Redonna Chandler (00:00:02):

Thank you all for joining us today. We have over 200 people registered to attend this afternoon, which will be filled with fantastic science. We're thrilled to welcome everyone. And we are also very grateful to our presenters who are going to be providing us with findings as well as advances that they have been afforded by having the opportunity to be awarded an Avant-Garde Award, which is one of our most prestigious awards at NIDA. I'm pleased to have the deputy director of NIDA join us for the afternoon, and to give some welcoming remarks, and so I don't want to take up any more time so we can get on to our great talk. So Dr. Wilson Compton is the deputy director of NIDA, and he also began early in his career as an HIV investigator himself, so I know that the intersection of drug abuse and HIV is a topic that has always been near and dear to his heart. So we're pleased to have him to kick us off with a welcome.

Wilson Compton (00:01:09):

Well, thanks very much, Redonna, and I really am glad to be here today. Certainly understanding and addressing drug-use related risks and the interactions with HIV has been the center of NIDA's HIV research program for over 30 years. And I personally can speak to this because I started my research career on two HIV projects that were funded to Washington University in St. Louis to Linda Cottler, and worked with that group for the following 13 years until I joined NIDA in 2002. Now our HIV research program here at NIDA is the second largest at NIH, and it certainly has made seminal contributions to the science of HIV prevention treatment, and now HIV elimination. Now today's symposium is designed to highlight an important component of this research effort, the NIDA Avant-Garde Award program. Since 2008, the NIDA's Avant-Garde Director's Pioneer or DP1 Award has been a flagship initiative of the NIDA HIV research program.

Wilson Compton (00:02:16):

This is a highly prestigious award that allows us to fund investigators of exceptional creativity, proposing innovative high-risk, high impact research. Now, since its start in 2008 and including our newest 2021 awardees, NIDA has awarded 36 Avant-Garde grants. Today's presenters include from 2009, Dana Gabuzda, from 2012, Davey Smith, from 2014, Melanie Ott, from 2015, Don Des Jarlais and Julie Overbaugh, and from 2016, Stuart Lipton. So we have a wonderful lineup from throughout the history of our Avant-Garde program. Now please join me in congratulating doctors, Linda Chang and Alex Shalek, who are the newest members of this exclusive group, the NIDA Avant-Garde awardees for 2021. Dr. Chang from the University of Maryland School of Medicine plans to explore the use of MR-guided focused ultrasound as a tool to eradicate CNS viral reservoirs and to promote neurogenesis in the HIV-infected brain. Low-intensity MR-guided focused ultrasound will also be applied to target brain regions involved in addiction for treatment of substance use disorders.

Wilson Compton (00:03:38):

Dr. Shalek from the Koch Institute for Integrative Cancer Research at MIT will work to better define the impact of drug use on immune function and fitness against HIV-1. His work will seek to define at unprecedented resolution how substance use disorders influence immune function and response to HIV and other pathogens. He will develop and apply innovative single cell and bulk profiling and perturbation tools. Now receiving this award means we are investing particularly in you as a scientist, of course, as well as in your innovative research ideas. So this is a combined award that's focused on the individual, as well as the innovative science. Now let's turn to some of the business for the day. Please join me in thanking those responsible for this symposium, which has a remarkable number of

participants. Something over 200 people are watching us today. Our NIDA AIDS Research Program team includes Redonna Chandler, Vasundhara Varthakavi or Kavi, Trishma Smith-Winston, and Andrea Zekowski.

Wilson Compton (00:04:47):

Now our coordinators from LMCI, our support contractor, have also helped us today. And I really want to thank everyone who's assisted in coordinating this event, Kavi and Redonna and our contract staff, Aaron Newman, Caitlin Dudevoir, and Jeremy Brundage. It really takes an entire village to pull off a successful Zoom meeting, and I'm very grateful for everyone's effort today. Now finally, you all may be wondering, "How come Wilson Compton's doing this today?" Now of course, my background as an HIV researcher in the past makes me particularly excited to be here today. But the main reason is that Dr. Volkow is unavailable today. She's with her family this week. It's the first time in over 15 months that she's been able to travel back home to Mexico. And she asked me to cover this event for her. But she particularly wanted you to know how much she appreciates this symposium.

Wilson Compton (00:05:40):

She congratulates the new awardees and is disappointed to miss the presentations. She really looks forward to hearing about your findings in the future. And I know both Redonna and I and Kavi will all be speaking to Nora to tell her what you teach us today. So we have to pay close attention so we can be sure to explain it to our director. Now, let me turn over the virtual podium to my colleagues from our AIDS Research Program, who will take it from here, as we learn about some of the exciting scientific discoveries and the new leads from our NIDA-supported Avant-Garde grantees. I'm not sure whether it's Redonna or Kavi, but I turn it back to you now.

Vasundhara Varthakavi (00:06:22):

Thank you Wilson for the really great welcome and introduction remarks. And I want to also take this opportunity to thank all the panel members, invitees, actually, to agreeing to participate in the symposium. I'm really excited and looking forward to your talks. So I'm going to get this kick-started with the first speaker in the lineup, Dr. Stuart Lipton, who comes to us from The Scripps Research Institute. He will be talking to us about TCA cycle compromise due to aberrant protein S-nitrosylation in NeuroAIDS, methamphetamine use, and neurodegenerative disorders. Thank you. Dr. Lipton.

Stuart Lipton (00:07:15):

I'm trying. Hold on. There we go. Great. Well, thank you so much for organizing this and for the award and my fellow awardees. It's kind of a life-changing award, lets you do what you want to do, and I'll take you through some of that. I'm going to talk today about aberrant protein S-nitrosylation. I'll tell you about that post translational and modification and how their networks of these apparently affected proteins, not only in HIV and drug use, but also in other neurologic disorders. And a lot of them are in common, and that's going to be a theme in what I'm going to tell you today. So here we go. As way of background, approximately 40% of AIDS patients manifest some form of NeuroAIDS or the one we mostly study is abbreviated HAND, HIV-Associated Neurocognitive Disorder. Most severe form can result in dementia, although we see a lot less of that with modern cART therapy. Worldwide, some 25 million people use methamphetamine, as you know, and in several studies, as many as 50% in some territories of HIV-positive individuals are meth users. So you can see there's a tremendous overlap with these. So what I'm going to tell you about today is that in HAND or NeuroAids and methamphetamine, there are

pathways to neuronal damage that involve in part metabolic insults, and particularly in the mitochondria.

Stuart Lipton (00:08:47):

Mitochondria are particularly important in producing energy for neurons. We'll talk a little bit about that, as many groups have shown. And then kind of strikingly, the insults that we found in HAND and methamphetamine brains at post-mortem in many respects resembled damage we've seen in Alzheimer's disease and Alzheimer's disease related disorders. And this CNS damage is in part due to aberrant post-translational redox reactions that I'll talk about on proteins that involve accessibly generated reactive oxygen and reactive nitrogen species that become toxic to neurons and the support cells in the CNS as well. And under the Avant-Garde, I was able to take a new approach to this using a different kind of mass spec analysis to look at these redox-altered proteins in human brains obtained very shortly after post-mortem through brain banks here at UCSD and elsewhere, not only in HAND and methamphetamine, but through a series of supplements and AD and ADRD. And as I said, strikingly, there's an overlap in some of the major proteins that are aberrantly S-nitrosylated that affect mitochondrial function. Going to tell you a little bit of that story right now. So let me tell you what the Avant-Garde has done for me. Not only as it supported 31 publications during the lifetime of the award. Today, I'm going to really talk about two very recent publications, one in science, one in general neurovirology talking about these mitochondrial deficits that we see in an overlap between HAND, meth use and in AD/ADRD.

Stuart Lipton (00:10:29):

I want to shout out to obviously the program directors at NIDA who have been very, very helpful to me, particularly John Satterlee who was kind of held my hand throughout the award. So this is what we knew kind of coming into the award 2015, 2016 from our group and many groups. And the CNS-HIV infection came into infected macrophages and microglia, which produced a series of chemokines and cytokines that gave off neurotoxins and viral proteins, such as GP120 and others. They would incite astrocytes that would have aberrant function to affect neurons, so you can have a direct microglial neuronal interaction, but also the astrocytes. We knew that neuronal glutamate would be affected by this, either coming from astrocytes or microglia that would engage NMDA receptors and other glutamate receptors, increased calcium and increased reactive nitrogen and oxygen species, particularly reactive nitrogen species, such as nitric oxide.

Stuart Lipton (00:11:29):

Subsequent work has shown that both human microglia and human astrocytes also produce a lot of reactive nitrogen species although certainly there are other pathways involved. These are major pathways to the damage in neurons. Now it's interesting because in a whole other set of work done by others, reactive nitrogen species had been... And redox effects of those reactive nitrogen species have been found in meth and other kinds of drug use. Okay, I need to tell you a little bit about reactive nitrogen species and what they do to proteins. So my colleagues and I discovered this post-translational modification about 20 years ago that chemists would call it S-nitrosation. We call it S-nitrosylation to kind of make it analogous to phosphorylation. You can also trans-nitrosylate that as donating a NO group from one protein to another. And it goes onto a critical cysteine or thiol. The chemistry involved is the protein is R, you deprotonate your thiol. We'll talk a little bit more about the chemical action in a minute.

Stuart Lipton (00:12:33):

The RS- reacts with something akin to NO+, although it's not free NO+, and you get what I affectionately call a SNO protein. NO on the sulfur. It's funny. When I moved to LA Jolla from Boston a number of years ago, I thought it would never snow again, but you'll see it's SNOing these proteins. And we and our colleagues have published many papers on this as have others. Now, chemists such as Michael Marletta have looked more carefully at the chemical reaction here. As we're taking a minute doing this, because you'll see in a subsequent slide, it's really helped us to codify and quantify these reactions in the human brain. So you know nitric oxide is NO-dot, an electron in the outer pi molecular orbital, but there are other forms of NO, depending on how many electrons are in that outer pi molecular orbital.

