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Paving the path to HIV neurotherapy: predicting SIV CNS disease

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Abstract

HIV-induced damage to the CNS remains a major challenge for over 30 million people in the world despite the successes of combined antiretroviral therapy in limiting viral replication. Predicting development and progression of HIV-associated CNS disease is crucial because prevention and early intervention could be more effective than attempts to promote repair. The SIV/macaque model is the premier platform to study HIV neuropathogenesis, including discovery of predictive factors such as neuroprotective host genes and both blood and CSF biomarkers that precede and predict development of SIV CNS disease. This report details the role of macaque MHC class I genes, longitudinal alterations in biomarkers in the circulation, and expression of inflammatory and neuronal damage markers in CSF using samples from SIV-inoculated pigtailed macaques collected during acute, asymptomatic, and terminal stages of infection.

Keywords

SIV; HIV; CSF; platelets; pigtailed macaque; MHC class I; IL-6; CCL2

1. Introduction

In addition to causing acquired immune deficiency syndrome (AIDS), human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) also damage multiple organs including the central nervous system, peripheral nervous system, lung, and heart. In the central nervous system (CNS), macrophages and microglia are the primary targets for infection and, in turn, produce infectious virus (Chakrabarti et al., 1991; Gabuzda

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et al., 1986; Zink et al., 1997). Combined antiretroviral treatment (cART) has significantly reduced the incidence of severe HIV dementia, but the cumulative prevalence of HIV-induced neurocognitive impairment (NCI) has nonetheless increased in the cART era (McArthur et al., 2003). The broad spectrum of neurologic disease associated with HIV-1 infection is known collectively as HIV-associated neurocognitive disorders (HAND), a term that encompasses clinical presentations ranging from the mildest form (asymptomatic neurocognitive impairment) to the most severe (HIV-associated dementia [HAD]) (Antinori et al., 2007). Before the advent of effective cART, HAD was recognized as a relatively common condition in HIV-infected individuals, especially those with low CD4+ T-cell counts and high viral load. After introduction of effective cART, the incidence of HAD drastically decreased along with the incidence of AIDS (Antinori et al., 2007; Heaton et al., 2010). Unfortunately, mild to moderate forms of HAND persist despite viral suppressive cART, and amongst the mildest forms of HAND, the overall incidence of NCI has actually increased in the post-cART era (Heaton et al., 2010). For instance, the 2010 CHARTER study reported that, overall, 52% of HIV-1 infected individuals had some level of NCI; of these, only 7% were diagnosed with HAD, 12% had mild NCI, and 33% had evidence of asymptomatic NCI (Heaton et al., 2010).

Despite the recognition of persistent neurologic disease as an important manifestation of HIV infection, the pathogenesis of HAND in successfully cART-treated individuals continues to be elusive. There are numerous possible explanations, including the legacy effect (viral or inflammatory-mediated damage that develops prior to initiation of therapy), ongoing CNS inflammation, immune restoration disorder, host genetic factors, potential cART neurotoxicity, and poor CNS penetrance of cART (Levine et al., 2012; Mothobi and Brew, 2012). Most likely, HAND is the end result of multiple pathophysiologic factors that contribute to a complex neurobehavioral outcome. The continued high prevalence of HAND in the post-cART era is especially concerning because affected individuals have poorer clinical outcomes, with higher mortality, lower quality of life, and worse adherence to treatment (Mothobi and Brew, 2012).

2. Non-human primate models of neuroAIDS

SIV infection of macaques produces an excellent model of systemic and CNS disease induced by HIV (Letvin and King, 1990; Murray et al., 1992). The neuropathological changes induced by SIV closely resemble those present in HIV-infected individuals, with characteristic postmortem histopathologic findings consisting of multifocal perivascular cuffs of macrophages and multinucleated giant cells harboring replicating virus (Sharer et al., 1988). Collectively, these lesions, along with glial responses, typify SIV encephalitis. Animal models are particularly useful in studying the neuropathogenesis of HIV because CNS samples can be obtained from multiple time points throughout infection, ranging from acute through asymptomatic to terminal disease. Biological fluids may be collected from the same animal at various post-infection time points and compared with a pre-infection baseline. Additionally, adherence to treatment may be a challenge in HIV patient cohort studies, while animal models of cART-treated HIV infection allow consistent and verifiable adherence to treatment protocols. Most primate models of AIDS use rhesus macaques (*Macaca mulatta*), and most primate models of neuroAIDS use either SIV_{mac239} or

SIV_{mac}251, both R5-tropic viruses that cause acute viremia (Williams et al., 2008). Only SIV_{mac}251 causes encephalitis and glial activation in the neuropil at a high frequency and both viruses are capable of inducing meningitis (Williams et al., 2008). Although SIV_{mac}251 provides an excellent model for HIV neuropathogenesis, there are several limitations: only approximately one quarter of infected rhesus macaques will actually progress to develop encephalitis, and the delay until the development of AIDS is long and inconsistent, taking two years or more (Williams et al., 2008). For this reason, several accelerated models of SIV CNS disease have been established.

In one model, classic lentiviral encephalitis can be induced in up to 90% of SIV-infected rhesus macaques by administering monoclonal antibodies that deplete CD8+ cells (Ratai et al., 2011; Schmitz et al., 1999; Williams and Burdo, 2012). Although this model highlights the importance of cytotoxic T lymphocyte (CTL) immunologic control in the development of HIV encephalitis, it is limited because two important subsets of CD8+ immune cells, CD8+ T cells and NK cells, are both eliminated. Another method that researchers have used to reliably induce SIV encephalitis is to develop neurotropic (able to infect cells in the CNS) and neurovirulent (able to induce neuropathology) strains of SIV. Macrophage tropism has long been known to play an important part in the development of HIV encephalitis (Brinkmann et al., 1992; Koyanagi et al., 1987); however, studies have shown that macrophage tropism alone is necessary but not sufficient to induce the development of neurologic disease (Mankowski et al., 1997). Studies with recombinant SIV indicate that portions of the *env* gene impart macrophage tropism, but that complete *env*, *nef*, and 3'LTR sequences are necessary for neurovirulence (Mankowski et al., 1997; Thompson et al., 2003).

Building on this understanding of the SIV determinants of neurovirulence, the Retrovirus Laboratory at Johns Hopkins University developed a non-human primate model of neuroAIDS by inoculating pigtailed macaques simultaneously with the neurovirulent molecular clone SIV_{mac}/17E-Fr and the immunosuppressive swarm SIV/DeltaB670. With this combination, approximately 60% of pigtailed macaques develop SIV encephalitis while all animals develop AIDS-defining criteria within three months (Mankowski et al., 2002a; Zink et al., 1999). Although most studies of SIV pathogenesis work with rhesus macaques, pigtailed macaques have thus proven to develop CNS disease more consistently when compared with rhesus macaques given the same SIV inoculum (Beck et al, 2015)

This SIV/pigtailed macaque model offers many parallels to HIV infection, including the development of characteristic CNS inflammation that correlates with high viral load in the brain, cognitive and motor deficits typical of HIV-associated dementia, and the typical lesions of HIV/SIV encephalitis, including neurodegeneration and neuronal apoptosis (Brew et al., 1997; Ellis et al., 1997; Mankowski et al., 2002b; McArthur et al., 1997; Weed et al., 2003; Zink et al., 1999).

