Immune_Activation_in_Treated_HIV_Infection_20181009_Hunt_360p.mp4

[00:00:01] Welcome to Physicians Research Network. I'm Jim Braun the course director of the monthly meetings of PRN in New York City. Since our beginning in 1990, PRN has been committed to enhancing the skills of our members in the diagnosis, management, and prevention of HIV disease as well as its coinfections and complications. We hope this recording of Peter Hunt's presentation 'Immune Activation in Treated HIV Infection: Does it Still Matter?' will be helpful to you and your daily practice. And invite you to join us in New York City for our live meetings in the future. PRN is a not for profit organization dedicated to peer support and education for physicians, nurse practitioners, and physician assistants. Membership is open to all interested clinicians nationwide at our website PRN.org. And now allow me to introduce Peter Hunt Associate Professor of Medicine in the Division of Experimental Medicine at the University of California, San Francisco.

[00:00:56] Thanks Jim for the invitation. And I'd like to start by just addressing what life expectancy looks like in the modern treatment era for people living with HIV in red, compared to the general population in blue. And what you can see is that while life expectancy has been increasing in the modern era, there is still about a 12 year gap in life expectancy despite the fact that most people are able to achieve and maintain viral suppression if they get access to medications. And the gap really narrows even further to about six years if you restrict to people with HIV who had a CD4 nadir above 500 at the time they started therapy, and exclude smokers, drinkers, or people with viral hepatitis. That sort of narrows the pool significantly, but it does demonstrate the value of early initiation of antiretroviral therapy to increase longevity.

[00:02:08] But it's also important to point out that life expectancy is really dramatically reduced by advanced disease prior to ART initiation and sort of delaying ART to a low T cell count. And this point was made in the NA-ACCORD Study several years ago now, where they just followed the life expectancy of your average 20 year old starting therapy over the early 2000s. And regardless of what CD4 count you started ART at, above 350 in orange and less than 315 green, there was a measurable increase in life expectancy over the decade, which is good. But there's this persistent gap between the two, it's 20 years lower in people who started at a low CD4 count. And that's a pretty dramatic difference and that's important because of the 20 million or so people who are currently on ART globally. The vast majority of them have started at a CD4 count below 350 and so the vast majority of the treated population globally fits this profile and is expected to have a strikingly reduced life expectancy compared to the general population.

[00:03:32] And it's not just decreased life expectancy, but there are many age associated morbidities that have also been shown to be increased in the context of treated HIV infection. Cardiovascular disease, non-AIDS cancer, bone fractures, COPD, liver disease, kidney disease, cognitive decline, non-AIDS infections. Intermediate-stage macular degeneration actually has also increased in the context of HIV infection, particularly people with a history of AIDS there is about a four-fold increased risk of this. So this is a new addition to the list of morbidities. And of course frailty, a syndrome of multi morbidity that we normally think about in elderly populations, we see younger people living with HIV particularly those who started ART at very low CD4 counts.

[00:04:25] And it's not just single morbidities, but a confluence of multiple morbidities that we see particularly in older people living with HIV. This is from the AGE HIV cohort in Amsterdam and they had a well-matched HIV infected and uninfected groups, stratified by

age. And you can see that once you get over the age of 50 among people living with HIV, you see a lot more people with at least two or more comorbidities as compared to the HIV uninfected population. And so it's multi morbidity that we're seeing in many of our patients.

[00:05:08] And so one may ask whether HIV accelerates aging? Well not exactly. And I think the best illustration of this is by focusing on cancer, because it's not all cancers that are increased. But infection-related cancers are really dramatically increased in the context of HIV, regardless of age stratum. Here in red is the incidence of infection-related cancers in HIV and the general population is down here, and you could see across all age strata there is an increased risk. A similar pattern is seen for smoking-related cancer, although not quite as dramatic. The purple line reflects the relative risk in HIV compared to the general population and you'll see that with advancing age, the relative risk starts declining as the risk in the HIV-negative population starts climbing. And so this is not accelerated aging, this is just accentuated aging. HIV is increasing the risk across all age groups, it's not accelerating the age-associated increase in cancer. And the other point to make about cancer is that it's not all cancers that are increased in the context of HIV. If you look at very common age-related cancers like prostate, breast cancer, and colon cancer they are not increased in HIV. In fact some of the earlier studies, and part of this may reflect a surveillance bias, but they they showed actually decreased risk of colon cancer and breast cancer and prostate cancer in the context of HIV. But in the modern era, in this population-based study from the Danes, you see really just almost identical risks in HIV positive versus HIV negative populations. So HIV does not increase the risk of all cancers, including colon cancer. I think that was another one of the questions.