Stuart Lipton (00:13:18):

One reaction mechanism that we think occurs is that ferric heme, or it can be copper transition metal, can catalyze this nitrosation and nitrosylation, and the way it does it is the electron is accepted by the transition metal. So it's now NO+ and it's donated to a protein producing the SNO protein. Another way of doing this, as I mentioned, is trans-nitrosation or trans-nitrosylation. In this case, thiolate anion attacks by nucleophilic attack a nitroso nitrogen and basically swaps the NO. Here, the NO was on protein number two. Here, it's on protein number one. So we think these are the major reactions that occur in the brain and elsewhere in the body, how you nitrosylate or nitrosate proteins. There are other potential reactions, but we have evidence these are the major ones, and we'll come back to this.

Stuart Lipton (00:14:07):

Okay. For under the Avant-Garde, we took about 50 human brains. Usually the very short post-mortem times for brain banks, control brains, brains with HAND, brains with HAND and methamphetamine. We also did with HIV without HAND or without HIV-Associated Neurologic Disease. We homogenized the tissue and went through this series of reactions in order to look for nitrosylated proteins by a new mass spec method, which I'll show you on the next slide. We actually used two methods. First method is an organomercury resonant enrichment method, who my colleague and collaborator Harry Ischiropoulos at the University of Pennsylvania has really pioneered. The second method is called a SNO trap method. It uses a triarylphosphine, a chemical that actually traps the NO. So the NO remains there, and that was pioneered by chemist Steve Tannenbaum at MIT. Both of these groups, we work very closely with.

Stuart Lipton (00:15:02):

And the first in the enrichment, the NO was actually removed by reduction by the mercury. And then the protein that had the NOs attached to agarose beads, we can further analyze it. And then in the trap method, we put the trap and hooked it on Biotin, so we have a tag on it. It's now tagged with the NO is actually still there. I looked at the triarylphosphine, then we can submit these for mass spec. We can actually do not only protein identity, but site mapping to see exactly what cysteines are nitrosylated. We can even quantify the degree of nitrosylation of each protein or peptide that we look at. Now, when we do that, this is firstly, the pen method. We use both methods in all of these. I'm going to show you samples. And I can say that the MIT method or the SNO trap gives you more nitrosylated proteins.

Stuart Lipton (00:15:50):

It's a little more sensitive, but many of the proteins overlap with the two methods. So we got several hundred proteins in our first runs in control, HAND, and methamphetamine brains. Interestingly, there's a unique repertoire of aberrantly nitrosylated proteins, and methamphetamine compared to control or HAND alone, and there are some that overlap. Now I should say that nitrosylation, like phosphorylation,

is also a normal event, and certain proteins are regulated. Their functions are regulated by nitrosylation. That's what we discovered some 25 years ago, actually initially on the NMDA type of glutamate receptor. However, with rising levels of NO in the brain during neuroinflammation or other times of insult, then you begin to aberrantly nitrosylate proteins. And that's what we're seeing here when we compare to the controls to the HAND, meth, or HIV brains. Now strikingly, when you look at go terms, we found that mitochondria function was really affected right up near the top of the list, particularly the TCA cycle and respiratory electron transport chain.

Stuart Lipton (00:16:56):

We'll come back to that in a minute. When we did a string pathway analysis, all the members, all the top hits were in the TCA cycle, interestingly. Okay, let's turn now to Alzheimer's disease. Again, 40 human post-mortem brains. Here, we're able to do a male and female. We had enough of both and we could do sex and age match controls. This was the SNO trap method. Again, we have more proteins, although again, we've done both methods in both.

Stuart Lipton (00:17:23):

Here, we get about 1500 proteins nitrosylated. It's been estimated from a motif I'll show you in a minute that there may be as many as 12 or 13,000 proteins that are S-nitrosylated, so it rivals phosphorylation, or in fact, might even be more ubiquitous than phosphorylation. Now strikingly, when we took a look at the Alzheimer's disease male and females compared to the controls and looked at the interactome, again, the very top hits just like in HAND and methamphetamine use were mitochondrial targets. Mitophagy or autophagy of mitochondria, TCA cycle, respiratory electron transport chain. I don't think this is a coincidence. It looks like there's some commonality between these. So we wanted to study that further.

Stuart Lipton (00:18:11):

So here's the TCA cycle, and what we've done is look at the effect of nitrosylation on each of the enzymes. Now under normal conditions, a connotate and isocitrate dehydrogenase are mildly nitrosylated, we think that's normal regulation. Other of the enzymes can be nitrosylated, but the major one nitrosylated in each of these conditions, interestingly, is alpha-Ketoglutarate dehydrogenase shown here. That's the first step that produces NADH, which is the important energy transfer to the electron transport chain into complex I, so you can make ATP. Neurons are exquisitely poised on disaster. They need a lot of ATP to maintain their synapses. Basal activity of neurons is thought to rely on the TCA cycle, although they can use glucose and glycolysis under certain circumstances during intense stimulation for basal activity. And if you've got dementia, you've obviously have it for years.

Stuart Lipton (00:19:08):

Your basal activity as a neuron is very important. So you need this NADH to go in, and we think there are other points of NADH production farther down in the TCA cycle. But if you've disrupted here, you can't get there. Now I won't show you today, but we have evidence for metabolic flux experiments that alpha-Ketoglutarate dehydrogenase is definitely compromised. So we came up with a way that we might be able to bypass this. One way would be to supply succinate, the next step, as dimethyl succinate, which is somewhat permeable as a kind of a pro-drug to see if we could bypass the cycle. Now, as you may remember from your biochemistry, complex II of the TCA cycle is the same as succinate dehydrogenase in the TCA cycle. So by supplying succinate or succinate derivative, we're actually not only jump-starting the rest of the TCA cycle, but supplying to complex II of the electron transport chain.

Stuart Lipton (00:20:06):

So we have some evidence that that kind of approach can work. So this uses the seahorse platform to look... This happens to be iPS neurons from Alzheimer's disease and isogenic controls, but we've also done it on iPSCs insulted with methamphetamine and HIV GP120. Very similar results. And what I want you to concentrate on here is oxygen consumption rate, particularly where we look at something called the respiratory reserve. This is when neurons are stressed, how good are they going to do? Are they going to be able to supply energy to the synapses that it maintain? We do that by using an uncoupler to see how much energy was there in reserve. And what we see compared to wild type, these are the AD neurons, but as I said, meth, or GP120 insult, very similar. Tremendously compromises your reserve. But if we give dimethyl succinate, we're not back to normal, but we're statistically protected.

Stuart Lipton (00:21:02):

Now we're looking to see if we can protect synapses with these kinds of rescues. And that work is ongoing. I want to pivot a little bit now and tell you why energy is so important to neurons and other ways that aberrant nitrosylation disrupts it. Really the synapses, which is the only pathological correlate to dementia and any of these diseases. It's not misfolded proteins, even though they're present. And they're important in triggering the events. The number of synapses correlates to how dementia [inaudible 00:21:32]. And you need a lot of energy as I've mentioned earlier to maintain these synapses. It's really what makes us uniquely human, why we're having this symposium today. Now the synapses are lost both in Alzheimer's disease, Alzheimer's disease-related dementias, HAND and meth use, and other insults to the central nervous system. As I mentioned, neurons particularly are sensitive and need the TCA cycle.

Stuart Lipton (00:21:55):

So we believe any disease modification of those diseases is going to need synaptic protection. Now there's another kind of injury to mitochondria apart from the TCA cycle that I want to share with you that kind of came as a kind of surprise. As you all know, in the last 15 or 20 years, we've discovered that mitochondria are not these cookie cutter looking little organelles that you learned about in high school. They actually undergo dramatic fission and fusion. And this is an elegantly regulated process by a series of GTPases and their associated proteins. The one that usually causes fission is called Drp1, or Dynamin-Related Protein, monitor GTPase that clips that mitochondria. Very regulated, we know these are important in neurological function in particular because there are rare genetic diseases that have a mutation of these GTPases such as an OPA1, which is a fusion protein giving you a form of dominant optic atrophy, certain forms of ALS and Drp1 mutations.

Stuart Lipton (00:22:57):

Charcot-Marie-Tooth disease also has mutation. So we know what can affect the brain. Now, what we discovered is that this mitochondrial fission and fusion not only is important in the brain, but it's very susceptible to aberrant nitrosylation. And what we found some years ago initially now, is that Drp1, which normally mediates vision is accessibly activated when it's nitrosylated. And what nitrosylates it? Aberrant or oligomerized amyloid beta in Alzheimer's disease, glutamates overstimulating NMDA receptors, HIV and meth insults can do it. They generate a lot of reactive nitrogen species as I intimated on prior slides. And we get this aberrant nitrosylation on a particular site on Drp1, which causes excessive activation of the GTPase, excessive multimerization of Drp1, and excessive cleavage and fragmentation of the mitochondria with dramatic bioenergetic failure resulting in synapse injury. Here's the ATP, we can measure ATP.

Stuart Lipton (00:24:01):

It falls off quickly when you give that oxygenase source of NO, S-nitrosocysteine, if you give GP120 with or without methamphetamine, which makes it worse, or you give a bit of oligomers, all of this cause a reduction of about 50% and a persistent reduction in ATP. Now we have other ways of looking at nitrosylated proteins. I want to bring this up because the subsequent slide, it becomes important. In addition to our mass spec approach, we have a modified immunoblot that we use that was pioneered by Sam Joffrey in Solomon Snyder's lab a number of years ago using a chemical trick that Jonathan Stamler and I had published. And what we find is that an Alzheimer brain, but not control brains, would get a lot of nitrosylated Drp1 just like in the meth and HIV brains. Parkinson's disease, not so much, but these are very late stage Parkinson's where we've already lost the substantia nigra early in Parkinson's that may actually occurred as well.

Stuart Lipton (00:24:55):

And the referees made us look at many AD brains. We see it in every brain. There's substantial nitrosylation of Drp1. So by site-directed mutagenesis, we looked at Drp1 and we found one particular cysteine residue at position 644 was nitrosylated. We've produced crystal structure of that with colleagues, and it has what we've called a partial motif that we've discovered that seems to facilitate, it's not required, but it facilitates nitrosylation and it surrounds that cysteine. So that's probably why that cysteine is susceptible to high levels of NO. Now, what does this do in terms of synaptic damage? Let's look at that for on this slide, spine density, which is kind of one half of the synapsis. It's the post synaptic side. You can actually label it up with GFP or YFP as shown here. These little protuberances are half the synapse. If you expose those to A-beta and many, many labs have shown this, oligomerized A-beta or HIV and GP120 will do it, or methamphetamine will do it. You lose within a day about half of your synapses.