3. Importance of host genetics in non-human primate models of neuroAIDS

Because of the considerable genetic complexity seen in macaques, including the polymorphic and polygenic nature of MHC class I alleles and associated wide range of host

responses to viral infection, non-human primate models have contributed substantially to our understanding of the importance of immunogenetics in HIV disease progression. Certain MHC class I alleles have been identified in humans infected with HIV that are associated with altered progression to AIDS (Sauermann, 2001), including *HLA-B*57* in elite suppressors. In rhesus macaques, the MHC class I alleles *Mamu-A*01* and *-B*17* have been correlated with decreased viral load and increased survival time (Muhl et al., 2002; O'Connor et al., 2003; Pal et al., 2002; Yant et al., 2006). The immunodominant SIV epitope restricted by *Mamu-A*01* (CM9, CTPYDINQM₁₈₁₋₁₈₉) is in the same highly conserved region of Gag capsid as HIV TW10, an epitope that is restricted by *HLA-B*57* (Allen et al., 1998; Gotch et al., 1993; Miller et al., 1991). This provides an invaluable tool to study CTL-mediated immunologic control; however, the supply of rhesus macaques for research is limited, and it has become increasingly difficult to obtain *Mamu-A*01* -positive rhesus for research (Cohen, 2000; Smith et al., 2005a).

Pigtailed macaques also show similar MHC class I allele-associated viral recognition. The allele *Mane-A1*084* (formerly *Mane-A*10*) presents the immunodominant SIV Gag capsid epitope KP9 (KKFGAEVVP₁₆₄₋₁₇₂), a region that overlaps with HIV Gag KF11 (an immunodominant epitope recognized by *HLA-B*5701*) (Smith et al., 2005a; Smith et al., 2005b). Furthermore, MHC class I-mediated immune pressure at that epitope rapidly induces the formation of a canonical lysine to arginine escape mutation (K165R). Studying the accelerated model of neuroAIDS in pigtailed macaques, our lab has demonstrated a neuroprotective effect of MHC class I allele expression; animals that express *Mane-A1*084* are much less likely to develop SIV encephalitis than animals that do not (Mankowski et al., 2008; Queen et al., 2011) (Table 1). This finding explains why, although all dual-inoculated animals go on to develop high viral loads and progress to AIDS, a substantial fraction of animals do not develop encephalitis. To the authors' knowledge, this is the first time that HIV/SIV-induced organ-specific disease outcome has been shown to be altered by MHC class I allele expression.

Interestingly, in *Mane-A1*084* positive macaques with the highest CNS viral loads, we found the prototypic K165R Gag escape in the CNS (Mankowski et al., 2008), suggesting that escape mutations play a role in the development of SIV encephalitis. Longitudinal sequencing of plasma and CSF in these animals also showed that the prevalence of the SIV K165R Gag escape mutation increased over time, and that the development of this mutation is driven by KP9-specific CTL pressure (Queen et al., 2011). In addition, SIV K165R Gag escape mutations were archived in latent proviral DNA reservoirs, including the brain, in animals receiving cART that suppressed viral replication (Queen et al., 2011). Replication-competent SIV Gag K165R escape mutations were present in the resting CD4⁺ T cell reservoir in cART-treated SIV-infected macaques, indicating that escape develops during the decaying phases of viremia prior to suppression and then persists in tissues, including the brain (Queen et al., 2011). By selecting for or against neuroprotective MHC class I alleles, pigtailed macaque-based SIV studies can be greatly refined with significant reduction in the size of animal groups.

4. Predictive hematologic markers of HIV-associated CNS disease

Although various hematologic markers have been studied in human and animal studies as potential markers of AIDS severity and disease progression (Chauhan et al., 2011; Etzerodt and Moestrup, 2013; Kanekar, 2010; Pandrea et al., 2012; Tasca et al., 2012; Zoufaly et al., 2014), there is a pressing need to identify markers that can be easily used in the clinical setting to predict and identify HIV-associated CNS disease. This is especially critical because early identification of HIV-infected individuals at high risk of developing CNS disease could allow therapeutic intervention prior to the development of neurologic symptoms. We have identified several hematologic markers that serve as prognostic markers of CNS disease in HIV/SIV infection.

4.1 Hemoglobin level

Anemia has long been recognized as a common complication of HIV/AIDS. Severity of anemia is considered a prognostic indicator of HIV-associated disease progression, and successful control of HIV replication with cART is associated with decreased severity of anemia (Chauhan et al., 2011; Kowalska et al., 2007; Mocroft et al., 1999). Despite this, hemoglobin level has rarely been evaluated in the context of HIV-associated CNS disease. Along with advanced clinical stage and low CD4+ T cell count, low hemoglobin level was identified as one of the major risk factors for the development of HAND in HIV-infected individuals in sub-Saharan Africa (Njamnshi et al., 2009). In the SIV model, infected pigtailed macaques consistently show greater decreases in hemoglobin from baseline compared to uninfected controls, especially in the asymptomatic and chronic stages of disease (Fig. 1A). Of SIV-infected macaques, animals that progressed to develop SIV encephalitis had greater decreases in hemoglobin compared to SIV-infected macaques that did not develop CNS disease (Fig. 1A). This is consistent with previously reported data from a smaller study by our group (Wachtman et al., 2006). These data clearly indicate the potential utility of hemoglobin levels as potential prognostic indicators for HAND, although additional studies are warranted in HIV positive cohorts.

4.2 Platelet count

One of the most predictive circulating hematologic markers of retroviral-associated CNS disease is decline in platelet count. Platelet decline during asymptomatic infection has been associated with significantly increased risk for the development of HIV-associated dementia (Wachtman et al., 2006) or with decreased brain gray matter in HIV+ patients (Ragin et al., 2011). In our cohort of SIV-inoculated pigtailed macaques, we observed pronounced declines in platelet count during the acute (d7–14 p.i.) phase of infection and again in asymptomatic and terminal infection (Fig. 1B). After day 28 p.i., animals that later developed SIV encephalitis during terminal infection had a greater decrease in platelet count compared to animals that did not develop SIV encephalitis, though the magnitude of platelet loss during acute infection was similar between SIV-infected animals that progressed to SIV encephalitis and those that did not (Fig. 1B). These data are consistent with previously published studies by our group (Wachtman 2006 & Metcalf Pate 2013) and emphasize the importance of considering absolute change from baseline platelet count as a prognostic indicator of increased risk for the development of HIV-associated CNS disease. This

predictive platelet loss represents the combined effect of decreased platelet production (Metcalf Pate 2014), increased platelet destruction (Metcalf Pate 2013), and increased activated platelet associations with monocytes and endothelium (Metcalf Pate unpublished data), and may reflect the fundamental role of platelets in the pathogenesis of lentivirus-associated CNS disease.

4.3 Monocyte count

As HIV infection of monocytes and macrophages is integral to the development of HIV-associated CNS disease (Williams et al., 2012), circulating monocyte levels may be correlative of the development of brain pathology. Indeed, increasing levels of circulating monocytes were predictive of the development of HIV-1 associated progressive encephalopathy in a subset of pediatric patients (Sanchez-Ramon et al., 2003). In our model, we have found that the increase in the number of circulating monocytes from baseline is greater longitudinally in SIV-infected macaques compared to uninfected procedural controls, but that there is no difference in total monocyte count between animals that eventually develop SIV encephalitis and those that do not (Fig. 1C). This demonstrates that circulating monocyte numbers alone may not be valuable as a predictive marker for the development of HIV-associated CNS disease (Wachtman et al., 2006). Increases in subsets of activated circulating monocytes may be a more predictive biomarker for the identification of CNS inflammation. Increased numbers of circulating activated CD14⁺ CD69⁺ monocytes has been reported in adults with HIV-associated dementia compared to HIV-infected individuals without CNS disease (Pulliam et al., 1997). In a pigtailed macaque model of SIV infection, changes in circulating activated monocytes were not predictive of the development of encephalitis (Bissel et al., 2006). Clearly, additional work is needed to determine the extent that circulating activated monocytes can be used to predict the development of lentivirus-associated encephalitis.