[00:07:21] The other point to make about the diseases that are really profoundly increased by HIV relates to the timing of antiretroviral therapy initiation. So what everyone remembers from the START Trial just published a few years ago, is that early initiation of treatment in some of the high CD4 count profoundly decreases the risk of AIDS events on the left. It also decreases the risk of non-AIDS events, although somewhat less dramatically. But if you take a look under the hood at the non-AIDS events that were reduced by early treatment initiation, they were infection-related morbidities primarily. TB, bacterial infections, Kaposi's sarcoma, lymphoma, non-AIDS cancers. But a lot of these were infection-related cancers that were reduced. And if you looked at cardiovascular disease, neurocognitive dysfunction, and pulmonary dysfunction really no evidence for a benefit of early versus slightly delayed antiretroviral therapy. It was primarily in these infection-related outcomes where you saw the biggest impact. And the other thing is that it wasn't just that early initiation of treatment dramatically reduce the risk, but there were still a persistently increased risk of infectious complications compared to what you would expect in the general population. For example, there was a 1 percent risk of AIDS in people who had immediate ART initiation in START. I think there was some KS for example, even in people who started ART at a high T cell count suggesting that there may be there is some subtle immunodeficiency that may still be at play.

[00:09:20] And even more compelling observations to support this notion came from the Temprano Study. This came at around the same time, this was a treatment strategy trial done in Western Africa, Cote d'Ivoire where people were randomised to either immediate or delayed ART plus or minus 6 months of isoniazid prophylaxis. And again what everyone remembers from this, was that early initiation of treatment dramatically reduced the risk of morbidity and mortality mostly from TB in this setting. But even if you look out here, at the people who got immediate ART at a cell count, there was still a five to seven percent risk of severe morbidity and mortality most of which is from TB out at 30 months. That's an extraordinarily high rate of morbidity and mortality, much higher than you'd expect in the

general population. And in this same study isoniazid prophylaxis actually reduced mortality, six months of INH reduced mortality in this study regardless of CD4 count. When I was a college student, before a medical school, in the summers I worked as a phlebotomist and I converted my PPD and I took INH for six months. Never in my right mind did I think that that was going to reduce my mortality over the next 30 months, but it does in Western Africa if you have HIV.

[00:10:53] And so that's pretty profound that there's really a significant increased risk of infectious, complications even in people who start early. Further underscored by data from South Africa, Gupta and colleagues published several years ago, where they saw an over five-fold increased risk of tuberculosis in ART-suppressed HIV infected individuals compared to the general population. And this continues to be the case, a three-fold higher risk even when you restrict to people with a CD4 count above 700, which is normal in that setting. So there appears to be this continued risk of infectious complications, even despite early treatment initiation.

[00:11:47] That's not really the case for other morbidities as I've tried to allude to. Cardiovascular disease in this particular case. I mentioned that in the START Trial there was really no evidence for a benefit on cardiovascular events with immediate versus delayed treatment. But when you plot the incidence of cardiovascular events from START and from the earlier SMART Trial, which was also a treatment strategy trial of intermit versus continuous ART guided by a CD4 count among chronically infected patients with lower nadir CD4 count, you saw much higher rates of cardiovascular disease here and you also saw that interrupting ART in that setting really increased the risk of cardiovascular events. And the curve started separating in that trial by six months, very different than what we saw here. And so there may be something about having a low nadir T cell count that increases the risk of cardiovascular disease in the context of HIV. And I think cardiovascular disease is likely this way and there may be many other morbidities perhaps pulmonary disease, perhaps neurocognitive dysfunction, where these diseases are really low CD4 nadir diseases. Not that all people living with HIV have this increased risk, primarily people with low CD4 nadirs.