Stuart Lipton (00:26:03):

If you block neuronal nitric oxide synthase, you can protect many of them. Now, this is the most important part of this. If you put a point mutation and produce a non-nitrosylatable mutant of Drp1, statistically, you totally protect the synapses. So that means that Drp1 was very important in injuring mitochondria and synaptic failure, and as I said, we can now start to rescue that with bioenergetic compromise. Okay. So the story gets a little bit more complicated. Because of our mass spec methods, we realized that there were a whole series or network of previously unknown nitrosylated proteins that interact with one another and they're in different biochemical cascades. No one would ever think they ever interacted. So Uch-L1, which is a ubiquitin ligase, Cdk5, a kinase, Drp1, a GTPase, no one thought they interacted.

Stuart Lipton (00:27:04):

It turns out they trans-nitrosylate one another. And we found this either after oligomerized A-beta or HIV GP120 and methamphetamine insults. And we also found it in human brains with Alzheimer's disease or HAND meth, and not in the controls. So let me delve down a little bit on this pathway and show you how we're able to study it. So, first of all, we do use mass spec. This is Uch-L1. We can tell from these precise molecular weights of masses, we can see exactly what cysteine is nitrosylated because it's altered by the additional weight of NO. And we can identify that cysteine. We can actually quantify it. Again, Uch-L1 also has one of those motifs for nitrosylation and the crystal structure that we've looked at. Okay. Now going back to the blot method, why would we want to do blots if we have mass spec?

Stuart Lipton (00:27:56):

Well, I'm going to show you an interesting facet of the blots that we've been able to take advantage of. So we found, for example, Uch-L1, not only in HIV, methamphetamine brains, but in AD brains and also Cdk5, the downstream also in AD brains. And we wanted to know how these were related. So to assess trans-nitrosylation from one protein to the other in a kinetic experiment, we can do this in vitro. What we can do is we can artificially nitrosylate Uch-L1, and then add it to Cdk5 and Drp1. And what we see over the course of about 25 minutes is initially Cdk5's nitrosylated, then about 10 minutes later, Drp1 is nitrosylated. If however you add to the mix, nitrosylated Cdk5, the reaction does not go in the other direction.

Stuart Lipton (00:28:44):

We never see Uch-L1 nitrosylated. It's always driven toward Drp1. Now I realized looking at these blots, we could be much more quantitative because we can quantify the density of the bands. So if you think about it, a nitrosylation is an oxidation. You're losing an electron. We have total protein, which is the reduced and oxidized protein. We can also use a chemical trip to just look at the reduced protein. So now we can look at the oxidized and reduced protein. Why is that important? Well, it turns out that reaction that I had shown you shows you that when you get nitrosylated, you lose an electron.

Stuart Lipton (00:29:26):

When you get denitrosylated, you gain an electron. Now someone a lot smarter than I am named Walther Nernst discovered that he could quantify this about 150 years ago. And he produced something you all know called the Nernst equation. We use it to look at membrane potential, but that's not why he did it. He was looking at electrons, where electrons went, and I was coming back from a meeting flying at 36,000 feet, probably was oxygen deprived, and I realized, "Oh my goodness, we could use the Nernst equation to quantify these redox reactions just by quantifying those bands on the blot." And when we did that, now we can look thermodynamically and see in a human brain over the course of several years of dementia what's really going on. And when we plug that in to this modified Nernst equation that I've come up with, which looks at transfer of NO, or an oxidation from one protein to the other, you get an amazing reaction.

Stuart Lipton (00:30:22):

So those of you who took physical chemistry will know that a Gibbs free energy can be obtained from the electromotive force of the Nernst equation. -18.35 kilojoules per mole. So when you get to -20, this is a tremendously driven reaction. It goes to completion. We are driven to destruction by reactive nitrogen species because of this thermodynamic consideration. And so what happens? Well, if you put A-beta, you get, on iPS cells for example, you see a lot of formation as I showed you before of SNO-Uch-L1. If you look at iPS-derived neurons from an Alzheimer disease patient, you lose many synapses. I showed you this before, but if you put in a point mutation, then Uch-L1, the upstream member now cannot be nitrosylated, you protect the synapses as shown in here and here. And we've also done this in vivo using lentiviral vectors.

Stuart Lipton (00:31:23):

We can put in a non-nitrosylatable form of Uch-L1. We've done this in AD models, we're doing it in HAND, methamphetamine models. And this was actually quantified blindly by Eliezer Masliah, who is now extramural director over at NIA. And what he found is these pre-synaptic markers. And synaptophysin, the red dye, you can see it's bleached out in an Alzheimer transgenic mouse made by

Leonard Mooky. And if you put in a non-nitrosylatable Uch-L1, not only do you totally rescue the number of synapses, you actually rescue normal aging loss of synapses. These mice are about a year old and they start to lose synapses on their own. Even more if they have this Alzheimer amyloid precursor protein, and we can rescue them. So what I've shown you in summary then is that A-beta oligomers, other misfolded proteins, I haven't shown you, but we've looked at HIV insults, methamphetamine, normal aging, to some extent, other causes of neuroinflammation, increased reactive oxygen and nitrogen species.

Stuart Lipton (00:32:23):

They are produced. And I didn't show you this today, but there's a lot of evidence for this in glial cells, both astrocytes and microglia, the iNOS and neurons would be nNOS. These glial cells that are stimulated by HIV produce a lot of glutamate, which engages extra synaptic NMDA receptors. We'll come back to that in a minute, which actually turns on the neuronal nitric oxide synthase. NO then can nitrosylate TCA enzymes and shut down early events in the TCA cycle, compromising energy. And it can also produce this whole network of nitrosylation. I believe there are many members we still don't know. These are three of the members that end up fragmenting mitochondria, compromising energy, and therefore compromising our synapses and producing cognitive impairment. Now I want to end on a happy note. The good news is there are ways to intervene. I told you, we're trying to intervene at the TCA cycle.

Stuart Lipton (00:33:17):

That's a work in progress. One way we've been able to intervene, I mentioned this very briefly in the talk is one of the first nitrosylation sites ever, the first actually ever discovered by our group in the early '90s was the NMDA receptor. This is a normal nitrosylation site that knocks down excessive NMDA receptor activity. We're going to take a drug amendment team, which I have developed and Marshall through the FDA approval process for Alzheimer's disease, attach it to a piece of nitroglycerin producing a new drug, and we can shut down extra synaptic NMDA receptors. And to a large degree, that shuts down all these subsequent events. So we think there are ways we can interact and protect these synapses, both from HIV- related and methamphetamine damage, but at the same time, other forms of.

PART 1 OF 6 ENDS [00:34:04]

Stuart Lipton (00:34:03):

... HIV related and methamphetamine damage, but at the same time, other forms of neuro-degeneration, such as Alzheimer's disease and Alzheimer's disease related dementias. So I've had a tremendous amount of help. I don't have time to mention everyone by name, but both in Boston and in LA Jolla and others have helped me so tremendously. And I am so appreciative to NIDA for giving me this award. And I just want to say that a lot more work remains to be done, and I hope you all come visit us when COVID is over in our beautiful new building for drug discovery, Scripps research. I'm glad to answer any questions. Thank you.

Vasundhara Varthakavi (00:34:36):

Thank you, Dr. Lipton. Attendees, if you have questions, please do enter those in the Q and A panel.

Speaker 1 (00:34:58):

Kavi, at least for me, the Q and A panel is not open for questions. So if the logistics contractor can check that and make sure it's open for everyone.

Aaron (00:35:13):

I will check that now.

Vasundhara Varthakavi (00:35:15):

So Aaron, can someone unmute the participants who have questions? It looks like there are a couple of attendees.

Stuart Lipton (00:35:22):

It actually says there's a message to me that you can use the chat to ask questions.

Vasundhara Varthakavi (00:35:26):

You can use chat as well, whichever works. There you go. It is open. The attendee says it's open.

Aaron (00:35:33):

We do have a few folks with their hands up. Kumutsing, we'll go ahead and allow you to unmute to ask your question.

Stuart Lipton (00:36:00):

He's still muted.

Kumutsing (00:36:01):

Sorry. I didn't have a question. I think, I by mistake I put the hands up. Thanks for the nice presentation. Thank you.

Aaron (00:36:12):

How about Paul Semi Perry Assami? We'll go ahead and unmute your line.

Shilpa Butch (00:36:27):

Oh, it's Shilpa Butch, sorry. Hi Stuart, excellent talk. So my question is, can you talk about the iron overload and the pool in all these neurodegenerative disorders, because showed us the Fenton reaction prior to the nitrosylation?

Stuart Lipton (00:36:44):

[inaudible 00:36:44] different reaction, but it's related. It doesn't have to be iron, it turns out it can be copper in many of these systems too, or any other transition metal. And you don't need a lot of it. And so most neurons have enough of it. As you probably know, a ferroptosis as a form of apoptosis using iron is very popular now in more [inaudible 00:37:04], such as stroke. And so we're becoming more and more aware that these transition metals are in neurons. So, that's usually not what we in our view is rate limiting.

Shilpa Butch (00:37:15):

But do you think that, that could be an initiating factor that then goes on to do what you've talked about? Is that one of the key [crosstalk 00:37:22]-

Stuart Lipton (00:37:22):

So there's a huge argument and the argument's been going on for 150 years of how you get NO onto a protein. And so I'm not going to pretend to know, but we think that is one of the mechanisms. There could be a radical reaction. There's other possible reactions, but once it's on a protein, there's this whole trans-nitrosylation network and in a sense, NO is a life of its own. It doesn't need NOS anymore. It's transferring it from protein to protein, and there are at least 3000 proteins that do this. And this is an untapped ... We're about 50 years behind our phosphorylation colleagues and the chemistry is much more complicated. So we are really ignorant about these networks. We're working on them.

Shilpa Butch (00:38:03):

Any of these things affecting the tat protein, you think? The tat, vital tat?

Stuart Lipton (00:38:10):

So I haven't studied tat as much as GP 120, but TAT, many of these proteins that induce any kind of inflammation will produce a lot of reactive nitrogen species. So the answer is undoubtedly yes.

Shilpa Butch (00:38:25):

Yes. Thank you. Very good.