4.4 Soluble CD163 (sCD163)

In addition to direct quantitation of circulating activated monocytes with flow cytometry, systemic monocyte activation may be measured by CD163, a monocyte/macrophage scavenger receptor that is shed as soluble CD163 (sCD163) in body fluids (Burdo et al., 2011). Recently, plasma sCD163 has gained interest as a biomarker of HIV activity and immune activation; in HIV-infected individuals, sCD163 decreased in the plasma with effective cART but did not return to baseline pre-infection levels, suggesting persistent underlying immune activation (Burdo et al., 2011). In addition, sCD163 in the plasma was higher in HIV-infected individuals with more severe neurophysiologic impairment, even in the context of fully suppressive cART (Burdo et al., 2013). Similarly, in the SIV pigtailed macaque model, our group has shown significantly higher levels of sCD163 in the plasma of SIV-infected macaques that develop encephalitis compared to macaques that do not (Fig. 1D).

5. Plasma and CSF viral RNA as a predictor of HIV-associated CNS disease

Although HIV replication is considered to be a risk factor for the development of neurologic disease, it remains unclear if and how actual viral replication in the CNS is directly

responsible for CNS injury. Successful control of virus replication with cART tends to improve cognitive performance outcomes in both HIV positive patients and non-human primates (Marcondes et al., 2009; Winston et al., 2010). In the pigtailed macaque model of SIV-associated CNS disease, both plasma and CSF SIV RNA levels are elevated during asymptomatic and terminal infection in animals that progressed to develop SIV encephalitis compared to those that did not (Zink et al., 1999) (Fig. 2). In the setting of effective cART, the association between HIV RNA concentrations in the CSF and impaired cognitive function is often weak (Cysique et al., 2005; McArthur et al., 2004; Sevigny et al., 2004). One explanation for this disparity is the possibility that viral RNA levels in the CSF are not closely representative of viral levels in the brain, but debate continues (Nightingale et al., 2014). Nonetheless, interest has turned to biomarkers of inflammation and neuronal damage in the CSF as more sensitive prognostic indicators of CNS disease.

6. CSF markers of neuroinflammation

6.1 Chemokine (C-C motif) ligand 2 (CCL2)

Increased CCL2 (or monocyte chemoattractant protein 1 [MCP-1]) levels in the CSF have been associated with the development of CNS disease in HIV patients (Sevigny et al., 2004) and in SIV positive pigtailed macaques (Mankowski et al., 2004; Zink et al., 2001). Beginning during asymptomatic infection at day 28 p.i., CSF CCL2 levels are significantly higher in macaques that progress to SIV encephalitis (Fig. 3A). SIV-infected animals that do not develop SIV encephalitis experience an acute spike in CSF CCL2 that subsequently drops, and remains no different from uninfected animals during the asymptomatic and terminal phases of disease, consistent with our previously published reports (Mankowski et al., 2004; Zink et al., 2001).

6.2 Interleukin 6 (IL-6)

Similar to CCL2, the pro-inflammatory cytokine IL-6 is significantly increased in the CSF during the asymptomatic and terminal phases of infection in pigtailed macaques that develop SIV encephalitis (Fig. 3B). Again, animals that do not develop SIV encephalitis have a sharp spike in CSF IL-6 at day 7 p.i. which then normalizes to the uninfected baseline by day 14 p.i., consistent with our previously published work (Mankowski et al., 2004). In the pigtailed macaque model of SIV encephalitis, increases in the pro-inflammatory cytokines in the CSF in asymptomatic and chronic stages of infection are highly predictive of the development of CNS disease.

6.3 Neopterin

Because macrophages and monocytes produce neopterin in response to interferon gamma stimulation, neopterin is considered to be a good marker of immune activation as well as generally prognostic of poor clinical outcome in many settings (Murr et al., 2002). CSF neopterin is produced by activated monocytes/macrophages in the brain in a variety of disorders, including multiple sclerosis and HIV (Fredrikson et al., 1987; Fuchs et al., 1989; Hagberg et al., 2010). CSF neopterin is also considered to be an excellent biomarker of HIV-associated CNS disease (Fredrikson et al., 1987; Fuchs et al., 1989; Hagberg et al., 2010), and, unlike CSF viral RNA, has been shown to persist despite effective cART

(Yilmaz et al., 2013). To our knowledge, CSF neopterin has not previously been reported as a biomarker of CNS disease in SIV-infected macaques. Animals that developed SIV encephalitis showed substantial increases in CSF neopterin in asymptomatic and chronic stages of infection, though we were able to detect only negligible amounts of CSF neopterin throughout the course of infection in SIV-infected pigtailed macaques that did not progress to develop SIV encephalitis (Fig. 3C). Thus, in our highly consistent pigtailed macaque model of SIV encephalitis, CSF neopterin is a very specific biomarker of inflammation in the CNS compared to the periphery.

7. CSF markers of neuronal damage

7.1 Human cartilage glycoprotein 39 (YKL-40)

YKL-40 is expressed by chondrocytes, synoviocytes, neutrophils, and monocytes in several chronic inflammatory and neoplastic conditions (Bonneh-Barkay et al., 2008). CSF YKL-40 was shown to be differentially upregulated in the CSF of SIV-infected pigtailed macaques that developed encephalitis and correlated with an increase in CSF viral load (Bonneh-Barkay et al., 2008). These results were supported by findings from our group, in which CSF YKL-40 levels were markedly higher in pigtailed macaques that developed SIV encephalitis, while SIV-infected macaques that did not develop encephalitis maintained low, consistent baseline levels of CSF YKL-40 throughout the course of infection (Fig. 4A).

7.2 Neurofilament light chain (NF-L)

Neurofilament light chain is a major neuronal cytoskeletal protein that, when increased in the CSF, is associated with neurodegeneration and neuroaxonal damage (Pranzatelli et al., 2014; Tortelli et al., 2014; Tortelli et al., 2012; van Eijk et al., 2010). In the context of HIV, CSF neurofilament light chain decreases with cART and increases with interruption of cART treatment, implying that cART is able to interrupt the neurodegenerative processes associated with chronic HIV-1 infection (Gisslen et al., 2005; Mellgren et al., 2007). CSF neurofilament light chain has been rarely reported in SIV primate models of HIV infection. In our pigtailed macaque model, treatment with fluconazole/paroxetine after SIV infection was shown to be neuroprotective, as evidenced by decreased CSF neurofilament light chain and decreased amyloid precursor protein (APP) accumulation in axons, although viral loads and markers of neuroinflammation were unchanged (Meulendyke et al., 2014). In our untreated SIV-infected pigtailed macaques, neurofilament light chain levels in the CSF spike at day 7–14 p.i. in animals regardless of whether they progressed to SIV encephalitis; however, CSF neurofilament light chain drastically increases during in the asymptomatic and chronic stages of infection in animals that develop SIV encephalitis and drops in animals that did not progress to SIV encephalitis (Fig. 4B). This confirms that increased neuroaxonal damage occurs in animals that develop encephalitis, and suggests that CSF neurofilament light chain may be added to the list of valuable predictive biomarkers for the development of lentiviral-associated CNS disease.

8. MicroRNAs (miRNAs) in the CSF

MicroRNAs (miRNAs) are considered to be a promising class of biomarker due to a packaging-conferred stability in the extracellular space and the potential to reflect the state

of diseased cells and tissues (Pritchard et al., 2012; Reynoso et al., 2014; Swaminathan et al., 2014). Our group has previously reported on miRNA expression in brain (Liu et al., 2013) and on the differential regulation of 45 plasma small RNAs during acute phase infection in our model, comparing expression at 10 dpi with a pre-infection baseline for each subject (Witwer et al., 2011). Furthermore, the differential expression of a set of upregulated plasma miRNAs (miRs-21, -34a, -125b, -130b, -146a, and -1233) tracked with eventual CNS disease severity (Witwer et al., 2011). At time points following acute infection—as the initial cytokine storm abates (Witwer et al., 2009)—these miRNAs generally return to baseline levels ahead of disease development (unpublished findings).

