Published in final edited form as:

J Neuroimmune Pharmacol. 2014 June ; 9(3): 269–276. doi:10.1007/s11481-014-9545-1.

Cellular Interactions and Signaling in NeuroAIDS: Emerging Issues Colloquium

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Abstract

On May 23, 2013 scientific leaders in the neuroAIDS community met at the University of Nebraska Medical Center to discuss cellular interaction and signaling for the third annual human immunodeficiency virus and neuroAIDS colloquium. The meeting continues a series of contemporary scientific issues related to how virus effects the nervous system. In 2011 the focus was on animal models and in 2012 in biomarkers. Here, our 2013 meeting featured ten presentations from outstanding scientists examining how inter- and intra-cellular processes contribute to neuropathogenesis. Talks highlighted emerging issues, findings, and potential therapies, followed by a panel discussion in which controversies in the field and gaps in our current knowledge were identified. The panel discussion was transcribed into the article and published as a field perspective. A link is available where all of the presentations and the concluding discussion can be seen and heard.

The third annual University of Nebraska Medical Center (UNMC) colloquium on current issues in neuroAIDS was held on May 23, 2013. Following the presentations, which can be viewed at <http://www.unmc.edu/pharmacology/CISN.htm>. A panel discussion ensued. This discussion raised important topical issues. To disseminate this information, a transcript is provided below.

Dr. Howard Fox: First, let me thank once again all of our speakers, everyone at UNMC who helped organize the meeting, our third annual colloquium, and all the attendees both in person and on the internet. Thus far it's been a wonderful day and we've learned a lot of

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new things and have started quite a few fruitful discussions that I'd like now to continue in this discussion. In addition, if any of the attendees have any topics or questions you would like addressed please let us know.

I'd like to start off this discussion with the effects of therapy. Kelly Jordan-Sciutto brought this up in her talk on the effect of the drugs themselves on neurons, and Howard Gendelman in his on novel formulations for long-lasting antiretrovirals. In addition there is currently an ongoing debate concerning brain-penetrating antiretrovirals: do we need them? I think the debates pretty irrelevant outside of countries that have access to current antiretroviral treatment regimens, but here is a concern for health care providers and infected individuals. So let me open that up. What are your thoughts on brain penetrating antiretrovirals?

Dr. Kelly Jordan-Sciutto: One of the reasons I actually started this project was an interest in the field on whether the CNS reservoir for HIV could be cleared by highly CNS-penetrant antiretroviral drugs. I wondered whether there would be increased neurotoxicity due to CNS penetration of antiretrovirals since we know that peripheral neuropathy and some other toxicities are caused by a subset of antiretrovirals and brain cells tend to be more vulnerable than peripheral cells (Akay et al., 2014; Zhang et al., 2014). Currently, reports in the literature are controversial on the benefit of CNS penetrating treatment for HAND. One of the main variables could be the length of treatment; initially CNS penetrating drugs may be beneficial by lowering viral titers, but over the long-term studies may not show significant cognitive improvement and may actually show cognitive decline due to toxicities. Although I wasn't at CROI this year, an update was given on a prospective study looking at drugs with increasing CNS penetration. It is important to consider both the short and long term effects on neurocognitive performance as we move forward with antiretroviral therapy. If there are side effects, but they are beneficial virologically, can we find things to mitigate these side effects? Also as we progress perhaps could we develop better drugs that don't have the side effects.

Dr. Fox: Thanks. The effect of these drugs on neurons and CNS function is important, certainly the blood brain barrier exists for a reason, it keeps a lot of things out of the brain that could damage it.

Dr. Jordan-Sciutto: It's good to have a blood brain barrier.

Dr. Dennis Kolson: Yes, I was at CROI but I want to defer that question about the CPE efficacy and outcome to Howard Gendelman. He was also at that session and asked the question directly so and I'll let him answer that; I note that I happen to agree.

Dr. Howard Gendelman: Dennis, thank you. They're two points to this query. The first is that the best central nervous system (CNS) penetrating drugs are commonly the most toxic (Abers et al., 2014). Common adverse events include nausea and vomiting, headache, peripheral neuropathy, neutropenia and anemia, lactic acidosis, hepatomegaly with steatosis, oral and esophageal ulcers and pancreatitis. The second and perhaps even more significant issue is that these antiretroviral medicines are amongst the least efficacious. Many are used either not at all or infrequently and include, for example, zidovudine, didanosine, zalcitabine and abacavir. The class of drugs is within the nucleoside reverse transcriptase inhibitors.