Stuart Lipton (00:38:27):

There's a question I want to answer in here in the chat box, let me answer it. They asked if I did meth alone. No, we were not able to get enough human brains to do meth alone. So that's something we'd like to do, but I don't have data on meth alone in human brains. We do in a dish. I mean, we've done it. And we see kind of additive effects of, for example, GO 120 and methamphetamine but not in the human brain. I don't have that data.

Vasundhara Varthakavi (00:38:51):

Thank you. There's also a question about, do you see the same sort of effect with cocaine or have you tested?

Stuart Lipton (00:39:00):

I have not tested. Not personally or scientifically.

Vasundhara Varthakavi (00:39:08):

So there's another question, I think, does antioxidant drugs such as NAC might be helpful?

Stuart Lipton (00:39:17):

Yeah. So my take on antioxidant drugs is you can't get them to the right place in sufficient quantities. We've taken other approaches to this. The approach we've taken, and it's a whole different talk, but important is to activate the NRF-2 pathway, which is an antioxidant anti-inflammatory transcriptional

pathway as you all know. We can do it in cells that are undergoing oxidative or nitrosative stress. And so we have a whole team looking at that kind of approach, and that can be successful.

Stuart Lipton (00:39:47):

I see another quick question. I don't want to take up too much time, but about synaptic damage key to neuro pathogenesis. Absolutely. This is true in every major dementing disease. And we are so tied up in these misfolded proteins we lose sight that the misfolded proteins do not correlate with how demented you are. And so we've done serial, we, meaning [Elysium Maslias 00:40:10] and several other, Bob Terry, others have done it. What they've done is serial neuro-psychological tests. So we know your cognitive function post-mortem exam. And the only finding at post-mortem that we're aware of that correlates with how demented you are as the number of senses.

Stuart Lipton (00:40:28):

So we need to protect synopsis. That doesn't mean the misfolded proteins or methamphetamine or GP 120 isn't triggering the damage, but there's a life of their own. Once you get neuro inflammation going, we need to treat that, we need to protect the synopsis. And I think many of us neuroscientists have lost sight of that, unfortunately.

Vasundhara Varthakavi (00:40:47):

Dr. Lipton, thank you so much. That's all the time we have for the questions. So we'll move on to the next talk by Dr. Dana Gabuzda. She comes to us from Harvard Medical School. She'll be talking to us about marijuana use, inflammation and comorbidities in people with HIV. Dana.

Dana Gabuzda (00:41:12):

Can you see my screen?

Vasundhara Varthakavi (00:41:13):

Yes.

Dana Gabuzda (00:41:16):

So marijuana use is highly prevalent in people with HIV with up to 20% to 30% of HIV positive individuals using marijuana for medical or nonmedical purposes. Given that marijuana is known to have anti-inflammatory effects and the fact that HIV infection is associated with chronic inflammation and inflammation related co-morbidities, we wanted to know whether marijuana has anti-inflammatory effects in people with HIV in natural settings, or in particular groups of HIV positive individuals, which could potentially have benefits for inflammation related complications.

Dana Gabuzda (00:42:07):

We also wanted to know what is the impact of heavy marijuana use, whether beneficial or detrimental on co-morbidities in people with HIV. So in my talk today, I'm going to talk about three aspects of our work. I'm going to talk about our analysis of longitudinal cohort data to understand effects of marijuana on the clinical course and health outcomes in people with HIV. I'll then talk about a new study on smoke related exposures detected in marijuana compared to tobacco smokers. And then I'll end with a new study on effects of marijuana compared to tobacco on inflammation markers in a large cohort of HIV positive individuals.

Dana Gabuzda (00:42:50):

In terms of background, it's complicated and there are many discrepancies between different studies. There are studies that have reported reduced activated immune cells or inflammatory biomarkers associated with marijuana use in HIV positive individuals. But there are other studies that find no significant association or even modest pro-inflammatory effects suggesting that these studies in humans, particularly complicated populations, such as those with HIV and trying to understand immunomodulatory effects that marijuana might be having in these individuals is very challenging. There are similar challenges in studying the impacts on co-morbidities in people with HIV, which today are still not well-defined with different results reported by different groups.

Dana Gabuzda (00:43:40):

And these results are likely to vary considerably depending on cohort characteristics that affect these outcomes. These questions are significant in order to know how can we optimize benefits while reducing risks in people using marijuana for common reasons that are very common in HIV positive individuals, such as chronic pain, sleep disorders, anxiety, stress, nausea, or vomiting, and cancer related symptoms, or for nonmedical reasons. I'd like to begin by highlighting some of the highlights of my Avant-Garde Award, which I received in 2009. These are older studies. We published 18 papers, and I chose six to highlight here.

Dana Gabuzda (00:44:25):

We were one of the first to identify a common biomarker signature of immune activation that detected immune activation, not only in viremic subjects, but also in aviremic subjects. And these included soluble CD 14 and CXCL 10, also known as IP 10. So we were one of the first to identify IP 10 as a very good biomarker of immune activation, including in virally suppressed subjects. We also published a paper characterizing monocyte activation markers in CSF associated with HIV related neurocognitive disorders shown here that also included IP 10.

Dana Gabuzda (00:45:04):

And part of our project had an emphasis on using metabolomics and other types of 'omics and big data to try to understand the pathogenesis of immune dysfunction and comorbidities in HIV positive individuals with substance related substance use disorders. So these are some of the first metabolomic studies we published. We were the first group to study sort of a global un-targeted metabolomics profile in a cohort of HIV positive individuals, and to find lipid modules shown here that were inversely related to high inflammation markers, bile acid abnormalities, indicating liver dysfunction, or to indicators of microbial translocation such as LPS and LBP.

Dana Gabuzda (00:45:56):

And we then went on to study metabolomics profiles in subjects with depression and identified a metabolite profile that included markers of altered tryptophan and dopamine metabolism, as well as mitochondrial energetics in HIV positive individuals with depression, many of which were chronically using crack cocaine. We then went on to reconstruct the [Mex 00:46:21] public dataset to do longitudinal analysis of substance use disorders in relation to health outcomes and in a sort of an all by all substance use patterns versus a number of comorbidities, we sort of hit on the ones that were easiest to find first and published one study where we did longitudinal modeling of depressive trajectories in HIV negative and positive individuals, and found a very strong relationship between crack

cocaine use and depressive profiles in the HIV positive individuals, much more so than in the HIV negatives.

Dana Gabuzda (00:46:55):

And then we also did a study of marijuana use in relation to cardiovascular events and other co-morbidities that I'll be talking more about in my next slides. And one of the most important new directions that resulted from my NIDA Avant-Garde Award was learning how to analyze longitudinal data from large well-characterized cohorts, including the MACS, the ALIVE, the NNTC, HNRC and CHARTER. And through this, we learned a lot of key data strategies that made it possible for us to do more sophisticated modeling of relationships between substance use and disease trajectories and health outcomes. And these are some of the many lessons that we learned here.

Dana Gabuzda (00:47:39):

And we use longitudinal data even for our cross-sectional studies, because it provides such an advantage in terms of characterizing levels of use of a substance over time, associations with polysubstance use and syndemic factors that can also impact outcomes. So this was probably the most risky and difficult thing that I did with my NIDA Avant-Garde Award, which is to basically take the entire MACS cohort public dataset from 1984 to 2014, and reconstruct the entire data set into a local SQL so that we could use this for anything we wanted.

Dana Gabuzda (00:48:17):

This was a very challenging project because there's 7300 subjects followed for five years or longer, typically with 6000 variables, it was a total of 140000 visits and over 30 million data points. However, this turned out to be extremely valuable in allowing us to look at multiple different data types and integrate them into new and interesting findings. So one of the first things we looked at was the effect of heavy marijuana use over time on disease progression and health outcomes. We started with a cohort from the MACS. This is from the MACS public dataset of serial converters, and then another cohort of chronic HIV, which were carefully selected so that at the baseline visit, they had to have a suppressed viral load on ART. And they had to have CD-4 counts greater than 300.

Dana Gabuzda (00:49:10):

Another aspect of this study that was important to reach our conclusions was minimizing confounding. And one of the approaches we took was we excluded all HCV positives, and we also excluded other types of heavy drug use, such as crack cocaine and heroin use to end up with a better match, a cleaner cohort in which we could examine the effects of heavy marijuana use over time on health outcomes. So we did a technique that we call lasagna plots, actually, somebody else called it lasagna plots, which allowed us to look at patterns of substance use over time. So shown here on the X axis is about a six and a half year followup.

Dana Gabuzda (00:49:50):

And then we can code at each visit for each individual subject. So each row here is visit level exposure data for individual subjects. Reporting daily marijuana use shown in red, the weekly are shown in orange, occasional use is shown in yellow, the less frequently use is light blue or dark blue. And through this approach, we are able to identify chronic heavy users using daily or weekly for years. And in this case, we have a cohort where the average use was at least four years of heavy use. And we do the same thing for the tobacco users characterizing the level of heavy smoking relative to non-smoking over time.

So this allows us to develop well-defined groups. Another approach we've taken is to look at sort of an all by all generalized pairs comparison. I'm not going to go into this in detail, but what this lets you do is take a big picture view of sort of the all by all pairs so that you can not only appreciate differences between groups in marijuana smoking, for example, here the marijuana users are shown in red and occasional users in green, and then the non-users in blue. What it lets you do is appreciate differences and in this case, it's more tobacco smoking and more heavy binge alcohol in the heavy marijuana users, but depressive symptoms were actually fairly similar between groups.

Dana Gabuzda (00:51:18):

We see a depression in CESD, depression scores with age, and it also lets you to get even a little more granular depending how you design this so that you can look under the hood and see, well, what about people with high education who are also smoking tobacco and marijuana? Are there any differences in their patterns? So we do this for any of our large scale substance use studies. So now that we have our groups, we looked at disease trajectories according to HIV disease markers, viral loads, CD-4 counts, CD-8 and CD-4, CD-8 ratios. And in both the serial converters and in the chronic HIV group that we selected, we saw no significant difference by level of marijuana use in any of these HIV related disease markers.

Dana Gabuzda (00:52:05):

In contrast, we found that tobacco and duel tobacco marijuana smoking in both cohorts was associated with significant elevations in white blood cell counts, predominantly representing expanded neutrophil counts, but still within the normal physiological range, which studies by other groups have demonstrated is an indication of systemic inflammation, even in people without HIV who are chronically smoking tobacco. And this pattern is important because it's been linked to some of the inflammation related outcomes in tobacco smokers without HIV, including cardiovascular events.