To assess the relationship between plasma and the CNS, we also profiled miRNAs in longitudinal, plasma-matched cerebrospinal fluid samples. Cell-free CSF has a different miRNA profile from plasma, as well as a lower total RNA concentration; nevertheless, it has been used successfully as a source of potential small RNA biomarkers (Baraniskin et al., 2011; Burgos et al., 2013; Cogswell et al., 2008), including in one study of HIV encephalitis (Pacifci et al., 2013). Individual miRNA profiling platforms can certainly influence results (Mestdagh et al., 2014; Watson and Witwer, 2011). However, with qPCR microarrays and individual hydrolysis probe-based assays, following a carefully selected and optimized protocol for CSF RNA extraction (McAlexander et al., 2013), we are able to achieve consistent, reliable detection of approximately 40 miRNAs per sample (unpublished findings). Two miRNAs that are differentially regulated in both plasma and CSF over the course of SIV infection and disease development are miR-125b and miR-146a (Fig. 5). The miR-125b acute response appears to be more pronounced in CSF than in plasma (Witwer et al., 2011). Both miRNAs have known roles in the response to viral infection. The miR-125 family has been reported to target matrix metalloproteinases and cell death-related proteins (Sun et al., 2013). miR-146a increases in HIV-1-infected microglial cells, has been found to target CCL8 (Rom et al., 2010), and also binds to the SIV genome (Sisk et al., 2013). As we continue to explore the potential of miRNAs as CSF and plasma biomarkers, we have begun to focus on specific carriers of miRNAs—such as extracellular vesicles (Raposo and Stoorvogel, 2013; Witwer, 2014)—that may provide more information on specific cells of origin than “total” biofluids profiling, which reflects mostly general responses and disease states.

9. Conclusions

With HIV-infected individuals living longer in the age of cART, the importance of managing complications of chronic HIV infection, such as HIV-associated CNS disease, is becoming even more urgent. There is a vital need to identify non-invasive and practical clinical measurements that can be used as reliable biomarkers for risk and development of HAND. It is likely that a panel of markers from both the blood and CSF will provide the most complete picture of ongoing neuropathology. Blood plasma biomarkers, such as hemoglobin level, platelet count, and sCD163, have the advantage of relatively easy technical collection, while CSF markers of neuroinflammation and neuronal degeneration may provide insight into the degree of neuroaxonal damage and immune activation in the CNS. As our ability to use biomarkers to identify patients with increased risk for the

development of HAND improves, so will our ability to implement neuroprotective therapeutic measures to provide optimal patient care.

As the brain is one of the most difficult organs to study in HIV-infected individuals due to limited sample availability, non-human primates remain the most representative and best-characterized animal model for HIV neuropathogenesis to date. The pigtailed macaque model of accelerated SIV-associated CNS disease provides an opportunity to study and develop prognostic markers of HAND for HIV-infected individuals in a controlled setting. In addition, similar to what has been observed in cART-treated HIV-infected patients, there is compelling evidence of ongoing neurodegenerative changes in pigtailed macaques that are cART-treated and virus-suppressed. Biomarkers of HAND provide us with the ability to readily track the neurological status of our SIV-infected macaques longitudinally throughout our studies, allowing for maximal optimization of an effective and useful panel for predicting the development of HAND to be used in the clinical setting.

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References

- Allen TM, Sidney J, del Guercio MF, Glickman RL, Lensmeyer GL, Wiebe DA, DeMars R, Pauza CD, Johnson RP, Sette A, Watkins DI. Characterization of the peptide binding motif of a rhesus MHC class I molecule (Mamu-A*01) that binds an immunodominant CTL epitope from simian immunodeficiency virus. *J Immunol.* 1998; 160:6062–6071. [PubMed: 9637523]
- Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, Gisslen M, Grant I, Heaton RK, Joseph J, Marder K, Marra CM, McArthur JC, Nunn M, Price RW, Pulliam L, Robertson KR, Sacktor N, Valcour V, Wojna VE. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology.* 2007; 69:1789–1799. [PubMed: 17914061]
- Baraniskin A, Kuhnhen J, Schlegel U, Chan A, Deckert M, Gold R, Maghnouj A, Zollner H, Reinacher-Schick A, Schmiegler W, Hahn SA, Schroers R. Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. *Blood.* 2011; 117:3140–3146. [PubMed: 21200023]
- Beck SE, Kelly KM, Queen SE, Adams RJ, Zink MC, Tarwater PM, Mankowski JL. Macaque species susceptibility to simian immunodeficiency virus: increased incidence of SIV central nervous system disease in pigtailed macaques versus rhesus macaques. *J Neurovirol.* 2015; 21(2):148–158. [PubMed: 25672885]
- Bissel SJ, Wang G, Trichel AM, Murphey-Corb M, Wiley CA. Longitudinal analysis of activation markers on monocyte subsets during the development of simian immunodeficiency virus encephalitis. *Journal of neuroimmunology.* 2006; 177:85–98. [PubMed: 16797085]
- Bonneh-Barkay D, Bissel SJ, Wang G, Fish KN, Nicholl GC, Darko SW, Medina-Flores R, Murphey-Corb M, Rajakumar PA, Nyaundi J, Mellors JW, Bowser R, Wiley CA. YKL-40, a marker of simian immunodeficiency virus encephalitis, modulates the biological activity of basic fibroblast growth factor. *The American journal of pathology.* 2008; 173:130–143. [PubMed: 18556781]
- Brew BJ, Pemberton L, Cunningham P, Law MG. Levels of human immunodeficiency virus type 1 RNA in cerebrospinal fluid correlate with AIDS dementia stage. *The Journal of infectious diseases.* 1997; 175:963–966. [PubMed: 9086160]
- Brinkmann R, Schwinn A, Narayan O, Zink C, Kreth HW, Roggendorf W, Dorries R, Schwender S, Imrich H, ter Meulen V. Human immunodeficiency virus infection in microglia: correlation