[00:13:21] So I'll come back to that concept toward the end. So why is all this happening? Well people living with HIV are more likely to smoke or use drugs which may contribute to risk. There may be direct toxicity to the drugs, but many of us have focused in on the role of persistent inflammation in driving these complications.

[00:13:43] And we've thought this because of an important clue from nature and many of you have seen these types of talks before and have been introduced to the Sooty Mangabey monkey before, on the left. It's found in Sub-Saharan Africa, naturally infected with SIV, experiences high levels of virus replication comparable to if not higher of them we see HIV infected people, yet it lives a normal lifespan and doesn't get AIDS. But you take the same virus and put it in a different monkey, in this case the Rhesus Macaque on the right, has comparable levels of virus replication but progress is very rapidly to AIDS and death. And the difference between the two scenarios is not the virus, the virus is exactly the same. But rather it's the response of the immune system to the virus that determines how rapidly the monkeys progressed. The monkey that doesn't get sick has very minimal levels of immune activation in the chronic phase of the infection, whereas the monkey on the right experiences massive levels of immune activation. And the more of it they have, the more rapidly they progress. And it's not just the T and B cells that are responding to specific HIV antigens or SIV antigens in this case, it's the global T and B cell populations that are getting non-specifically activated. The natural killer cells are getting

activitated, dendritic cells, monocytes, macrophages are getting activated. So non-specific innate and adaptive immune activation that seems to predict increase clinical progression.

[00:15:27] And the same thing is seen in HIV infected people. And we and others showed many years ago that during treatment mediated viral suppression in green, that some markers of this immune activation process, in this case activated CD8 T cells, declined significantly compared to untreated individuals. But remains significantly abnormally elevated when compared to HIV uninfected individuals in blue, despite years of treatment mediated viral suppression.

[00:16:06] And others have demonstrated that innate markers of immune activation and inflammation are also persistently elevated in the context of treating HIV infection. And what's more those markers of innate immune activation strongly predict the subsequent development of disease. And so these are data from the INSIGHT Network combining the control arms from three different clinical trials, SMART, ESPRIT, and SILCAAT Trials where they just combined a single measurement of IL6, an inflammatory biomarker, and the coagulation marker D-dimer, into sort of an inflammatory score. And those in the top quartile, the highest levels of that score, had about a 20 percent or higher risk of a serious non-AIDS event or death over the next decade. Compared to just about five percent of people in the lowest two quartiles. So this is a really profound difference in the risk of events. It's a much stronger effect than we would observe in say HIV uninfected elderly individuals, where inflammation also predicts disease. Suggesting to many of us that inflammation is likely playing a more important role in contributing to morbidity and mortality in the context of HIV than it is in the general population. But the other thing to point out from this is that these curves are continuing to separate over time, suggesting that there are likely to be some people living with HIV that have high inflammatory setpoints, if you will, that may be continuing to be at risk over time. Whereas others in the bottom quartiles here are maybe at minimal risk and maybe not in need of additional interventions. And interestingly the same types of relationships are observed in the START trial as presented recently. In fact almost the identical hazard ratio for predicting risk of disease was observed in this trial compared to these earlier ones, even though the event rates were much much lower because it was a much less sick population with high T cell counts. So inflammation strongly predicts disease, whether you start treatment early or late.

[00:18:38] And our group, many other groups have done studies linking the persistent inflammatory state to several disease complications, many of the diseases that I've shown you in the earlier slide have now been linked to the inflammatory state in treated HIV infection. And I've listed, this is an incomplete list at this point, but just giving you the sense that many of these diseases have been linked to the inflammatory state.

[00:19:11] So how can we reduce the inflammatory state and morbidity and mortality now? Well the first thing to point out, because we don't yet have approved medications to reduce the inflammatory state that also have been shown to reduce morbidity and mortality, other than ART alone, is to stress the importance of lifestyle interventions. So Keri Althoff presented this at CROI last year demonstrating that the population attributable risk for myocardial infarction is really high for traditional risk factors in the treated population of people living with HIV. So smoking, high blood pressure, high cholesterol all explaining a significant portion of the risk for heart disease. Whereas HIV related factors like CD4 count, viral load, history of AIDS really explain only a small piece of the risk. So really the bread and butter stuff are people living with HIV may be at increased risk. But what you do about it is really primarily focusing on the traditional risk factors.