Dana Gabuzda (00:52:46):

So we then did Kaplan-Meier Analysis of long-term outcomes by level of marijuana use. And the heavy users are shown in red, the non-users or occasional users in blue. We found no difference in the rates of AIDS diagnoses for between ages 30 to 60, mortality or cancer diagnosis. And this is important because I'd like to mention, we have not found any evidence of association between cancer outcomes and marijuana use in any cohorts we've studied so far, but in contrast, we found a significant increase in rates of cardiovascular events. And this is a merged cohort aged 40 to 60. And this is important because it's easier to detect this pattern in these mostly middle-aged men than it is if you expand the age range, like to go out to age 70 or 75, when other factors commonly have more impact.

Dana Gabuzda (00:53:41):

And these are cardiovascular events either by just tobacco smoking with these tobacco smokers shown in yellow or a three-way stratification by marijuana only shown in green or marijuana plus tobacco shown in red. Showing these effects were detectable in the HIV positive individuals, but were not significant in the HIV negative counterparts, possibly suggesting that the already elevated risk of cardiovascular disease in HIV positive individuals may bring out the added effect of marijuana and tobacco smoking on these outcomes.

Dana Gabuzda (00:54:21):

These are adjusted logistic regression models, showing that in adjusted models, including other risk factors, viral load and CD-4 counts and age, we detected about a twofold odds ratio in either marijuana

users or cigarette smokers for cardiovascular events in middle-aged men in the MACS. These studies suggest that heavy marijuana smoking and when I say heavy, I'm thinking about those red lasagna plots with four plus years of daily or several times a week use is associated with an increased rate of cardiovascular events in HIV positive men ages 40 to 60 beginning with some events occurring at relatively young ages, including myocardial infarctions or strokes under age 50.

Dana Gabuzda (00:55:09):

This may explain a portion of the elevated cardiovascular risk in some HIV positive populations that is not attributable to tobacco smoking and traditional risk factors. We then went on to do a similar type of study using the MACS public dataset. And this time, we took an even wider time range from 1996 to 2014, where we compared the effects of marijuana smoking on pulmonary disease in HIV infected and uninfected men. We did a matched group design where we first excluded other types of heavy drug use. And then we used a matching software to identify matched groups of HIV negative and positive participants matched for age and race at the baseline visit.

Dana Gabuzda (00:55:58):

And in this study, marijuana smoking was defined of at least one year of daily or weekly use. And we used a time updated variable with a sliding window of a two year average to identify heavy marijuana or tobacco smoking over time. The major findings from this study were a significant impact of heavy marijuana use shown in blue, tobacco use shown in yellow or the combination of using both marijuana and tobacco on self-reported chronic bronchitis in HIV positive individuals or on a composite of infectious pulmonary diagnoses that included viral pneumonia, influenza, bacterial pneumonia, quote, other causes of pneumonia, which presumably was mycoplasma.

Dana Gabuzda (00:56:47):

And that was associated also with ineffective marijuana on a composite of infectious pulmonary outcomes between ages 30 to 65. However, we did not find similar findings in the HIV negative group matched for similar age and race at baseline. And also we did not find associations with other pulmonary outcomes, including CLPD or lung cancer in terms of the marijuana exposure. We then wanted to know more about the mechanisms through which marijuana smoking or heavy marijuana smoking in particular might lead to elevated rates of cardiovascular and pulmonary comorbidities.

Dana Gabuzda (00:57:32):

And to accomplish this, we performed a study in collaboration with the tobacco and volatiles branch group at the CDC in which we measured 33 plasma and 28 urine metabolites of nicotine, THC and various smoke exposure related chemicals, such as polycyclic aromatic hydrocarbons, VOCs and other smoke related chemicals using mass spectrometry. This was done in a longitudinal cohort study of 245 participants all over age 40, 76% were HIV positive and the subjects were enrolled mostly in the NNTC cohort or the HNRC and CHARTER cohorts. And we had about 40 subjects included from the Chicago site of the MACS. We then evaluated these findings in relation to exposures and health outcomes.

Dana Gabuzda (00:58:27):

This is the overall characteristics. The median age at baseline was 53. The groups were pretty well matched for age. Most of the characteristics are fairly well matched, but interestingly, there were more cardiovascular diagnoses within five years prior to endpoint, particularly in the tobacco smokers and to a lesser extent than the marijuana users. So, these are the lists of the metabolites that were measured in

plasma and urine. We compared levels both by geometric means and T tests as well as in these box plots with nonparametric tests. And shown here are the most notable findings.

Dana Gabuzda (00:59:11):

So the four groups are shown in different colors where blue are the non-smokers, the green are the marijuana only smokers, yellow are the tobacco only smokers and the red are the dual users. And as shown here, we detected good levels of THC in just about all of the marijuana users for in the dual users who are both using marijuana and tobacco. And the marijuana users had essentially no nicotine metabolites, so they were clean. And that was partly by our study design. And then we looked at the other metabolites and the significant findings in terms of marijuana exposure, where that marijuana smoking was associated with elevated levels of plasma naphthalene, here's one example shown here as well as other metabolites, including O-cresol sulfate, but most notably were the acrylamide and acrylamide shown here, AAMA and GAMA and acrylonitrile metabolites, CYMA and CYHA, which were elevated in marijuana smokers who are not using tobacco shown here in green compared to the non-smokers.

Dana Gabuzda (01:00:20):

But these elevations were generally not as great as those seen in tobacco smokers. And this was generally true across the board that the highest levels were seen in the tobacco smokers or the dual users with relatively modest elevation seen in the marijuana only users. Now contrary to our original hypothesis, we did not find additional elevations in the dual users who were smoking both marijuana and tobacco. Their levels were relatively similar to those of the tobacco only smokers. And these are heat maps of the same data organized by group, the non-smokers, the tobacco only smokers, the ... Sorry, these are the marijuana only smokers in green with the THC and then low levels of some of these smoke related chemicals.

Dana Gabuzda (01:01:06):

These are the tobacco only smokers with high levels of these smoke related chemicals. And then these are the dual smokers with similarly high levels of these chemicals. And I wanted to bring your attention to HPMA, which is the major metabolite of acrolein, which is considered one of the most toxic chemicals in tobacco smoke and show you here that acrolein is high in the smokers, only detected in a few marijuana smokers, but at levels that were really no different from the non-smoking controls. So you may wonder where is this acrolein coming from in these non-smokers well it's because there are other sources, particularly dietary sources related to fried foods and charred meat.

Dana Gabuzda (01:01:47):

And acrolein is thought to be one of the major reasons why fried foods are unhealthy for cardiovascular risk, because it is a reactive aldehyde, the major source is derived from combustion of tobacco and other combustible products, but it can also be derived from food sources, especially fatty foods and it's reactive. So it can cause reactive modifications of proteins, for example, in vessel walls. And it can also activate neutrophils and monocytes by its reactivity, and it's been associated, we and others have associated it with tobacco smoking shown here, the correlations with nicotine metabolites and in adjusted models, we associated high acrolein levels with cardiovascular risk adjusting for other factors, either in the total cohort or just in the HIV positive subset.

Aaron (01:02:42):

Dr. Gabuzda?

Dana Gabuzda (01:02:45):

Yes.

Aaron (01:02:45):

A few more minutes before the Q and A.

Dana Gabuzda (01:02:45):

Thank you. So anyhow, this shows that acrolein exposure is increased by tobacco smoking, but not explicit marijuana smoking and contributes to cardiovascular disease in tobacco smokers. Since I'm running short on time, I'll just briefly talk about our profiling of inflammation markers in a cohort of 317 individuals, 232 had HIV. They were from NNTC, CHARTER and HNRC and 95% of them had plasma viral load suppressed below 200 copies. The marijuana users were all using for at least one year, and they had detectable THC in urine or plasma at the sample visit.

Dana Gabuzda (01:03:28):

This is another G pairs plot. And I'll basically give you the punchline since I'm running short on time. When we looked overall at the total cohort, we really did not see any significant effects of marijuana use on inflammation markers, such as gamma, interferon IL-1, IL-6 and CRP. However, we did detect an anti-inflammatory effect on these markers if we focused on the HCV positive subset, and it's well-known that co-infection with HCV further augments inflammation levels in HIV positive individuals. And in these individuals, we could detect an anti-inflammatory effect of marijuana use on inflammation markers.

Dana Gabuzda (01:04:11):

And we looked at this in adjusted models, both logistic regressions, and linear regressions where marijuana's effects alone were not remarkable. But when we included an interaction term of marijuana with HCV serostatus, we could detect an inhibitory or negative effect of marijuana use on the levels of these three inflammation markers, interferon, gamma, IL-6 and CRP, which had the strongest findings, suggesting that you need to look at these cohorts very carefully in terms of context. And this is a population at greater risk of inflammation. And in this subgroup, we can detect anti-inflammatory effects.

Dana Gabuzda (01:04:54):

So to conclude, our studies together with findings from other studies in humans suggest that the anti-inflammatory effects of marijuana in people with HIV are context dependent. Modest effects on inflammation markers, particularly IL-6 and CRP are detectable in some natural settings where inflammatory activity is high, such as HCV co-infections when the study design accounts for co-variants such as tobacco smoking, BMI, viral suppression, et cetera. We've also shown that long-term heavy marijuana use is associated with elevated rates of cardiovascular events, infectious pulmonary diagnoses, and chronic bronchitis in men with HIV independent of tobacco smoking. In contrast, however, we did not detect significant associations of heavy marijuana use with rates of progression to AIDS, mortality, cancer, COPD, or cognitive impairment.

Dana Gabuzda (01:05:50):

We've also shown that marijuana smoking is associated with exposure to some of the smoke related chemicals that are also associated with other types of smoking, including acrylonitrile and acrylamide metabolites, but the exposures are lower in comparison to tobacco smoking and the health risks of these exposures remain unclear. So in sum, given these findings, the high prevalence of marijuana use and increasing legalization, there is an urgent need for well-designed research to better understand potential benefits and risks of various marijuana products with different potencies and different routes of deliveries and different ratios of CBD to THC, which might have different effects on inflammatory profiles and to determine the safest routes of delivery.