- between cells infected in the brain and cells cultured from infectious brain tissue. *Annals of neurology*. 1992; 31:361–365. [PubMed: 1586136]
- Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, Rosenberg ES, Ellis RJ, Williams KC. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. *The Journal of infectious diseases*. 2011; 204:154–163. [PubMed: 21628670]
- Burdo TH, Weiffenbach A, Woods SP, Letendre S, Ellis RJ, Williams KC. Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. *AIDS*. 2013; 27:1387–1395. [PubMed: 23435298]
- Burgos KL, Javaherian A, Bomprezzi R, Ghaffari L, Rhodes S, Courtright A, Tembe W, Kim S, Metpally R, Van Keuren-Jensen K. Identification of extracellular miRNA in human cerebrospinal fluid by next-generation sequencing. *RNA*. 2013; 19:712–722. [PubMed: 23525801]
- Chakrabarti L, Hurtrel M, Maire MA, Vazeux R, Dormont D, Montagnier L, Hurtrel B. Early viral replication in the brain of SIV-infected rhesus monkeys. *The American journal of pathology*. 1991; 139:1273–1280. [PubMed: 1750503]
- Chauhan NK, Vajpayee M, Singh A. Usefulness of hemoglobin and albumin as prognostic markers for highly active antiretroviral therapy for HIV-1 infection. *Indian journal of medical sciences*. 2011; 65:286–296. [PubMed: 23422702]
- Cogswell JP, Ward J, Taylor IA, Waters M, Shi Y, Cannon B, Kelnar K, Kemppainen J, Brown D, Chen C, Prinjha RK, Richardson JC, Saunders AM, Roses AD, Richards CA. Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. *J Alzheimers Dis*. 2008; 14:27–41. [PubMed: 18525125]
- Cohen J. AIDS research. Vaccine studies stymied by shortage of animals. *Science*. 2000; 287:959–960. [PubMed: 10691568]
- Cysique LA, Brew BJ, Halman M, Catalan J, Sacktor N, Price RW, Brown S, Atkinson JH, Clifford DB, Simpson D, Torres G, Hall C, Power C, Marder K, McArthur JC, Symonds W, Romero C. Undetectable cerebrospinal fluid HIV RNA and beta-2 microglobulin do not indicate inactive AIDS dementia complex in highly active antiretroviral therapy-treated patients. *Journal of acquired immune deficiency syndromes*. 2005; 39:426–429. [PubMed: 16010165]
- Ellis RJ, Hsia K, Spector SA, Nelson JA, Heaton RK, Wallace MR, Abramson I, Atkinson JH, Grant I, McCutchan JA. Cerebrospinal fluid human immunodeficiency virus type 1 RNA levels are elevated in neurocognitively impaired individuals with acquired immunodeficiency syndrome. HIV Neurobehavioral Research Center Group. *Annals of neurology*. 1997; 42:679–688. [PubMed: 9392566]
- Etzerodt A, Moestrup SK. CD163 and inflammation: biological, diagnostic, and therapeutic aspects. *Antioxidants & redox signaling*. 2013; 18:2352–2363. [PubMed: 22900885]
- Fredrikson S, Link H, Eneroth P. CSF neopterin as marker of disease activity in multiple sclerosis. *Acta neurologica Scandinavica*. 1987; 75:352–355. [PubMed: 3618112]
- Fuchs D, Chiodi F, Albert J, Asjo B, Hagberg L, Hausen A, Norkrans G, Reibnegger G, Werner ER, Wachter H. Neopterin concentrations in cerebrospinal fluid and serum of individuals infected with HIV-1. *AIDS*. 1989; 3:285–288. [PubMed: 2504232]
- Gabuzda DH, Ho DD, de la Monte SM, Hirsch MS, Rota TR, Sobel RA. Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. *Annals of neurology*. 1986; 20:289–295. [PubMed: 3532930]
- Gisslen M, Rosengren L, Hagberg L, Deeks SG, Price RW. Cerebrospinal fluid signs of neuronal damage after antiretroviral treatment interruption in HIV-1 infection. *AIDS research and therapy*. 2005; 2:6. [PubMed: 16109178]
- Gotch F, Nixon D, Gallimore A, McAdam S, McMichael A. Cytotoxic T lymphocyte epitopes shared between HIV-1, HIV-2, and SIV. *Journal of medical primatology*. 1993; 22:119–123. [PubMed: 7692055]
- Hagberg L, Cinque P, Gisslen M, Brew BJ, Spudich S, Bestetti A, Price RW, Fuchs D. Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection. *AIDS research and therapy*. 2010; 7:15. [PubMed: 20525234]

- Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, Marcotte TD, Atkinson JH, Rivera-Mindt M, Vigil OR, Taylor MJ, Collier AC, Marra CM, Gelman BB, McArthur JC, Morgello S, Simpson DM, McCutchan JA, Abramson I, Gamst A, Fennema-Notestine C, Jernigan TL, Wong J, Grant I. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*. 2010; 75:2087–2096. [PubMed: 21135382]
- Kanekar A. Biomarkers predicting progression of human immunodeficiency virus-related disease. *Journal of clinical medicine research*. 2010; 2:55–61. [PubMed: 21811520]
- Kowalska JD, Mocroft A, Blaxhult A, Colebunders R, van Lunzen J, Podlekareva D, Hansen AB, Machala L, Yust I, Benfield T. Current hemoglobin levels are more predictive of disease progression than hemoglobin measured at baseline in patients receiving antiretroviral treatment for HIV type 1 infection. *AIDS research and human retroviruses*. 2007; 23:1183–1188. [PubMed: 17961102]
- Koyanagi Y, Miles S, Mitsuyasu RT, Merrill JE, Vinters HV, Chen IS. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. *Science*. 1987; 236:819–822. [PubMed: 3646751]
- Letvin NL, King NW. Immunologic and pathologic manifestations of the infection of rhesus monkeys with simian immunodeficiency virus of macaques. *Journal of acquired immune deficiency syndromes*. 1990; 3:1023–1040. [PubMed: 2213505]
- Levine AJ, Service S, Miller EN, Reynolds SM, Singer EJ, Shapshak P, Martin EM, Sacktor N, Becker JT, Jacobson LP, Thompson P, Freimer N. Genome-wide association study of neurocognitive impairment and dementia in HIV-infected adults. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2012; 159B:669–683.
- Liu J, Sisk JM, Gama L, Clements JE, Witwer KW. Tristetraprolin expression and microRNA-mediated regulation during simian immunodeficiency virus infection of the central nervous system. *Mol Brain*. 2013; 6:40. [PubMed: 24103357]
- Mankowski JL, Clements JE, Zink MC. Searching for clues: tracking the pathogenesis of human immunodeficiency virus central nervous system disease by use of an accelerated, consistent simian immunodeficiency virus macaque model. *The Journal of infectious diseases*. 2002a; 186(Suppl 2):S199–S208. [PubMed: 12424698]
- Mankowski JL, Flaherty MT, Spelman JP, Hauer DA, Didier PJ, Amedee AM, Murphey-Corb M, Kirstein LM, Munoz A, Clements JE, Zink MC. Pathogenesis of simian immunodeficiency virus encephalitis: viral determinants of neurovirulence. *Journal of virology*. 1997; 71:6055–6060. [PubMed: 9223498]
- Mankowski JL, Queen SE, Clements JE, Zink MC. Cerebrospinal fluid markers that predict SIV CNS disease. *Journal of neuroimmunology*. 2004; 157:66–70. [PubMed: 15579282]
- Mankowski JL, Queen SE, Fernandez CS, Tarwater PM, Karper JM, Adams RJ, Kent SJ. Natural host genetic resistance to lentiviral CNS disease: a neuroprotective MHC class I allele in SIV-infected macaques. *PloS one*. 2008; 3:e3603. [PubMed: 18978944]
- Mankowski JL, Queen SE, Tarwater PM, Fox KJ, Perry VH. Accumulation of beta-amyloid precursor protein in axons correlates with CNS expression of SIV gp41. *Journal of neuropathology and experimental neurology*. 2002b; 61:85–90. [PubMed: 11829347]
- Marcondes MC, Flynn C, Huitron-Rezendiz S, Watry DD, Zandonatti M, Fox HS. Early antiretroviral treatment prevents the development of central nervous system abnormalities in simian immunodeficiency virus-infected rhesus monkeys. *AIDS*. 2009; 23:1187–1195. [PubMed: 19455015]
- McAlexander MA, Phillips MJ, Witwer KW. Comparison of Methods for miRNA Extraction from Plasma and Quantitative Recovery of RNA from Cerebrospinal Fluid. *Front Genet*. 2013; 4:83. [PubMed: 23720669]
- McArthur JC, Haughey N, Gartner S, Conant K, Pardo C, Nath A, Sacktor N. Human immunodeficiency virus-associated dementia: an evolving disease. *Journal of neurovirology*. 2003; 9:205–221. [PubMed: 12707851]