[00:20:35] And this is further underscored by data from the same CROI presented by the D:A:D Study showing that among people living with HIV, who are able to quit smoking, that with increasing time since they quit smoking there is a progressively decreased risk of non-AIDS cancer, mostly non-AIDS cancers which is pretty dramatic. So it's not just cardiovascular disease, but also cancer you'll see a decreased risk. So it's really important to encourage people to quit smoking.

[00:21:15] And it's also clear that exercise improves health in a variety of ways. You feel better when you exercise, most of the time, but it also tends to decrease the inflammatory state and some of the key biomarkers that predict disease and treat HIV. And so in this study done in Italy, this is an uncontrolled trial of exercise and it wasn't that rigorous. So it was three times a week of brisk walking for 60 minutes. So a lot of people can do that. So if you have sedentary patients in your clinic, just getting them off the couch a little bit more and instituting something like regular brisk walking might actually make a big difference. Reducing D-dimer, IL6 levels and some of these biomarkers that predict disease. So that's important to do.

[00:22:17] So one might then ask if the relationship between inflammation and end-organ disease in all of these studies in HIV is just simply explained by health related behaviors? Again people living with HIV are more likely to smoke, they may be more likely to use injection drugs, perhaps may be more sedentary. But that's not the case actually. So there is a really well done study done in Copenhagen, over a thousand people living with HIV had a biomarker of immune activation assessed called soluble CD163, which predicted mortality in that setting. And they did a whole bunch of subset analyses in that study, because it was so big they had plenty of power for this. And it turns out that the relationship between immune activation and mortality was actually stronger in non-injection drug users than it was an injection drug users. Now I don't want to suggest for a moment that injection drug use is good for you, it's certainly not, IDU increases the risk of mortality. It's just that it's not doing that through the inflammatory state. IDU does contribute to inflammation, but the mechanisms by which IIDU increases the risk of mortality is not through inflammation, it is through other stuff. Right. The other thing to emphasize here though is that other drivers of the immune activation, like HIV itself and the indirect ways by which HIV contributes to immune activation, that seems to be more important in terms of driving the risk of disease. The same thing is seen in smoking, almost even more dramatic. Despite the fact that smoking increases immune activation, the relationship between immune activation and mortality is stronger among never smokers than among smokers. So it's not just that people with HIV are more likely to smoke that explains this relationship between immune activation and mortality, the relationships are stronger among never smokers non-IDU. But I think this also suggests that the root drivers of the inflammatory state may make a difference.

[00:24:51] So what about initial therapeutic strategies, beyond just getting people to quit smoking and lifestyle modification? Are there are medications we can offer? And so the field, we don't have them yet, but this is the current approach that the field has been taking. I call this the Low-Lying Fruit strategy to test commonly used drugs with anti-inflammatory properties to see if in pilot trials they reduce surrogate markers of immune activation. And if they do, they would move on to clinical endpoint trials. So there have been a number of such studies, ACE inhibitors have been tried, angiotensin receptor blockers have been tried, and really not been shown to be beneficial in randomized controlled trials and they haven't really progressed on to clinical endpoint studies.

[00:25:46] Aspirin is another commonly used medication with anti-inflammatory properties that we studied in the ACTG and it also failed to do anything beneficial on the biomarkers that we care about in HIV. It clearly reduced thromboxane levels, a direct biomarker of cyclooxygenase inhibition. So we know that people in that trial, whether it's high dose or low dose aspirin, took their medicine. But it did very little to soluble CD14. In fact, the placebo arm seemed to improve somewhat more in soluble CD14, this marker of immune activation, than the lower high dose aspirin groups. And there was absolutely no effect on D-dimer, this coagulation biomarker, that we thought we would see something there but really no evidence for an effect. There were no evidence for an effect on endothelial function by FMD or really any other inflammatory pathway of interest. So it really didn't seem to work.

[00:26:55] What does seem to work in reducing immune activation is statins. So on the left is a study from Grace McComsey's group of rosuvastatin which significantly reduced soluble CD14 levels, this marker of monocyte macrophage activation. It also reduced cellular markers of monocytes activation in that study. Janet Lo and Steve Grinspoon's group soon after published a study showing that atorvastatin caused a plaque regression in the aorta. So really having a direct cardiovascular benefit as well.

