson SD, Winston A.

Changes in cerebral function parameters in HIV-1 infected subjects undergoing a treatment simplification to darunavir/ritonavir: a randomised, prospective study.
HIV-10, Glasgow, November 2010
Garvey L , Higgs C, Mohammed P, Nelson M,Winston A

Do antiretroviral combination therapies with greater central nervous system (CNS) penetration prevent the development of CNS opportunistic diseases?
17th Conference on Retroviruses and Opportunistic Infections (CROI), San Francisco. Abstract #427
Garvey L, Winston A, Sabin C on behalf of UK CHIC

Effect of Acute HCV infection on the Central Nervous System in HIV-1 infected subjects
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Does Acute HCV Infection Affect the Central Nervous System in HIV-1-infected Individuals?
16th Conference on Retroviruses and Opportunistic Infections (CROI), Montreal. Abstract #465
Winston A, Garvey L, Scotney E, Allsop J, Thomson E, Grover V, Main J *et al.*

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