- McArthur JC, McClernon DR, Cronin MF, Nance-Sproson TE, Saah AJ, St Clair M, Lanier ER. Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain. *Annals of neurology*. 1997; 42:689–698. [PubMed: 9392567]
- McArthur JC, McDermott MP, McClernon D, St Hillaire C, Conant K, Marder K, Schifitto G, Selnes OA, Sacktor N, Stern Y, Albert SM, Kieburtz K, deMarcaida JA, Cohen B, Epstein LG. Attenuated central nervous system infection in advanced HIV/AIDS with combination antiretroviral therapy. *Archives of neurology*. 2004; 61:1687–1696. [PubMed: 15534180]
- Metcalf Pate KA, Lyons CE, Dorsey JL, Shirk EN, Queen SE, Adams RJ, Gama L, Morrell CN, Mankowski JL. Platelet activation and platelet-monocyte aggregate formation contribute to decreased platelet count during acute simian immunodeficiency virus infection in pig-tailed macaques. *J Infect Dis*. 2013; 208(6):874–883. [PubMed: 23852120]
- Metcalf Pate KA, Lyons CE, Dorsey JL, Queen SE, Adams RJ, Morrell CN, Mankowski JL. TGFβ-Mediated Downregulation of Thrombopoietin Is Associated With Platelet Decline in Asymptomatic SIV Infection. *J Acquir Immune Defic Syndr*. 2014; 65(5):510–516. [PubMed: 24220290]
- Mellgren A, Price RW, Hagberg L, Rosengren L, Brew BJ, Gisslen M. Antiretroviral treatment reduces increased CSF neurofilament protein (NFL) in HIV-1 infection. *Neurology*. 2007; 69:1536–1541. [PubMed: 17923616]
- Mestdagh P, Hartmann N, Baeriswyl L, Andreasen D, Bernard N, Chen C, Cheo D, D'Andrade P, DeMayo M, Dennis L, Derveaux S, Feng Y, Fulmer-Smentek S, Gerstmayer B, Gouffon J, Grimley C, Lader E, Lee KY, Luo S, Mouritzen P, Narayanan A, Patel S, Peiffer S, Ruberg S, Schroth G, Schuster D, Shaffer JM, Shelton EJ, Silveria S, Ulmanella U, Veeramachaneni V, Staedtler F, Peters T, Guettouche T, Wong L, Vandesompele J. Evaluation of quantitative miRNA expression platforms in the microRNA quality control (miRQC) study. *Nat Methods*. 2014; 11:809–815. [PubMed: 24973947]
- Meulendyke KA, Queen SE, Engle EL, Shirk EN, Liu J, Steiner JP, Nath A, Tarwater PM, Graham DR, Mankowski JL, Zink MC. Combination fluconazole/paroxetine treatment is neuroprotective despite ongoing neuroinflammation and viral replication in an SIV model of HIV neurological disease. *Journal of neurovirology*. 2014
- Miller MD, Yamamoto H, Hughes AL, Watkins DI, Letvin NL. Definition of an epitope and MHC class I molecule recognized by gag-specific cytotoxic T lymphocytes in SIVmac-infected rhesus monkeys. *J Immunol*. 1991; 147:320–329. [PubMed: 1711081]
- Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, Pradier C, d'Arminio Monforte A, Ledergerber B, Lundgren JD. Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. *AIDS*. 1999; 13:943–950. [PubMed: 10371175]
- Mothobi NZ, Brew BJ. Neurocognitive dysfunction in the highly active antiretroviral therapy era. *Current opinion in infectious diseases*. 2012; 25:4–9. [PubMed: 22156897]
- Muhl T, Krawczak M, Ten Haaf P, Hunsmann G, Sauermann U. MHC class I alleles influence set-point viral load and survival time in simian immunodeficiency virus-infected rhesus monkeys. *J Immunol*. 2002; 169:3438–3446. [PubMed: 12218167]
- Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. *Current drug metabolism*. 2002; 3:175–187. [PubMed: 12003349]
- Murray EA, Rausch DM, Lendvay J, Sharer LR, Eiden LE. Cognitive and motor impairments associated with SIV infection in rhesus monkeys. *Science*. 1992; 255:1246–1249. [PubMed: 1546323]
- Nightingale S, Winston A, Letendre S, Michael BD, McArthur JC, Khoo S, Solomon T. Controversies in HIV-associated neurocognitive disorders. *The Lancet. Neurology*. 2014; 13:1139–1151. [PubMed: 25316020]
- Njamnshi AK, Bissek AC, Ongolo-Zogo P, Tabah EN, Lekoubou AZ, Yepnjo FN, Fonsah JY, Kuate CT, Angwafor SA, Dema F, Njamnshi DM, Kouanfack C, Djientcheu Vde P, Muna WF, Kanmogne GD. Risk factors for HIV-associated neurocognitive disorders (HAND) in sub-Saharan Africa: the case of Yaounde-Cameroon. *Journal of the neurological sciences*. 2009; 285:149–153. [PubMed: 19631349]

- O'Connor DH, Mothe BR, Weinfurter JT, Fuenger S, Rehauer WM, Jing P, Rudersdorf RR, Liebl ME, Krebs K, Vasquez J, Dodds E, Loffredo J, Martin S, McDermott AB, Allen TM, Wang C, Doxiadis GG, Montefiori DC, Hughes A, Burton DR, Allison DB, Wolinsky SM, Bontrop R, Picker LJ, Watkins DI. Major histocompatibility complex class I alleles associated with slow simian immunodeficiency virus disease progression bind epitopes recognized by dominant acute-phase cytotoxic-T-lymphocyte responses. *Journal of virology*. 2003; 77:9029–9040. [PubMed: 12885919]
- Pacifici M, Delbue S, Ferrante P, Jeansonne D, Kadri F, Nelson S, Velasco-Gonzalez C, Zabaleta J, Peruzzi F. Cerebrospinal fluid miRNA profile in HIV-encephalitis. *J Cell Physiol*. 2013; 228:1070–1075. [PubMed: 23042033]
- Pal R, Venzon D, Letvin NL, Santra S, Montefiori DC, Miller NR, Trynieszewska E, Lewis MG, VanCott TC, Hirsch V, Woodward R, Gibson A, Grace M, Dobratz E, Markham PD, Hel Z, Nacsa J, Klein M, Tartaglia J, Franchini G. ALVAC-SIV-gag-pol-env-based vaccination and macaque major histocompatibility complex class I (A*01) delay simian immunodeficiency virus SIVmac-induced immunodeficiency. *Journal of virology*. 2002; 76:292–302. [PubMed: 11739694]
- Pandrea I, Cornell E, Wilson C, Ribeiro RM, Ma D, Kristoff J, Xu C, Haret-Richter GS, Trichel A, Apetrei C, Landay A, Tracy R. Coagulation biomarkers predict disease progression in SIV-infected nonhuman primates. *Blood*. 2012; 120:1357–1366. [PubMed: 22653975]
- Pranzatelli MR, Tate ED, McGee NR, Verhulst SJ. CSF neurofilament light chain is elevated in OMS (decreasing with immunotherapy) and other pediatric neuroinflammatory disorders. *Journal of neuroimmunology*. 2014; 266:75–81. [PubMed: 24342231]
- Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: approaches and considerations. *Nat Rev Genet*. 2012; 13:358–369. [PubMed: 22510765]
- Pulliam L, Gascon R, Stubblebine M, McGuire D, McGrath MS. Unique monocyte subset in patients with AIDS dementia. *Lancet*. 1997; 349:692–695. [PubMed: 9078201]
- Queen SE, Mears BM, Kelly KM, Dorsey JL, Liao Z, Dinoso JB, Gama L, Adams RJ, Zink MC, Clements JE, Kent SJ, Mankowski JL. Replication-competent simian immunodeficiency virus (SIV) Gag escape mutations archived in latent reservoirs during antiretroviral treatment of SIV-infected macaques. *Journal of virology*. 2011; 85:9167–9175. [PubMed: 21715484]
- Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J Cell Biol*. 2013; 200:373–383. [PubMed: 23420871]
- Ratai EM, Pilkenton S, He J, Fell R, Bombardier JP, Joo CG, Lentz MR, Kim WK, Burdo TH, Autissier P, Annamalai L, Curran E, O'Neil SP, Westmoreland SV, Williams KC, Masliah E, Gilberto Gonzalez R. CD8+ lymphocyte depletion without SIV infection does not produce metabolic changes or pathological abnormalities in the rhesus macaque brain. *Journal of medical primatology*. 2011; 40:300–309. [PubMed: 21463330]
- Reynoso R, Laufer N, Hackl M, Skalicky S, Monteforte R, Turk G, Carobene M, Quarleri J, Cahn P, Werner R, Stoiber H, Grillari-Voglauer R, Grillari J. MicroRNAs differentially present in the plasma of HIV elite controllers reduce HIV infection in vitro. *Sci Rep*. 2014; 4:5915. [PubMed: 25081906]
- Rom S, Rom I, Passiatore G, Pacifici M, Radhakrishnan S, Del Valle L, Pina-Oviedo S, Khalili K, Eletto D, Peruzzi F. CCL8/MCP-2 is a target for mir-146a in HIV-1-infected human microglial cells. *Faseb J*. 2010; 24:2292–2300. [PubMed: 20181935]
- Sanchez-Ramon S, Bellon JM, Resino S, Canto-Nogues C, Gurbindo D, Ramos JT, Munoz-Fernandez MA. Low blood CD8+ T-lymphocytes and high circulating monocytes are predictors of HIV-1-associated progressive encephalopathy in children. *Pediatrics*. 2003; 111:E168–E175. [PubMed: 12563091]
- Sauermann U. Making the animal model for AIDS research more precise: the impact of major histocompatibility complex (MHC) genes on pathogenesis and disease progression in SIV-infected monkeys. *Current molecular medicine*. 2001; 1:515–522. [PubMed: 11899095]
- Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Raciz P, Tenner-Raciz K, Dalesandro M, Scallon BJ, Ghayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science*. 1999; 283:857–860. [PubMed: 9933172]