Dana Gabuzda (01:06:39):

These are the people in my lab who did the work, particularly David Laurens, Vikas Misra and Deb Johnny, who did most of the work that I'll present today. And these are our collaborators at the NNTC, the MACS and the CDC. And I'd like to end by thanking you for attention and be happy to answer any questions.

Vasundhara Varthakavi (01:06:59):

Dana, that was a beautiful talk. Thank you very much. We have some questions that have come in. So do you have any insights on the differential impact of smoked versus vaped cannabis?

Dana Gabuzda (01:07:14):

I couldn't hear you. I'm sorry.

Vasundhara Varthakavi (01:07:16):

Do you have any insights or data on differential impact of smoked versus vaped?

Dana Gabuzda (01:07:23):

That is a very important question. We would love to have been able to dissect our data in terms of the smoking versus the vaping versus different ways of smoking, whether it's a joint or a pipe or a water pipe, et cetera. As we suspect there probably are differences, but we did not have the data available to do this. And one thing I would like to encourage is for these well characterized observational cohorts to get some kind of additional funding for new questionnaires that could be added onto these existing cohorts so that we can collect that data, not only about the roots, the smoked versus the vaped, but what products are they using? What kind of strain is ...

PART 2 OF 6 ENDS [01:08:04]

Dana Gabuzda (01:08:03):

But what products are they using? What kind of strain? Is it the high-CBD variants? And I think that would be incredibly useful data because I suspect we will find differences, but right now there is virtually no data to address any of those questions for marijuana use.

Vasundhara Varthakavi (01:08:19):

So we can tell you from my program perspective, [inaudible 01:08:23] program staff are working with the NICHD, now managing MACS/WIHS cohort in trying to introduce some of these instruments to get more clarity on types of drug use and the [inaudible 01:08:36] of use. We are met with little bit of

pushback because already the visits are so long with so many different instruments that the participants have to take, but we are pushing for it. So hopefully it will happen pretty soon. We have more questions here. Okay. So, were you able to look at any data comparing other routes of marijuana? Which you just answered.

Dana Gabuzda (01:09:09):

On pulmonary disease, that's one of the first questions. Yeah. Other ways of smoking, does that affect pulmonary disease? I suspect there will be differences between the smoked, the vaped, the non-inhaled routes that there will be differences, but right now I don't have any data to address this. And I'm not aware of even data in the general population to address that question. I think those studies are awaited.

Vasundhara Varthakavi (01:09:35):

Any data by gender? What are the consequences in women versus-

Dana Gabuzda (01:09:41):

That's a good question. In the HIV positive cohorts, we tried to look at that, but we did not have enough females to say whether there's a difference. But in many studies, females are more prone to high levels of inflammation, especially if it's combined with female with high BMI and other risk factors, lifestyle factors. But so, it would be very interesting to look at that question, but we didn't have the statistical power to be able to do that. I'm not aware of data from other cohorts that have strongly addressed that question, but it's a good question.

Vasundhara Varthakavi (01:10:13):

Right. We have-

Dana Gabuzda (01:10:16):

Oh yeah. So I'm going to answer the last one, because that's an easy one. So anonymous attendee. Do you find IL-6 has an exclusive pro-inflammatory impact in your work? We did not yet try to tie IL-6 data to what is the impact. Do people with higher IL-6 in our cohort of 300 people have more cardiovascular disease or more of anything else? We would like to do that, but we haven't done that yet. And given all the confounders that I showed you in those giant grids, those will need to be carefully adjusted models to be sure that the outcome is related to one factor and not another.

Dana Gabuzda (01:10:52):

But I would like to emphasize that of all the biomarkers we looked at, IL-6 and CRP seemed to be the ones that had the best signal to noise. And I think are ones that we're going to continue to focus on. IL-6 is also interesting because of the so-called inflammaging story. And we also see trends of higher IL-6 with aging and CRP too. So those are good biomarkers to look for a number of outcomes, including frailty and aging related effects.

Vasundhara Varthakavi (01:11:19):

That was part of my question I was going to ask. But, are you looking at COVID?

Dana Gabuzda (01:11:26):

Oh. So I'm not, I'm not looking at COVID and I knew somebody was going to ask about COVID. So I looked at the literature, including I even looked into the BioRX unpublished stuff that's posted on. Has anybody looked at marijuana smoking or vaping to say whether there's more risk of bad pulmonary outcomes in COVID? I cannot find any real data that's looked at that. And I think most of the studies have looked at tobacco smoking and vaping and yes, there is a higher risk for COVID related outcomes, and that's why they got prioritized for vaccine groups in some of the states. But for marijuana use, I can't find any data on that. And I think it's a very important question. I think a lot of these hospital-based and EHR-based databases just didn't have enough data for the people doing the studies to be able to quickly answer those questions.

Wilson Compton (01:12:15):

The paper in molecular psychiatry that Dr. Volkov has coauthor on, and there's a companion paper in world psychiatry addresses cannabis use disorder in the huge IBM dataset. So that's where it-

Dana Gabuzda (01:12:32):

Thank you. Thank you very much.

Wilson Compton (01:12:33):

It does show in association with [inaudible 01:12:35]. It's kind of universal for all the substance use disorders. It's an association with COVID-19 diagnoses. Opioid use disorders at the top, but virtually all of them are significant risk factors adjusting for multiple other possibilities.

Dana Gabuzda (01:12:51):

Okay. So I'm dying to answer this question from Monique Brown because it's really important. I am surprised there was no effect on cognitive impairment. Does marijuana use usually affect cognitive impairment?

Dana Gabuzda (01:13:02):

So we have looked at cognitive data in almost every cohort that I mentioned because a lot of them have a focus on cognitive outcomes and you can get T-scores and depression scores, and you can get diagnoses of the so-called hand related diseases that Stuart Lipton was talking about. The ANI and MND, different types of cognitive outcomes. To date, we have not seen either a positive or negative relationship between heavy marijuana use and either T-score data or hand related diseases. We haven't really drilled down to start looking prospectively, or like five years later, or in an aging cohort. But I think it's an important question because different cohorts report different results.

Dana Gabuzda (01:13:47):

So Ron Ellis in Southern California has found potentially beneficial effects. And Igor Grant as well, saying that marijuana use was associated with lower incidents of HIV related cognitive disorders in their cohorts. And then there are studies on the East Coast that have said the opposite and have said that they find higher rates of cognitive impairment, particularly memory disorders, in people who are reporting heavy marijuana use. And I think part of this is exactly like one of the attendees that just said depends how much you use, routes of exposure, other co-factors like tobacco smoking or not. And potentially, even the products that are available in the regions where the studies are being done. The products on the Southern California might be different from what people are getting either on the

streets or in the dispensaries and other states. So I think, it's very complicated. And I think the different studies in their context could be correct, but they need to be viewed in the context and the setting and the products that are being studied in that study.

Dana Gabuzda (01:14:47):

So we have not detected positive or negative effects, but I think we'll stay tuned and keep looking. Somebody said, you would think there would be impairment. But when we study people at the study visits, we assume most of them are not coming in very impaired from any recent drug use. Otherwise, they might not be coming in to do the entire neurocognitive battery. So I think, when they're looking in the neurocognitive batteries, presumably they're mostly looking at the more long-term effects rather than the effect of an acute exposure, but anyhow remains to be determined.

Vasundhara Varthakavi (01:15:19):

Thank you, Dana. That was a wonderful talk. And I think that's all the time we have with questions.

Dana Gabuzda (01:15:25):

Thank you. But feel free to answer them if you can, in the Q and A, if you have time. And let's move on to the next talk by Dr. Don Des Jarlais. He's from School of Global Public Health, New York University. He will be talking to us about Combined Prevention of Initiation into Injecting Drug Use. It is... Trying to find them. Okay, there you go. Don, you are on. Do we have to man the slides or are you okay with?

Don Des Jarlais (01:16:19):

My medical center put a very heavy firewall on my computer that does not allow me to do any screen sharing. There's great concern about the confidentiality of data within the medical center. So I do have that problem. Okay. So if we could go back to one slide. Okay.

Don Des Jarlais (01:16:49):

So I'll be talking about Reducing Initiation and Relapse into Injecting Drug Use. Next slide, please. Okay. I'd like to acknowledge the team members from New York City and also from Tallinn, Estonia. This has been a long standing international collaboration, looking at HIV among people who inject drugs.

Don Des Jarlais (01:17:18):

In New York City, we were basically studying a Suburban Opioid Epidemic. And in Eastern Europe, we were studying an HIV Epidemic, a longstanding HIV epidemic among people who inject drugs. Next slide, please.

Don Des Jarlais (01:17:37):

Okay. The problem of injecting drug use is becoming universal throughout the world. It's a very cost-effective method of administering drugs. You get a very intense drug effect at low cost because you're using almost all of the drug. Even compared to smoking, you use most of the drug and get an intense effect and that's an immediate effect. So in terms of habit formation and behavior change, an immediate effect can be quite powerful.

Don Des Jarlais (01:18:15):

There are however many adverse individual and societal consequences of injecting drug use, which everybody is familiar with. Blood-borne viruses, bacterial infections, increased likelihood of overdose, and more rapid development of dependence and substance use disorders. These, however, tend to be delayed effects. They may take months to even years to development. So again, in terms of setting up habits and behavior modification, the delayed effects are likely to have less importance to a person who is contemplating injecting, beginning to inject drug use. Next slide, please.

Don Des Jarlais (01:19:03):

Okay. In this avant-garde study, we use the logic of combined prevention that's similar to the logic of combined ART treatment. For HIV infection, you don't use just one drug, you use a combination of drugs to get maximal effect. It's also the same logic in preventing HIV infection, among people who use drugs. That when you use multiple interventions, such as needle syringe programs, ART, and medication assisted treatment, you can see actual elimination of HIV in population of people who inject drugs.

Don Des Jarlais (01:19:51):

For the problem of beginning injecting drug use, we looked at programs to reduce initiation of new injectors by current injectors, programs to keep non-injecting drug users from seeking a first injection, programs to prevent relapse by injectors who have changed and non-injecting drug use. That's an interesting thing that has come up several times in the study, that many people who inject will decide they don't want to inject anymore, and they'll change to non-injecting forms of heroin or cocaine use.