Thus, even if you're able to get the drugs across the blood-brain barrier, is patients will not adhere to the medicines because of secondary adverse events and the antiretroviral responses are limited you will have, overall, a reduced therapeutic response. Third, is that it may not be necessary to have the drug reach the nervous system to treat nervous system disease. Virus is constantly trafficking from the periphery to the CNS through monocyte-macrophages and CD4+ T cells and can both enter and exit through a broad range of mechanisms. Thus, inhibiting peripheral infection may be sufficient to affect the viral burden in sanctuaries like the brain and spinal cord. We just don't have enough knowledge on the topic to be absolutely certain of cause and effect. This leads me to the final question on the topic is what is the future in eradication of HIV in its CNS sanctuary? What types of approaches are we seeking and should such approaches be CNS-centric? At the present the answer, at least to my mind, is not clear.

Dr. Lena Al-Harthi: Maybe I could just say something, and that is I think the field has been very remarkable in pushing a lot of the description of what happened in neuroAIDS. What are the important cells? But, one of the things that I think we should do is to dig a little deeper and start looking at mechanism. Mechanisms including interactions within the CNS, because that may not only drive what we're seeing in neuroAIDS or HAND, but also that just gives us a great deal of information about the biology in neuroscience. While we can study individual cells, the CNS works through interaction between cells. So even though such mechanisms and interactions are complicated, when it comes to HIV and CNS, some of information that we can unravel through mechanistic studies will be informative in other neurodegenerative diseases and just science for the sake of science.

Dr. Fox: I'd like people to amplify on what Lena just said. In particular, Jonathan I'd like you to comment as your talk clearly relates to other neurodegenerative disorders. One debate that often gets going is like the chicken and egg with neuroinflammation and neurodegenerative disease. Did it start there or did it not? In one sense it doesn't matter clearly in neuroAIDS whether it was with the old encephalitis or the current one where it's hard to pin down a characteristic pathology. There is immune activation. In HIV infected individuals there is chronic immune activation both in the body and the brain; this is one aspect in common between HIV, Alzheimer's and Parkinson's disease, in that in the brain one sees signs of immune activation (Amor et al., 2013). In Alzheimer's and Parkinson's diseases we don't know the cause in the vast majority of cases. But, in HIV dementia and HAND, we know the initial cause, it's HIV. So we actually have one advantage over those other diseases in that we have the inciting agent, but the manifestation of HAND has clearly shifted with time. But that has put us in a special situation now that if we could understand the mechanism of neurodegeneration, and perhaps a mechanism for neuroprotection, this could apply not only HIV but also to other disorders.

Dr. Jonathan Geiger: Well, you just said exactly what I was going to say in response to Howard Gendelman. My take on this is that we know the starting point. As an example, for many years Avi Nath and I received grants from the Alzheimer's Society as they were supporting studies on Alzheimer's and other related dementias. While there was no evidence that the two diseases were coming together at that time, we knew the starting point for AIDS dementia, the virus HIV-1. HIV infection was the start. For no other neurodegenerative

disease other than possibly head trauma do we know the initiating event (Canizares et al., 2014). So, I think this is a huge advantage and one that we should take advantage of. It may give us that edge at a time when such insights are very important.

Dr. Shilpa Buch: Lena has made a very valid point about understanding the mechanisms involved in cellular interactions of HAND. The current focus has to be on unraveling these pathways. Jonathan, I had question for you about the role of viral proteins. Have we systematically looked at what proteins exist in the CNS in the era of ART? What are the reservoirs in the CNS and do we know where the virus is lurking? It is important to understand whether these processes underlie the chronic activation observed in HAND. Focusing on effects of chronic activation and the presence of viral proteins and their source and eradication from the CNS are important areas of study.

Dr. Jordan-Sciutto: If I could add, and this sort of builds on Howard Gendelman's point earlier which is that some of the antiretrovirals are differentially effective in culture or in vivo on viremia, but I don't think that we actually know how effective they are against viruses in CNS cells. What is their efficacy in astrocytes and in microglia versus macrophages, monocytes and T-cell? And, so what cell types do we test antiretroviral activity? And, how does that differ in vitro versus in vivo in each of the tissues, in particularly, the CNS. And so, while we may be able to get these drugs across the blood brain barrier and have them persist in the brain, they still may not be the right tools to get rid of the virus in the cells of the CNS.