- Sevigny JJ, Albert SM, McDermott MP, McArthur JC, Sacktor N, Conant K, Schifitto G, Selnes OA, Stern Y, McClernon DR, Palumbo D, Kiebertz K, Riggs G, Cohen B, Epstein LG, Marder K. Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia. *Neurology*. 2004; 63:2084–2090. [PubMed: 15596754]
- Sharer LR, Baskin GB, Cho ES, Murphey-Corb M, Blumberg BM, Epstein LG. Comparison of simian immunodeficiency virus and human immunodeficiency virus encephalitis in the immature host. *Annals of neurology*. 1988; 23(Suppl):S108–S112. [PubMed: 2831797]
- Sisk JM, Witwer KW, Tarwater PM, Clements JE. SIV replication is directly downregulated by four antiviral miRNAs. *Retrovirology*. 2013; 10:95. [PubMed: 23988154]
- Smith MZ, Dale CJ, De Rose R, Stratov I, Fernandez CS, Brooks AG, Weinfurter J, Krebs K, Riek C, Watkins DI, O'Connor DH, Kent SJ. Analysis of pigtail macaque major histocompatibility complex class I molecules presenting immunodominant simian immunodeficiency virus epitopes. *Journal of virology*. 2005a; 79:684–695. [PubMed: 15613296]
- Smith MZ, Fernandez CS, Chung A, Dale CJ, De Rose R, Lin J, Brooks AG, Krebs KC, Watkins DI, O'Connor DH, Davenport MP, Kent SJ. The pigtail macaque MHC class I allele Mane-A*10 presents an immunodominant SIV Gag epitope: identification, tetramer development and implications of immune escape and reversion. *Journal of medical primatology*. 2005b; 34:282–293. [PubMed: 16128923]
- Sun YM, Lin KY, Chen YQ. Diverse functions of miR-125 family in different cell contexts. *J Hematol Oncol*. 2013; 6:6. [PubMed: 23321005]
- Swaminathan G, Navas-Martin S, Martin-Garcia J. MicroRNAs and HIV-1 infection: antiviral activities and beyond. *J Mol Biol*. 2014; 426:1178–1197. [PubMed: 24370931]
- Tasca KI, Calvi SA, Souza Ldo R. Immunovirological parameters and cytokines in HIV infection. *Revista da Sociedade Brasileira de Medicina Tropical*. 2012; 45:663–669. [PubMed: 23295865]
- Thompson KA, Kent SJ, Gahan ME, Purcell DF, McLean CA, Preiss S, Dale CJ, Wesselingh SL. Decreased neurotropism of nef long terminal repeat (nef/LTR)-deleted simian immunodeficiency virus. *Journal of neurovirology*. 2003; 9:442–451. [PubMed: 12907389]
- Tortelli R, Copetti M, Ruggieri M, Cortese R, Capozzo R, Leo A, D'Errico E, Mastrapasqua M, Zoccolella S, Pellegrini F, Simone IL, Logroscino G. Cerebrospinal fluid neurofilament light chain levels: marker of progression to generalized amyotrophic lateral sclerosis. *European journal of neurology : the official journal of the European Federation of Neurological Societies*. 2014
- Tortelli R, Ruggieri M, Cortese R, D'Errico E, Capozzo R, Leo A, Mastrapasqua M, Zoccolella S, Leante R, Livrea P, Logroscino G, Simone IL. Elevated cerebrospinal fluid neurofilament light levels in patients with amyotrophic lateral sclerosis: a possible marker of disease severity and progression. *European journal of neurology : the official journal of the European Federation of Neurological Societies*. 2012; 19:1561–1567.
- van Eijk JJ, van Everbroeck B, Abdo WF, Kremer BP, Verbeek MM. CSF neurofilament proteins levels are elevated in sporadic Creutzfeldt-Jakob disease. *Journal of Alzheimer's disease : JAD*. 2010; 21:569–576. [PubMed: 20555148]
- Wachtman LM, Tarwater PM, Queen SE, Adams RJ, Mankowski JL. Platelet decline: an early predictive hematologic marker of simian immunodeficiency virus central nervous system disease. *Journal of neurovirology*. 2006; 12:25–33. [PubMed: 16595371]
- Watson AK, Witwer KW. Do Platform-Specific Factors Explain MicroRNA Profiling Disparities? *Clin Chem*. 2011
- Weed MR, Hienz RD, Brady JV, Adams RJ, Mankowski JL, Clements JE, Zink MC. Central nervous system correlates of behavioral deficits following simian immunodeficiency virus infection. *Journal of neurovirology*. 2003; 9:452–464. [PubMed: 12907390]
- Williams DW, Eugenin EA, Calderon TM, Berman JW. Monocyte maturation, HIV susceptibility, and transmigration across the blood brain barrier are critical in HIV neuropathogenesis. *Journal of leukocyte biology*. 2012; 91:401–415. [PubMed: 22227964]
- Williams K, Burdo TH. Monocyte mobilization, activation markers, and unique macrophage populations in the brain: observations from SIV infected monkeys are informative with regard to pathogenic mechanisms of HIV infection in humans. *Journal of neuroimmune pharmacology : the*