Don Des Jarlais (01:20:32):

And the problem then is to keep them in the non-injecting mode of administration rather than relapsing back to injecting. And we also looked at medication for opioid use disorders, because if you can put somebody on buprenorphine or methadone, you can dramatically reduce, possibly even eliminate their injecting, eliminate the adverse consequences of injecting. And if you can get people onto MOUD before they start injecting, it may prevent initiation of injecting. Next slide, please.

Don Des Jarlais (01:21:13):

Okay. I mentioned that we did this in New York City and Tallinn, Estonia. In New York City, we worked mostly on Staten Island, which is the suburban borough of New York. It was in the midst of an opioid epidemic. People going from painkillers to heroin and from non-injecting to injecting drug use. It had very, very high rates of non-injecting drug use. And heroin was the primary drug for both non-injecting and injecting use.

Don Des Jarlais (01:21:50):

First, Estonia is a Baltic country near Russia. Tallinn is the major city. It is towards the end of an injecting drug use epidemic. Their injecting drug use epidemic started with the collapse of the Soviet Union, and they got up to HIV rates of 50% or more. They have low to moderate rates of non-injecting drug use. Interestingly, fentanyl and amphetamine are the primary drugs used. Fentanyl came into Tallinn and basically drove out heroin. So it was the place in the world where fentanyl has been used the most intensively. And for the longest period of time, it is traditionally had the highest rates of fatal overdoses of any place in the world. And the avant garde has allowed us to continue to study a fentanyl epidemic. Next slide, please. Okay.

Don Des Jarlais (01:23:02):

Illicit drug injection is a complicated, messy, and dangerous procedure. It's potentially fatal and almost everyone requires the assistance of an experienced injector for their first injection. It's not something that you just pick up by yourself. You typically would never have the skills to just start injecting by yourself. Next slide, please.

Don Des Jarlais (01:23:32):

Okay. This is a picture of somebody giving injecting to someone else. And I'll focus on both people in this situation, the person receiving an injection and the person giving an injection. Next slide, please.

Don Des Jarlais (01:23:52):

This avant guard was built on some previous research, particularly the Heroin Sniffer Project that we did in the 1980s. That was an intervention for heroin sniffers, intranasal heroin user. Both people who had never and injected and people who had formerly injected, they had stopped injecting. It was a small group intervention with trained group facilitators. It focused on providing information, motivation, and modeling of behavior. And it was a randomized clinical trial. Next slide, please.

Don Des Jarlais (01:24:34):

We did an eight month follow-up. We found that in the experimental group, 15% had injected during follow-up and in the control group, 33% had injected during follow-up. So [inaudible 01:24:49] ratio better than two, in terms of participating in the study, reducing the chances that you would inject drugs during follow-up. Injecting during follow-up was associated with the recency of last injection among the former injectors. If they had injected within six months of coming in to the study, they were much, much more likely [inaudible 01:25:14] ratio of about four to inject during follow-up. And it was also associated with close personal relationships with current injectors. That the more friends or family or sexual partners you had who were injecting, the more likely you were to inject during the follow-up period. Next slide, please.

Don Des Jarlais (01:25:37):

We adapted this intervention into what we call Avoid the Needle. We changed to one-on-one sessions with a booster one month later. If you've tried to work with people who inject drugs, getting them together for group sessions is a major logistical challenge so we moved to one-on-one sessions. We had slightly different versions for the people who had never injected and for people who had formerly injected.

Don Des Jarlais (01:26:08):

We focused on motivational interviewing to reduce ambivalence about injecting, emphasize concerns about immediate negative consequences, rather than long-term consequences. It was a strength-based intervention, where we try to develop their particular skills and we gave them skill practice in refusing offers to inject. We also provided information on naloxone where they could obtain it, given the overdose epidemic going on in both locations. And we provided information about safer injecting in case they did start injecting. Next slide, please.

Don Des Jarlais (01:26:55):

The components of Avoid the Needle is first, there was introduction then we reviewed people's current drug use and their history for their main drug. And we got them to think about one year in the future.

Most people who use drugs think about today and tomorrow and that's about it. So we tried to create a longer timeframe for thinking about their drug use.

Don Des Jarlais (01:27:22):

We've reviewed their knowledge of injecting, and many of the never injectors were pretty ignorant about injecting, their thoughts, feelings, and have they ever been in situations where they were close to injecting. We reviewed the health, social, emotional, and legal risks of injecting and discussed how to handle situation where injecting may be immanent. Next slide, please.

Don Des Jarlais (01:27:51):

We had role plays of refusing to inject in the face of social pressure from other people to inject. And we found that that was particularly useful to give them skills in refusing to inject. We also, as I mentioned, discuss safer injection and dealing with overdose, and then we had a wrap up of what they would take away from the session. Next slide, please.

Don Des Jarlais (01:28:22):

We recruited from SSPs in Tallinn and Staten Island. We had a pre-post design with a six month follow-up. We had the same pattern of results that we had in heroine sniffer with very, very low rate. Under 15% of people who had never injected starting to inject a very, very low rate of relapse among the long-term former injectors. But a high rate relapse among the short term former injectors. That if your previous last injection was within six months, you were very likely to go back to injecting during follow-up. But if you'd gotten past that six month period, people tend to form a stable pattern of not injecting drug use with very, very low chance of relapsing back to injecting. And we found that it was concerns with friends' and relatives' reactions to going back to, or starting to inject. That was the most important factor in whether or not they did inject during or avoided injecting during the follow-up period. Next slide, please.

Don Des Jarlais (01:29:45):

We did have a major finding that it was very difficult to recruit non injecting drug users at organizations. The non-injectors did not want to come to the Syringe Service Programs. They wanted to maintain confidentiality of their drug use. They didn't want other people to know that they might start injecting. Concern about track marks was an important factor in non-injecting.

Don Des Jarlais (01:30:17):

We also tried working with schools, drug treatment programs, and drug courts, and found that those organizations were basically not cooperative. They wanted to deny that any of the people they were working with use drugs. And if they admitted that people were using drugs, they preferred an abstinence only approach rather than a harm reduction orientation. That they wanted the people that they were working with to just totally stay away from drugs and not consider safer versus more dangerous types of drug use, sniffing heroin versus injecting. Next slide, please.

Don Des Jarlais (01:31:03):

We then restarted the study in New York City with street-based respondent-driven sampling and found many non-injectors were willing to come to an anonymous building. It was actually part of the dental school. So that if they could come to participate in the study without risking their confidentiality, we had

no problems recruiting non injecting drug users. Then COVID hit. And as part of the lockdown in New York, we had to shut the study down. We were not allowed to see research subjects in person. Months later, we were able to restart the study based on telephone interviewing. We mailed payment cards to subjects so that we could load money onto a Visa card after we interviewed them. But unfortunately, many subjects had moved. They changed phone numbers. Some had moved from various shelters due to COVID. But we formed a new hypothesis that the COVID pandemic conditions would accelerate transitions into and relapses into injecting drug use. That the stress of the pandemic would increase the likelihood that they would move on to injecting drug use. Next slide, please.

Don Des Jarlais (01:32:39):

But in the data we've collected so far, that hypothesis was not confirmed. None of the 42 subjects we've interviewed so far injected during follow-up. And we would have expected from our previous research that seven or more of them would have injected. So we had a clear rejection of a hypothesis that the pandemic would lead to worse forms of drug use. We started thinking about resilience among non-injecting drug users. A number of them told us that they had taken the pandemic as a time to re-examine their drug use, and trying to reduce their drug use and definitely staying with non-injecting drug use rather than moving on to injecting. As a side note, the COVID vaccination rate in this group was approximately equal to the rate in New York City as a whole. Next slide, please.

Don Des Jarlais (01:33:47):

Next, I'll talk about the person actually giving the assistance with the first injection. There was previous research on an intervention called Break the Cycle. It was originally developed by Neil Hunt in the United Kingdom. A second version using peers or people who inject rather than a trained counselors was developed by Carol Strike in Toronto. It's based primarily in motivational interviewing. And the studies have seen relatively large effect sizes with 50% or greater reductions in having people who are currently injecting, assisting with first injections in pre versus post design. Next slide, please.

Don Des Jarlais (01:34:38):

We wanted to update Break the Cycle. We first conducted qualitative studies of people currently injecting drugs in New York City. The qualitative studies gave us four different types of PWID with respect to assisting the first injection. There was a group that we called the Kantian moral imperatives. They just never, ever wanted to assist with the first injection. That was the large majority of people who were currently injecting. We also found a group we called the Situationalists. They generally do not assist, but they will assist if they believe that that will cause lesser harm to the non-injecting user who wants the first injection.

Don Des Jarlais (01:35:25):

We found a group we called the Opportunist who would assist for drugs or other considerations. And we found a small but worrisome group that we called the Evangelical Assisters. They were people who believe that injecting is by far the best way to use drugs. And they wanted to turn on people who were not currently injecting. Again, that was a small group but definitely a worrisome group. Next slide, please. Okay.

Don Des Jarlais (01:35:58):

We used a clinical trial research design for our updated test to Break the Cycle. Next slide, please.

Speaker 2 (01:36:07):

Doctor, just a few more moments before the Q and A, please.

Don Des Jarlais (01:36:12):

Okay. I will go through quickly. We used subjects. Okay. Next slide. I'll go on to... Okay. It was a pre post design in six months time periods. Next slide, please. I'll skip over the components of the updated intervention. I've mentioned, it's primarily motivational interviewing and skills training. Do you want to note that we did a role-play of refusing to assist if they were asked. Next slide, please.

Don Des Jarlais (01:36:52):

People often regretted their first injection often with intense emotion. Next slide, please. These subjects were very different in the two cities, particularly in their drug use and their ethnicity. Next slide, please.

Don Des Jarlais (01:37:10):

Okay. We did see very different patterns of injecting drug use. As I mentioned, it was fentanyl and amphetamines in Tallinn and heroin and cocaine in New York. Next slide, please. We look at injecting promoting behavior, whether or not people talked about positively about injecting or modeled injecting in front of non-injectors or offered to assist. Next slide, please.

Don Des Jarlais (01:37:47):

Okay. These were the outcomes in Tallinn. We saw a reduction in promoting behavior and in both sites, we saw substantial reductions in assisting with first injections. 80% reduction in Tallinn and a 60% reduction in Staten Island. Next slide, please.