Dr. Jay Rappaport: I think that when it comes to what Howard Gendelman was asking regarding what we should really be thinking of, in in terms of what we contribute. What we can contribute as a field are also in areas common to the overall HIV/AIDS field. I think to identify questions that are of major importance, we should think of topics not just in our field, but also in the HIV/AIDS field in general. We were talking earlier about reservoirs. Clearly, the CNS is a major reservoir, and the macrophages that invade the CNS are cellular reservoirs. When you look at brains from encephalitis patients, you see the macrophages in the brain and they make virus; whereas, macrophages don't produce virus in some of the other organs. So the CNS may be unique and I think we should be looking at mechanisms for suppression and eradication that pertain to the CNS. From what Kelly Jordan-Sciutto was saying earlier, I agree using antiretroviral drugs is not going to be the be all and end all to get rid of virus in the brain. A recent study reported that some regimens that have higher CNS penetration effectiveness actually impair cognition, so that I think it's clear antivirals are not the only way to go; we need to come up with other strategies.

Dr. Kolson: I'd like to emphasize what Jay just said about the point, "What can we contribute to the HIV treatment community in general." As an example, when we were beginning our studies on HAND, we had some leads on the heme oxygenase-1 (HO-1) story (Cross et al., 2011). The approach we're using with dimethyl fumarate and this class of compounds, which induce HO-1 expression, has broader implications for the HIV treatment community; the drug has immune suppressive effects in multiple sclerosis patients who are treated with it. I approached the head of our HIV clinical trials group at UPenn and I said, "Am I crazy to even think about pursuing this as a treatment in HIV-infected people if it's

going to be considered to be an immunosuppressive type of drug?” He got all excited and said, “No that’s exactly what we want!” He also said, “If you can do this in your model system and you have effects in the brain, that’s terrific, but I want to look at this to suppress systemic immune activation. We just did a cyclosporine study in HIV-infected patients with the goal of suppressing systemic immune activation to attempt to alter disease progression.” He then said, “You need to push these kind of things into pilot studies.” He was telling us that there’s something we (neuroAIDS researchers) can contribute to the field of HIV therapeutics, and all of this work in mechanisms of neurodegeneration can inform the rest of the HIV research field.

Dr. Al-Harathi: I also think it would be helpful to sort of define what is an acceptable degree of inflammation, because inflammation is not all bad. There’s a certain level of inflammation that is important in natural defense processes. So what is the balance and what can we tolerate in the CNS?

Dr. Kolson: That’s an interesting point. I am involved with the treatment of multiple sclerosis patients and, as many of you may be aware, there is a monoclonal antibody that we infuse monthly into patients. It’s called natalizumab, and it targets VLA-4 integrin on activated T cells and monocytes, and it is incredibly effective at limiting migration of activated T cells and monocytes into the CNS (Romme Christensen et al., 2014). It has profound prophylactic benefit in multiple sclerosis but it really shuts down immune surveillance in the CNS. Well, with that effect this drug is associated with a small, but significant risk of PML, progressive multifocal leukoencephalopathy. Thus the thinking is that it’s perhaps too effective at limiting the associated CNS inflammatory responses. But we think that’s an extreme case of completely shutting down CNS immune surveillance, although most of the other approaches for limiting CNS inflammation and immune activation are unlikely to be that profound. In the psoriasis literature there are patients with seventeen years of treatment with the class of immune-suppressing, anti-inflammatory compounds that dimethyl fumarate belongs to, oral compounds that show no increased risk of opportunistic infection or other infections despite a modest decrease in total circulating T lymphocyte counts. People in the rheumatology and cardiology fields are looking at this class of drugs because they think that these drugs can strike the right balance in suppressing excessive immune activation and inflammation while still allowing an essentially normal level of “good” inflammation.

Dr. Sabita Roy: It is interesting that parallelism exists in bacterial and viral clearance mechanism. Both extracellular bacteria and HIV inhibits phagolysosomal fusion thus compartmentalizing the pathogen leading to persistence and latency. Viral proteins such as TAT and gp-120 modulate lysosomal acidity and not only prevent viral eradication but may also sustain opportunistic infection such as TB or *S. pneumonia* (Dutta et al., 2012). In fact, Dr. Howard Gendelman used the same system to sustain and maintain viability of his nanoparticle antiretroviral therapy as well. Thus, one can target the delivery of the drug to the same compartment that concentrates the virus. As we know as reported by the more recent study that the reservoirs are formed very early following infection, and once integrated is difficult to eradicate, because of the extended half-life of these cells. Furthermore these infected reservoir cells can function in cell-to-cell transfer of the virus.