- official journal of the Society on NeuroImmune Pharmacology. 2012; 7:363–371. [PubMed: 22167311]
- Williams R, Bokhari S, Silverstein P, Pinson D, Kumar A, Buch S. Nonhuman primate models of NeuroAIDS. *Journal of neurovirology*. 2008; 14:292–300. [PubMed: 18780230]
- Winston A, Duncombe C, Li PC, Gill JM, Kerr SJ, Puls R, Petoumenos K, Taylor-Robinson SD, Emery S, Cooper DA. Does choice of combination antiretroviral therapy (cART) alter changes in cerebral function testing after 48 weeks in treatment-naïve, HIV-1-infected individuals commencing cART? A randomized, controlled study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2010; 50:920–929. [PubMed: 20146627]
- Witwer KW. Circulating MicroRNA Biomarker Studies: Pitfalls and Potential Solutions. *Clin Chem*. 2014
- Witwer KW, Gama L, Li M, Bartizal CM, Queen SE, Varrone JJ, Brice AK, Graham DR, Tarwater PM, Mankowski JL, Zink MC, Clements JE. Coordinated regulation of SIV replication and immune responses in the CNS. *PloS one*. 2009; 4:e8129. [PubMed: 20019816]
- Witwer KW, Sarbanes SL, Liu J, Clements JE. A plasma microRNA signature of acute lentiviral infection: biomarkers of CNS disease. *AIDS*. 2011; 204:1104–1114.
- Yant LJ, Friedrich TC, Johnson RC, May GE, Maness NJ, Enz AM, Lifson JD, O'Connor DH, Carrington M, Watkins DI. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with control of simian immunodeficiency virus SIVmac239 replication. *Journal of virology*. 2006; 80:5074–5077. [PubMed: 16641299]
- Yilmaz A, Yiannoutsos CT, Fuchs D, Price RW, Crozier K, Hagberg L, Spudich S, Gisslen M. Cerebrospinal fluid neopterin decay characteristics after initiation of antiretroviral therapy. *Journal of neuroinflammation*. 2013; 10:62. [PubMed: 23664008]
- Zink MC, Amedee AM, Mankowski JL, Craig L, Didier P, Carter DL, Munoz A, Murphey-Corb M, Clements JE. Pathogenesis of SIV encephalitis. Selection and replication of neurovirulent SIV. *The American journal of pathology*. 1997; 151:793–803. [PubMed: 9284828]
- Zink MC, Coleman GD, Mankowski JL, Adams RJ, Tarwater PM, Fox K, Clements JE. Increased macrophage chemoattractant protein-1 in cerebrospinal fluid precedes and predicts simian immunodeficiency virus encephalitis. *The Journal of infectious diseases*. 2001; 184:1015–1021. [PubMed: 11574916]
- Zink MC, Suryanarayana K, Mankowski JL, Shen A, Piatak M Jr, Spelman JP, Carter DL, Adams RJ, Lifson JD, Clements JE. High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. *Journal of virology*. 1999; 73:10480–10488. [PubMed: 10559366]
- Zoufaly A, Kiepe JG, Hertling S, Hufner A, Degen O, Feldt T, Schmiedel S, Kurowski M, van Lunzen J. Immune activation despite suppressive highly active antiretroviral therapy is associated with higher risk of viral blips in HIV-1-infected individuals. *HIV medicine*. 2014; 15:449–457. [PubMed: 24580846]

Figure 1. Longitudinal hematologic markers that predict the development of CNS disease in the pigtailed macaque model of neuroAIDS include hemoglobin, platelet count and sCD163

In contrast, circulating monocyte numbers are not predictive. Depicted are longitudinal median values for uninfected (open circle), SIV-associated encephalitis negative (SIVE-, grey square) and SIV-associated encephalitis positive (SIVE+, black triangles) pigtailed macaque groups. (A) Median change from pre-infection baseline of hemoglobin levels, (B) median change from baseline of circulating platelet counts, and (C) median change from baseline of absolute monocyte counts as measure by complete blood count. (D) Soluble CD163 (ng/mL) levels measured by ELISA (Trillium Diagnostics, Bangor, Maine) in plasma. Asterisks (*) denote significantly different blood biomarker levels in SIVE+ macaques compared to SIVE- macaques ($P = <0.05$, Mann-Whitney).

Figure 2. High viral load in the plasma and CSF during post-acute infection is associated with the development of SIVE post-acute infection

However, the magnitude of the difference in viral load is much more pronounced in the CSF (B) than plasma (A). Longitudinal median viral load values were measured by quantitative RT-PCR for SIVE negative (grey square) and SIVE positive (black triangles) groups.

Asterisks (*) denote significantly different viral RNA levels between SIVE+ and SIVE- macaques ($P = < 0.05$, Mann-Whitney).

Figure 3. Elevated CSF markers of neuroinflammation post-acute infection that precede development of SIVE in pigtailed macaques include CCL-2 (A), IL-6 (B), and neopterin (C) Longitudinal group median of (A) CCL2 (ng/mL), (B) IL-6 (ng/mL) and (C) neopterin (nmol/L) as measured by ELISA in the CSF of SIVE negative (grey squares) and SIVE positive (black triangles) pigtailed macaques. Asterisks (*) denote significantly different CSF biomarker levels in SIVE+ macaques compared to SIVE- macaques ($P = <0.05$, Mann-Whitney).

Figure 4. Elevated markers of matrix and neuronal damage in the CSF post-acute infection precede development of SIVE in pigtailed macaques

Longitudinal median of (A) human cartilage glycoprotein 39 (YKL-40) (ng/mL), and (B) neurofilament light chain (NF-L) (log 10 ng/mL) as measure by ELISA in the CSF of SIVE negative (grey squares) and SIVE positive (black triangles) pigtailed macaques. Asterisks (*) denote significantly different CSF biomarker levels in SIVE+ macaques compared to SIVE- macaques ($P = <0.05$, Mann-Whitney).

Figure 5. Elevated levels of miRNA -125b and -146a in CSF during acute infection associated with the development of SIVE in pigtailed macaques

Longitudinal median of (A) miR-125b (fold change over baseline), and (B) miR-146a (fold change over baseline) as measure by a stem-loop RT primer, hydrolysis probe qPCR assay normalized to a sample-specific internal control in the CSF of SIVE negative (grey squares) and SIVE positive (black triangles) pigtailed macaques.

Table 1
Host genetics influence development of SIV Encephalitis in pigtailed macaques

Expression of the MHC class I allele *Mane-A1*084* by pigtailed macaques is neuroprotective against SIV-induced CNS disease, demonstrating the importance of considering individual macaque MHC class I gene expression to refine SIV pathogenesis and vaccine studies. ($P = 0.0317$, one-sided Fisher's exact).

<i>Mane-A1*084</i> Expression	SIVE –	SIVE +	Total
Negative	11	25	36
Positive	9	5	14
Total	20	30	50

Table 2

Summary of predictive markers of lentivirus-associated neurologic disease.

	Biomarker	Findings in SIV	Findings in HIV
Blood	Hemoglobin level	↓ ¹	↓ ²
	Platelet count	↓ ¹	↓
	Absolute monocyte count	No change ¹	↑ ⁴
	Soluble CD163	↑ [*]	↑ ⁵
	Viral RNA	↑ ⁶	↑ ⁷
CSF Neuroinflammation	Viral RNA	↑ ⁸	No correlation ⁹
	CCL2	↑ ¹⁰	↑ ¹¹
	IL-6	↑ ¹²	Not reported
	Neopterin	↑ [*]	↑ ¹³
CSF Neurodamage	Human cartilage glycoprotein 39 (YKL-40)	↑ ¹⁴	Not reported
	Neurofilament light chain (NF-L)	↑ ¹⁵	↑ ¹⁶
CSF microRNA	miR-125b	↑ [*]	Not reported
	miR146a	↑ [*]	Not reported

* Previously unreported data

¹ Wactman et al., 2006² Njamnshi et al., 2009³ Ragin et al., 2011⁴ Sanchez-Ramon et al., 2003⁵ Burdo et al., 2011⁶ Marcondes et al., 2009⁷ Winston et al., 2010⁸ Zink et al., 1999⁹ Cysique et al., 2005; McArthur et al., 2004; Sevigny et al., 2004¹⁰ Mankowski et al., 2004; Zink et al., 2001¹¹ Sevigny et al., 2004¹² Mankowski et al., 2004¹³ Fredrikson et al., 1987; Fuchs et al., 1989; Hagberg et al., 2010¹⁴ Bonne-Barkay et al., 2008¹⁵ Meulendyke et al., 2014

¹⁶ Gisslen et al., 2005; Mellgren et al., 2007

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