[00:27:41] So as a consequence of these positive results from pilot studies, we've moved on to a large clinical endpoint trial called REPRIEVE. Which is now nearly fully enrolled, there are actually over enrolling beyond 6500 individuals, because the event rates have ended up being lower than they anticipated. And so this is going to still continue enrolling for a bit longer, but this will be a really important study because it will be the first clinical endpoint trial of an immune based strategy to reduce immune activation to see if it reduces cardiovascular events. And I think more interesting, people would not be too surprised if yet another study of statins show that it reduced heart attacks, but it would be surprising to a lot of people if it reduced cancer, if it reduced bone fractures, and that's something that I think it's plausible based on the observational data. And so that's what a lot of us are waiting to see from this study.

[00:28:52] But what if statins are not enough? So this is what I think about all the time. I don't think statins are going to be enough. I've lost several patients to premature cardiovascular disease who are already on statins, and I think there's more going on than just statins will be able to deal with. And to develop new interventions, I think we need to understand the root causes of the inflammatory state during antiretroviral therapy to come up with better interventions. And so the first obvious place to look is the virus itself. So although our patients now routinely become undetectable on modern ART, if you use an ultrasensitive assay so-called Single Copy Assay, to look for presence of virus in the plasma you can often find it below the hood and if you look in the tissues you can more readily find it. Most of this virus we think reflects release from infected cells in the absence of productive replication. And that's important to note because we currently lack interventions that block virus release from cells. All of our drugs block new rounds of replication, but they don't block HIV expression from cells and that's an area where I think we need new interventions to decrease the inflammatory state.

[00:30:20] And as I alluded to the situation is more significant in the tissues. And to give you a sense of some, I like this study from Tim Schacker's group where he did lymph node and gut biopsies in individuals who were interrupting their retroviral therapy, and were followed really closely in an analytical treatment interruption and sampled immediately when the virus first became detectable in the first few weeks after stopping therapy. So the viral load was still quite low in the peripheral blood at the time the biopsies are taken, but

yet when you looked in the tissues by in-situ hybridization you saw gobs and gobs of virus in the lymph nodes and in the gut, suggesting that likely the source of this rebound virus likely was in the tissues. And probably during antiretroviral therapy, there is slow leaching of virus out of cells it's happening all the time which may be enough to activate the immune system. It's important to think about that because HIV is preferentially releasing in the same anatomic location where adaptive immune responses are supposed to be developed. It's leaching out in the lymph nodes and then the inductive lymphoid tissues at the gut. And while HIV can infect myeloid cells and other tissues, brain, liver, and fat, this tends to occur later in the course of untreated HIV disease. That might spread the anatomic distribution of this potential source of persistent inflammatory state. Now I'll come back to why I think that's important at the very end.

[00:32:07] But there are other viruses that are likely contributing to the inflammatory state during HIV disease. CMV I think is one of them and there's a pilot randomised controlled trial that we did in San Francisco many years ago now of valgancyclovir among individuals with suboptimal CD4 recovery during antiretroviral therapy. And showed that valgancyclovir reduced CD8 T cell activation levels significantly more than placebo. An effect that was sustained for four weeks after they stopped the intervention. CMV shedding, by the way, remained suppressed for four weeks after we stopped, which is an interesting phenomenon that we also see in people without HIV that get treated for CMV shedding. Interestingly, valacyclovir a drug that gets other herpes viruses, valgancyclovir of course gets HSV 1 and 2, EBV, HSV, among other herpes viruses. Valacyclovir which gets some of those other viruses but has minimal anti CMV activity at the dosages used, failed to reduce immune activation for subsequent studies. So for a variety of reasons we think that this really was an effect on CMV but we hope to definitively answer that question in an upcoming clinical trial, which was just approved for development in the ACTG of a new CMV drug letermovir which is specific for that virus.