Dr. Fox: These are great points. I'd like to comment on two points. First, that the effect of the HIV on CNS cells and antiretrovirals on these cells can be clearly different than found in the immune cells typically studied, which are typically T cells, and hopefully sometimes peripheral macrophages. It is important that was mentioned because if we know some treatment is great against T cell carried virus in the blood or lymph node that doesn't mean that even if we can get it through to the brain it's going to do anything similar to the infected cell in the CNS. So that's an important issue and I hope we can elaborate on that further. The second point concerns IRIS – Immune Reconstitution Inflammatory Syndrome, it's a problems now in the clinic, and affects organs including the CNS (Bahr et al., 2013). I wonder if a fumarates may be an approach to treat their patients, in which we need to calm down the T cell storm and the same time also calm down activated macrophages and associated immune pathogenesis. Any comment on that idea? Let me start with Dennis.

Dr. Kolson: So the question is of what might be the effects of fumarates on T cells function and IRIS?

Dr. Fox: Yes. IRIS is characterized by T cells, their localization/concentration, activation and activity. So could fumarates, which tone down the T cells, be part of an adjunctive therapy?

Dr. Kolson: Yes. That question came up several years ago when I first started talking about this class of drug, fumarates. Someone said, "Well if you have a drug like this that's going to be approved for treatment of multiple sclerosis could it also be used to treat IRIS in MS patients that is caused by withdrawal of other MS drugs, such as natalizumab?" Currently there's no proven way to prevent or effectively treat IRIS. We do treat IRIS with steroids but we don't really have an effective treatment paradigm to prevent it when we withdraw people from the Natalizumab antibody therapy, which results in a high risk for IRIS. We know that this class of compounds (fumarates) inhibits migration of activated monocytes (CD14+, CD 16+) and T lymphocytes into the brain in experimental allergic encephalomyelitis (EAE) (Lee et al., 2008). Fumarates are also very effective at limiting CD4+ and CD8+ T cell infiltration into psoriasis lesions in the skin. So, with all of this information, one would think that this type of drug could have a prophylactic use in preventing IRIS, including that associated with HIV immune reconstitution in patients on ART. Along those lines, we've shown that dimethyl fumarate suppresses CCL-2 mediated monocyte chemotaxis and that it probably decreases expression of CCR2, so its pleiotropic effects hit multiple steps that might be relevant to IRIS and to HIV entry into the CNS compartment. We don't know if all of these pleiotropic effects are related to its ability to induce heme oxygenase-1 (HO-1) expression. One important question, however, is whether *withdrawal* of fumarate therapy would result in an increased risk of IRIS. We don't think that's going to be an issue with fumarates because none of the 2600 multiple sclerosis clinical trial patients treated for two years who came off the drug for one reason or another developed IRIS.

Dr. Fox: Did you address the question? Specifically, does fumarate have the potential be a treatment for IRIS?

Dr. Kolson: I think the answer is very likely yes.

Dr. Jordan-Sciutto: I would like to add a comment regarding Dennis' fumarate study. It targets several cellular mechanisms, but it does so by understanding the mechanisms and then harnessing the power of the cells' own machinery. What's nice about the fumarates is that the system allows for the cellular response to be shut down when it is not longer needed and keep the stress pathways in balance. Instead of using gross general anti-oxidants to try and to overcome oxidative stress or strong anti-inflammatories to just completely shut down the inflammatory system, fumarates are a more subtle tool that allows the cell to dampen the response when it's necessary but then also to have the feedback to enable the cellular mechanisms to turn on when needed, modulating HO-1 and other pathways that are still active even when you're in the presence of the fumarate compounds. I think that's an important advance that these compounds aren't just shutting something down or just trying to shut down one thing, they have pleiotropic effects and they do so in the context of cellular feedback mechanisms that allow them to respond appropriately or maintain some sort of balance or homeostasis.