[00:33:45] And we're particularly interested in looking at surrogate markers of cardiovascular disease in that upcoming trial, in part because of this observation that from the ICONA Cohort in Italy where CMV serostatus significantly predicted non-AIDS events. In particular are cardiovascular events where the hazard ratio is the highest. Now interestingly CMV serostatus did not seem to predict risk of AIDS-associated events. So some, but not all, complications that are increased in treated HIV disease seem to be related to CMV. Cardiovascular disease makes sense because CMV replicates in the vascular endothelium and contributes to transplant vasculopathy in the transplant setting we think. And we also think that this is likely to play a greater role in individuals with lower CD4 nadirs who have more profound immunodeficiency, allowing this other virus to replicate more.

[00:34:53] The third driver of the inflammatory state is microbial translocation and so-called "Leaky Gut" syndrome. So described by Jason Brenchley and Danny Douek now over a decade ago, where the top is a cartoon of a healthy gut epithelial barrier, the pink ribbon is a healthy gut epithelial lining, keeping those blue risotto-like particles out of systemic circulation, and those are bacteria that keep them out. And behind that brick wall of the epithelium you have an intact mucosal immune system to clear any invading microbes. But in the very first few weeks, actually the first few days of HIV infection we know from animal models, there is profound disruption of gut epithelial barrier function and profound loss of mucosal immunity, depletion of CD4 T cells and Th 17 cells thought to play an important role in maintaining barrier health. There's gut epithelial cell apoptosis, loss of tight junctions, all of this happens at the very earliest moments of HIV infection and it becomes even worse with advanced disease stages.

[00:36:17] And this point is drilled home by the work that we did in San Francisco with the gastroenterologist, Ma Samsouk, and working with Jake Astiz at the NIH where we perform many gut biopsies in HIV uninfected individuals, those with HIV who had restored their normal CD4 counts on suppressive antiretroviral therapy, and then those who are so-called immunologic non-responders with persistently low CD4 counts despite viral suppression. The brown stain is myeloperoxidase stain for neutrophils, and so this is just sort of the neutrophilic infiltration responding to a breach in the epithelial barrier. You see this even in people who are immuno restored, but you see a lot of it in people who started ART at a low nadir and ended up plateauing at low CD4 counts. And so there's probably consequences to microbial translocation for delaying ART initiation.

[00:37:22] What's more specific? Biomarkers of gut barrier dysfunction predict mortality in a study of individuals with a history of AIDS who are aren't suppressed, in the SOCA cohort study. And several markers of innate immune activation strongly predict an increased risk of mortality in that setting. These are hazard ratios comparing the fourth to the first quartile. These are just sort of extraordinarily strong associations.

[00:37:54] So you can ask, well how do we you know choose a specific interventional target here where so many biomarkers are related to mortality, have so many root drivers? Well I've started to think about immune activation as sort of like a tree in HIV. Where all the leaves are the various end-organ diseases that are increased in the context of HIV. And the various roots are there different route drivers of the inflammatory state, HIV reservoirs, CMV, microbial translocation. And the branches are the many immunologic perturbations that exist in treated HIV, adaptive immune defects, inflammation like IL-6, coagulation D-dimer, fibrosis the lymph nodes, each contributing potentially to different disease manifestations.

[00:38:50] But if we try and attack an individual root or an individual branch we may have the whack a mole problem. You go after this guy over here and another guy's going to pop up. And in fact we have several examples of this, in recent clinical trials we've tried inhibiting toll-like receptor responses, sort of an innate immune response, with drugs like chloroquine the anti-malarial that has anti-inflammatory properties. When given in treated HIV infection, it seems to decrease immune activation modestly. When given to people with untreated HIV, it actually increases HIV viral load because you're actually blocking a protective anti-viral response. And so it's whack a mole. So you have to be concerned that an immune-based strategy isn't going to make one of the root drivers of the inflammatory state worse.

[00:39:50] And so now the field is wrestling with lots of different other approaches. We're trying to find the tree trunk, if one exists. A common immunologic pathway that may be driven by multiple root drivers, that may give rise to multiple downstream inflammatory pathways. And so there is sort of an interesting time in the immune activation field because we're having many of these tree trunk interventions, if you will, that are going to be reported soon.