Dr. Johnny He: I want to go back to the very first question that Howard Gendelman brought up AIDS research or neuroAIDS research field in that what we can bring to the community? I think it is very important for us to continue to do what we have been doing. I think, as Lena pointed out the basic research is important. We know the NIH director Francis Collins is under great pressure to produce, meaning emphasis on translational research, thus I think this is represented in the Kelly's and Howard Gendelman's presentations on the antiretrovirals. We do have to think about the translational aspect of what we are doing, so we can really identify what we can bring back to the community. We all strive to improve the quality of the life of HIV-infected individuals. As we all know translational research at the NIH now has its own center, the National Center for Advancing Translational Sciences, NCATS. We can approach them for research ideas, and do need to put our efforts there. For example inflammation is a common pathway that can be targeted. We need to keep the translation aspect of our research in mind; I think that's very, very important.

Dr. Fox: I agree and let me just say a comment and then open this up. One problem is that the clinical trials are by their nature very expensive, even when done on a small-scale for neuroAIDS. Unfortunately the ones for neuroAIDS haven't born fruit, of course it is only now that some promising results are being achieved in the vaccine field but no one would doubt that we need a vaccine but people will say well why do a trial for neuroAIDS? What have we learned from those trials and other studies and where can we go translationally?

Dr. He: I think that's where we can probably spend lots of time to discuss this because we should ask ourselves, the neuroAIDS field as a whole, how much have we brought back to the community? I mean after twenty-thirty years we really need to ask ourselves about this. I understand that the basic research is very important. I'm a basic researcher and 100% for that, but again I think the translation aspect of that is probably where we need to put our brain power.

Dr. Al-Harthi: I absolutely agree with that, but at the same time I don't understand why that burden is being placed on the neuroAIDS field? When you look at Parkinson's, you

know there are no effective drugs to stop its progression, similar for Alzheimer's and many other diseases. That's my issue because sometimes I think that one can jump into clinical and translational studies without really understanding the basis of what's going on and then those studies end up being not productive and thus doing harm to the field because we're not very well informed about how we want to approach it. I think you just have to be targeted and I agree that if there's an opportunity to turn it into translational studies absolutely that's the way that we should all try to go.

Dr. Gendelman: This brings us to what is perhaps our most important question and that is what is our future? How can we best make an important contribution to the HIV/AIDS field? Where do we fit within the collective and what should be the topics and directives we need to focus on? To address this issue we must take into account the changing epidemiologic trends in neuroAIDS. As we all understand and appreciate it is much more a multifaceted disease complex where viral infection per se is one amongst many other disease inciting elements. These include co-morbid infections such as hepatitis, depression, abused drugs, age and aging, race and social issues amongst others. So past days when we considered the role of the virus as a primary driver has been replaced with a disease complex that is mild in severity and complex in pathogenic mechanism. Now substance abuse, metabolic syndromes, and long-term toxicities of antiretroviral therapies better define cognitive abnormalities as medicines used to treat HIV-1 become widely available across the globe and where compliance for medicines is the norm. So, we must be increasingly vigilant to best define the disease complex as it evolves, continuously look at ways to recognize it and intervene and consider the greater goals towards viral eradication. In this way we can, as a group, continue to make important contributions to the overall HIV AIDS effort.

Dr. Jialin Zheng: I want to add a few points and address what Johnny said about what we can bring to the table. What can we learn from neuroAIDS? Actually, we have learned much. The past 20–30 years of AIDS research, including that on the brain, have led to a significant accumulation of knowledge and a good foundation for how damage to the brain leads to neurodegeneration. I think this very important knowledge can be applied to the other neurodegenerative disorders. I think that is how we serve. The most important point is what is the next step, the next stage of investigation and what we should focus on in terms of a potential method or therapeutic means for repair. Kelly's work is related to how the therapy would affect the brain. Also, we need to examine the role aging will play. Fortunately, HIV infected people are now living longer and with a greatly improved quality of life. I think those are important questions and events that we should focus on

Dr. Jordan-Sciutto: Earlier, Howard Gendelman asked me what we should prioritize out of my studies. I would like to add that when I think of HIV disease, the patients have to take antiretroviral drugs, and so either we need to focus on getting better drugs or we need to focus on getting better adjunctive therapies. What we can't do is change that they are aging and right now we can't change that they are HIV infected. We can talk about the vaccine trials and we talk about our failures in neuroAIDS adjunctive therapies, but we still don't have a vaccine for HIV and we don't have a way to eliminate the virus. It is not like this isn't a problem we are not all trying desperately to solve, but there are some real things that

we can control and those things are the therapies we are giving them and the potential adjunctive therapies that we can create.

Understanding the mechanisms that underlie neurodegenerative diseases leads us to think that those adjunctive therapies for HAND would likely be applicable to Alzheimer's, to Parkinson's, and potentially even Huntington Disease, and some other genetic trinucleotide repeat diseases that also have some components that are common including misfolded proteins and oxidative stress. When you look at many of these things, some of the pathogenic mechanisms are quite similar. With neuroAIDS, the clinical trials haven't been successful at reducing HAND, but many of them have either been rather blunt tools such as over treating with antioxidants instead of for example inducing an enzyme that can be responsive when it's needed or pull away when it's not. So we have finer tools and we have a finer understanding now that it may not be effective to give those therapies when someone's already progressed to HAD or even probably when they are in minor cognitive disorders. Therapies have to be given when they're in the ill-named asymptomatic neurocognitive disorder phase. That's when we need to treat or maybe even before that. So, given the HIV-infected population, the question is can we design preventative therapies and test them in this population? I understand that would be a much broader and more expensive trial than treating people who are already down the path to cognitive decline, but I think that by the time they are down the path, unless we develop biomarkers, which obviously is another one of the areas that the field has been focusing on, this will be difficult and that development will take time. I think the only way for these preventive therapies to show efficacy is to do a clinical trial that allows for success instead of ones that set up for failure. I don't have a great answer as to how to get such things funded or implement them, because I'm not a clinician; but I do think that we set ourselves up in these clinical trials to fail by treating patients too late with large hammers instead of finer tools to treat the disease.

Dr. Fox: Many have talked about mechanisms, however we have many different potential mechanisms. We are in a bit of a dilemma, because as Howard Gendelman states HAND is evolving process. We certainly learned a lot from the era of HIV encephalitis and dementia. The question is, is that knowledge applicable now or is this a different disease? Or it's the same disease and those who go off or not adherent to their antiretrovirals can develop one related form of it, and certainly in resource poor countries without access to many of the treatment options there is another related form. Jonathan I'm now going to pick on you because you came with a good idea for a database. The question is, is it feasible? What should we put in it? And, how would it be used? I know it was an off-the-cuff remark you made as we were coming down the elevator but please elaborate on it a bit. I think it might have traction.

Dr. Geiger: Right, so let me say this though because both of Howard Gendelman's points, while excellent, have a real depressing side to them. As a result, we could either get depressed and take our ball and go home, but I've never known him to back off from a good challenge. I can't imagine him or any of us as well backing off from what we perceive to be important. Rather, we have to do things smarter, and follow the old adage that you go big or you go home. It wasn't so many years ago that on the basis of for example a single receptor binding assay that a drug went into clinical trials. I know people have thought that we should

have a more comprehensive bank of in vitro and in vivo assays to really get a more comprehensive picture before we put a drug into trials, and without it we set ourselves up for failure. So one of the comments I made in the elevator which I will preface it by saying this is maybe naïve, but in this day and age where people are making biological maps of various things maybe it's time that the neuroAIDS field starts putting together some of the signaling maps and this is the topic of our symposia today, signaling. Maybe, we should really try to identify the key signaling in the different cell types, the different organs that are really at play, and it maybe through that map the ACTG could better identify more comprehensive targets, even maybe criteria by which any compound going forward could be measured, and that way we could get the backing of the ACTG and the neuroAIDS community and might have a better chance of success at treatment. So, I think in this day of so many "omics" maybe we should take advantage of that.

Dr. Rappaport: But I think we should think about that as a Venn diagram, there's neuroAIDS and there's HIV/AIDS, and then there are some things in the middle that are common to both and represent the common pathways. These common areas of research, even if coming at them from different directions, will likely meet in the middle. If we pursue those areas, I think we would have the most impact. For example, a CD4 nadir seems to correlate with neurocognitive impairment and neurocognitive impairment is predicted mortality in HIV/AIDS in general, so clearly there's an association between neuroAIDS and AIDS. I believe determining the important connections will have greater impact than trying to work exclusively within the neuroAIDS compartment. Reaching across fields will find commonality.