[00:40:24] I'll tell you about canakinumab in just a second. We'll hear about the results of a ruxolitinib study, a JAK-STAT inhibitor, probably in the next couple of months around the time of CROI. And others are thinking about other alternative strategies. But when we do these studies it's important to keep on measuring these root drivers to make sure they're not getting worse as a consequence of our interventions.

[00:40:51] So what about canakinumab? So this really made headlines this last summer or a year ago now, a Paul Ridker study in the general population of people with cardiovascular disease got this IL-1 beta inhibitor, canakinumab, or a placebo to see if that it reduce cardiovascular events. And it did, it significantly reduced cardiovascular events in the general population, which is actually the first evidence that a direct pure anti-inflammatory drug reduced cardiovascular events. So a really big big time finding. It also reduced mortality from lung cancer too, interestingly. So really proof that inflammation can cause a chronic disease like this.

[00:41:44] So Priscilla Hsue in San Francisco did a pilot study, a non-randomized controlled trial. I think that was another trick question that I gave you, this is not randomized and placebo controlled. But she did show that it could appear to reduce immune activation, IL-6 and CRP levels, over a short period of time. And this we think is probably a clinically significant reduction in IL-6 levels.

[00:42:18] But is it a viable intervention in treated HIV? There was an increase in fatal infections and sepsis in the canakinumab arm in the original trial in HIV uninfected individuals. And so there is concern there might be negative consequences on immune function in treated HIV. And will all adaptive immune defects be reversed by this largely innate immune activation blocker? So it's not clear as of yet whether this is truly the tree trunk, if you will. I think it may be emerging that it's more of a branch.

[00:42:58] And so it brings me back to the tree, I've revised the tree analogy and now I'm thinking about it more like a Banyan tree, and there may be multiple tree trunks. And the really interesting thing about the Banyan tree is that all these parallel trunks here are actually not trunks. There are aerial roots that grow from the branches. You have primary trunk that comes up, develops some branches, and then these aerial roots go down and dig into the ground to establish new water supplies. And I actually think that's probably what HIV is doing. The primary trunk is HIV and the aerial roots are seeing the microbial translocation, these things that are made worse by HIV, but in and of themselves once they get established can be new drivers of immune activation resulting in new trunk's feeding new branches and leaves. And I think that that's probably what's happening in HIV.

[00:44:05] And to bring it home, I think it also depends on the CD4 nadir. I kept on pointing out earlier in that talk that some diseases haven't even started yet, if you start people very early in the course of their infection on ART. Cardiovascular disease, neurocognitive dysfunction, etc. And I think that may be because some of the root drivers of the disease that are there, these infections and cancers, may be HIV itself that's replicating or being expressed in lymphoid tissues where adaptive immune responses are being developed. Other things like microbial translocation, while they exist at the very early stages of disease, the extent to which it is irreversible really depends on the CD4 nadir. So you really don't see a severely irreversible significant gut damage until you start getting into lower nadir CD4 counts. And that can drive multiple morbidities as these microbial products go throughout the circulation. HIV infected in myeloid cells, which can exist in people with advanced disease prior to ART initiation can plausibly contributed to CNS, liver, and metabolic disease. I would expect this only to be a problem in people of low CD4 nadirs. Then CMV, as I mentioned, preferentially replicates not in the lymph nodes but in blood vessels and other mucosal tissues. So it may confer a different spectrum of disease patterns. That's a current theoretical model that we're working with.

[00:45:53] So to summarize, immune activation strongly predicts increased morbidity and mortality in treated HIV, particularly infectious complications and cancer even if people who start ART early. Lifestyle interventions are important, they will always be important, but it's all we have until we have developed new interventions. And to identify new interventions we need to prioritize studies that target those root drivers. We do have new agents for CMV that we need to test, but we desperately need interventions that block HIV expression from cells. I think that might actually end up being a useful strategy. And we've been really unsuccessful at trying to reverse microbial translocation, they need better interventions for that. We do hope to find the tree trunk, but it's possible that our tree is a Banyan, and we need to be prepared for that possibility. And if it is a Banyan, particularly in individuals with low CD4 nadirs, there may be a broader spectrum of disease manifestations and inflammatory drivers that we need to think about.

[00:47:02] So with that I'll stop and thank my multiple collaborators that contributed to a lot of the data you saw and take your questions.