Dr. Kolson: So, I'd like to make a follow-up comment. Howard Gendelman made the point that we have to answer the question what are we studying and what do we have to offer, and Lena made that we're held to a standard we have to come up with something in our treatment trials and failures are thrown back at us in comparison with AD and PD which haven't had, although there is one FDA-approved drug for Alzheimer's disease now but all told considerably more effort that the we have put in. I think it's as much we're a victim of the success of treatment of HIV/AIDS with antiretrovirals because I have plenty of people come up to me and say that AIDS dementia is gone now, it's done, we're not worried about it, there's nothing left. I think Howard Gendelman's point is that we really do have to define what we're studying. I personally think that we have to look at the classification of these patients and that key is the functional impairment that they still have and that's not getting across in many of our talks and presentations. The classification for HAND use now that Kelly talked about – asymptomatic neurocognitive impairment – means that there's dysfunction on at least two of these sophisticated tests, showing dysfunction from norms but no functional impairment in daily living. So it's considered by many clinicians to be insignificant so you have test functional impairment, but that middle group with such impairment is still thirty percent of antiretroviral treated individuals by very good data from San Diego. That's thirty percent, plus or minus a few percent that not only have the neuropsych test dysfunction but they have functional impairment in a number of daily activities that means they're living morbidities that affect their productivity. Likely long

term they will get worse with age. Even two percent that have dementia now, everyone recognizes that you don't want to have that.

But we have to be clear when we talk to people that's what we're studying. We're studying a disease that is truly a chronic disease. It's pathologically different now and there are a number of markers that are coming out that will let us track it. We have to have these; we can't do pathologic studies to define stages in living people. So how do we track them? A lot of work going into biomarkers is important and that has to continue the basic research there. The imaging is extraordinary now that many of us aren't that familiar with but I think the imaging techniques are showing us things that we can't detect any other way. That's what we have to offer in neuroAIDS. There isn't anything comparable to our neuroimaging studies that tell you in real-time what's changing in the bodies of HIV-infected individuals. Some applications of MR spectroscopy studies and blood flow studies of the bold fMRI-type are capable of showing disease state and response to therapies, so we're going to inform the HIV research community what antiretrovirals and adjunctive therapies do to the brain in real time.

We need to redefine as Howard Gendelman said in the era of antiretroviral treatment I think we have the tools to do that. As Kelly we've got to identify and treat those at risk prophylactically. The theme that from the last thirty years in multiple sclerosis treatments is that now we diagnose and treat people before they even have clinical diagnosis of MS. Why do we do that? We have imaging criteria that are very sensitive now and we treat because we want to treat before they have the disease and it's enormously effective. We have the CHARTER study that follows HIV-infected patients at multiple centers, longitudinally without selection criteria to follow their neurologic function long-term, we have newly diagnosed patients going on therapy and studying them will inform us on who we can identify as "at risk." (Marcotte et al., 2013). We already know that those with low CD4 nadirs are, but there is likely more. I think we have all the tools in place I think we have to voice this more clearly and be a little more rigid in our definitions and understanding that we do have a problem. Antiretrovirals are here to stay. They've done so much for patients. This is what we have to offer the rest of the community now. And there distinct potential benefits of what we're going to come up with in the brain targeted therapy or maybe those not even targeted just to the brain.

Dr. Fox: Dennis, I don't think I could summarize things any better. I wanted to add one comment, a very astute comment from the web. While it is true that HIV dementia and HIV encephalitis are greatly reduced in the US and Europe, more than half the world is still experiencing the same magnitude of HIV disease in individuals as we had in the nineties, and on a societal basis much more, as the percentage of infected people is much higher; while we have 1–2 million infected in North America, there are 20–25 million infected in Sub-Saharan Africa. Certainly we don't want to ignore the rest of the world, of course we have to justify it locally in order to get the funding, but these problems are quite real and of great magnitude, and the result of the rollout of antiretroviral treatment may not be the same as here for many, many reasons.

It has been a delightfully productive day and I want thank everybody greatly. This panel has been absolutely wonderful both in their talks and discussions. I'd like to thank the audience here in person and on the Web for their thoughtful and insightful questions and comments. And once again let me thank Robin Taylor and Sandy Amendola, and the rest of the UNMC Department of Pharmacology and Experimental Neuroscience for their help and support. Everyone have a safe trip home, and we all look forward to more productive interactions on these issues. I hope you can join us for our fourth annual colloquium, on the role and use of brain banks in neuroAIDS, other neurodegenerative disorders and mental health, to be held May 21, 2014, more information will follow.

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