Don Des Jarlais (01:38:11):

Ah, in red, we've looked at the baseline to follow up for the people who received the intervention and notice that we saw reductions in their own injecting, reductions in non-injecting drug use statistically significant. Next slide, please.

Don Des Jarlais (01:38:37):

So for additional studies, we've done another round of Break the Cycle in Tallinn, and we found equivalent results of 80% reduction, but evidence that the intervention was not sustained over a two year period. We were doing an RCT of Break the Cycle in New York to quantify reductions and assisting and reductions in people's own drug use that did get interrupted by the COVID pandemic also. We were doing an implementation of Break the Cycle at an SSP where we would be able to sustain it because the people doing the intervention would be staff members and they would get reimbursed for substance use counseling. Again, that was interrupted because of the COVID pandemic. On next slide, please.

Don Des Jarlais (01:39:37):

So in terms of our next step, we really want to get everyone vaccinated for SARS COVID. It is very, very, very difficult to do interpersonal interventions when COVID is not under control. Next slide, please.

Don Des Jarlais (01:39:58):

We want to strengthen the Break the Cycle intervention for reducing injection promoting behavior, further study the effects of Break the Cycle on participants' own drug behaviors, and try to identify the active ingredients in Break the Cycle and examine Break the Cycle for sustainability and the possible need for booster sessions. Next slide, please.

Don Des Jarlais (01:40:25):

For Avoid the Needle, we wanted increase the focus on interpersonal relationships because those seem to be critical for whether or not people start or relapsed to injecting. We need to find institutional homes for providing Avoid the Needle. We don't really have an infrastructure for providing this intervention. We want to develop a more specific intervention for people who've recently transitioned from injecting. If we can get them to six or more months of non- injecting drug use, we feel they're very, very unlikely to relapse to injection. And we want to try to increase access to a low threshold for MOUD. That has happened because of COVID. And we want to see if we can continue that. And next side, please.

Don Des Jarlais (01:41:20):

And thinking at a bigger level, we're considering working with PWID in Haiphong, Vietnam. We recently completed a study there where HIV incidents has been reduced to less than 0.1/100 person-years at risk, essentially eliminating HIV among people who inject in the city. But 45% of these people who inject heroin are smoking methamphetamine and about 5% have injected. So there's a potential massive spread of injecting methamphetamine among these people who are currently smoking. We're thinking-

PART 3 OF 6 ENDS [01:42:04]

Don Des Jarlais (01:42:03):

Among these people who are currently smoking, we're thinking in terms of population level, implementation of combined interventions to prevent transition from smoking to injecting methamphetamine. I think that this would really be need to be done at a population level that you can't really prevent epidemics of injecting by working with small groups. But if we can develop methods for preventing injection of methamphetamine, that would have great implications for the US as well. Okay. Thank you.

Vasundhara Varthakavi (01:42:44):

Thank you so much. That's a very interesting talk. So I'm trying to... So we went over time. [Crosstalk 01:42:55] so if you could please go ahead and answer any questions that might come up in the Q&A because it's so hard to hang on the zoom calls forever without a break. So we scheduled for a break beginning 2:45 to 3:00 PM, a 15-minute break. So Aaron do you have any announcements to how people should join back?

Aaron (01:43:21):

No. No announcements says noted. We were planning to return about three o'clock for the remaining presentations. I don't see any questions in the Q&A or the chat box. So if anybody has any for a doctor [Vizu Leewho 01:43:43] feel free to enter those in now.

Vasundhara Varthakavi (01:43:49):

Yeah, just stay on connected. You can just be unmute and turn off your video, so you don't have to use the link again to dial back in. Because there might be some problems sometimes. So we'll see you all at three for the next talk.

Vasundhara Varthakavi (01:44:04):

So I now introduce the next speaker is Dr. Julie Overbaugh. She's from University of Washington. She will be talking to us about profiling escape pathways for HIV antibodies. Julie?

Julie Overbaugh (01:44:23):

Thank you. I want to talk a little bit about one project that we did under the avant-garde mechanism. Our overall interest in this avant-garde proposal was to try to understand how HIV evolution and variation impacts the function of the envelope protein and also its antigenicity. And I'm going to specifically talk today about the second part of that, which is understanding how changes in HIV envelope impact antigenicity with the goal of more effectively targeting envelope for vaccines and prevention of HIV infection. So I think most of you here know well that HIV envelope can evade antibodies through a rapid mutation. That's a highly mutable virus. It also has other strategies for evading antibodies like altering confirmation and glycosylation. And so this presents major challenges because HIV is such a genetically diverse virus and it's hard to target the virus with antibodies. But of course the good news is... And I'm trying to find my advanced slot, my advanced thing.

Julie Overbaugh (01:45:36):

There we go. The good news is that there are broadly neutralizing antibodies that can neutralize diverse viral strains, and they're elicited fairly regularly in infection, but not always. And everybody considers these to be a critical component of an effective HIV vaccine so that you can actually target the globally circulating variants. And also these broadly neutralizing antibodies have been shown in the context of animal models to prevent infection and also to therapeutically suppressed viral replication. So these are for that reason considered important in both prevention through vaccines and also potentially as therapeutics. But even though these are broadly neutralizing antibodies, they still are subject to escape. So HIV can escape these broadly neutralizing antibodies. And if they do, this will compromise the efficacy that we hope to achieve with vaccines. And of course, we see this concept playing out with SARS-CoV-2 vaccines right now.

Julie Overbaugh (01:46:41):

So we wanted to understand the pathways of escape to try to develop approaches that might tip the balance in favor of the host over the virus. A lot of the traditional methods that have been used have been very useful in this regard. For example, people use structural studies where they look at complexes of the HIV envelope protein and an antibody. And they define how these two interact at the amino acid level. They define these contact residues, assuming that these are also going to be the amino acids in the envelope protein that could allow escape from the broadly neutralizing antibody. And then they use the structural studies to test this. And a number of mutations have been identified in this way, it's a very labor intensive process and not complete. And what we also don't know or didn't know was whether these contexts that you capture in a structural study actually give you a very complete picture of escape pathways.

Julie Overbaugh (01:47:41):

The other approach people use is just to make individual mutations, either guided by structure or other biological reasoning, but this is very labor intensive and certainly not comprehensive. So we set out to develop a more comprehensive method to define escape pathways. And for this we used Deep-mutational scanning. And this work I should say, was done in collaboration with Jesse Bloom, who had developed these methods in his lab at Fred Hutch for influenza virus. So Deep-mutational scanning is a method in which you mutate every possible amino acid in the protein of interest to every possible option. So that's just illustrated here with the wild type sequence shown on the top line. And you can see at one position, we mutate every possible amino acid. At the next position, we do the same thing, et cetera. So you create a library that may be 10,000, 15,000 different sequences that capture every possible way of the virus could evade and evolve.

Julie Overbaugh (01:48:53):

Okay. Let's see if this will go forward again. Okay. So the method that we applied this to is called mutational antigenic profiling. This is a method developed by Adam Dingens, who was a joint graduate student in my lab and Jesse's lab. And Adam created this library of mutants in the HIV envelope, protein. He generated this library in the context of replication competent virus. And then he put this virus library under antibody selection. So you have a library that is being challenged by antibody. And as a control, you have that same library of viruses used infect cells with no selection. You then look at the viruses that are successful in infecting the cells. And you see that those that were selected in the presence of antibody or those that were able to escape the antibody pressure. So you compare the sequence of the viruses that made it through this antibody bottleneck with the sequences of viruses that were not under selection.

Julie Overbaugh (01:49:56):

And you identify sequences that are enriched during this selection process. And that enrichment indicates that they are escape variance. So that's shown in the logo plot here where you can see, for example, at one position, N671, in the amino acid, you see that a large number of viruses with a three [Inin 01:50:19] at that position were able to be selected in the presence of antibody indicating that that is an escape variant. And you can see by the Loco plots that you can also not only see where escape occurs, but what amino acids are favored for escape. So you get a very granular view of escape pathways using this method. So the first thing Adam did was to apply this method to a well-studied HIV broadly neutralizing antibody. One that had been characterized structurally and with individual mutagenesis studies. And this is one called PGT151 that targets the fusion peptide and gp120/gp41 interface. And what Adam saw is shown in the graph at the bottom, which is that there were three regions where you can see indication of selection around 511, 611, and just beyond there in the envelope protein. So when you look at those positions in more detail and drill down to look at the escape pathways, you see that there are a number of individual amino acids within those regions that allow escape. And those are shown in the logo plots again, as I illustrated conceptually before. But I think what's particularly interesting about Adam's data is at the bottom of this Loco plot. You can actually see the sites that have been identified in previous studies. These were extensive previous studies, structural studies, mutagenesis studies, high profile studies. And I think what you can appreciate as well, we captured all of those positions as being important in antibody interactions with PGT151 and escape.

Julie Overbaugh (01:52:04):

We also captured quite a number that were not previously identified. One example I'll just highlight is this position 648, where residues that change charge in the protein seem to allow escape based on this analysis. Of course, we wanted to know whether or not those mutations actually were causing escape in this proof of concept study. So we introduced the individual mutations back into the virus, one by one as is traditionally done, and we examined their ability to be neutralized by PGT151. And I'm just showing you here some data for that position 648. And what you can appreciate is that the two mutations that were most enriched in the deep mutational scanning study, the lysine and arginine at that position were also the ones that most impacted neutralization compared to the wild type. And in fact, we looked at a number of mutations identified in this study and did the same thing across different amino acids.

Julie Overbaugh (01:53:10):

And what I'm showing you to the right in that graph is just the correlation between the results we generated using traditional neutralization with individual mutations in the virus, compared to what we saw with enrichment and deep mutational scanning. And you can see there's a very high correlation between these two readouts. So with that success with a known antibody, Adam went on to look at a collection of antibodies that are from the five major classes of broadly neutralizing antibodies for HIV, the ones that target the CD4 binding site, the V3 glycan the V2 Apex, the fusion peptide and MPER as shown in the chart. And I point out here with the purple circle that a number of these are actually in clinical use or being tested, I should say for clinical use. So these kind of analysis are not only important in understanding HIV replication and antibodies in tissue culture, but they really should apply to what we can understand from the scape in a clinical setting.

Julie Overbaugh (01:54:15):

I'm not going to show you all the local plots for these monoclonal antibodies, but I just want to show you a summary here and make a few points. So you can see the enrichment plots for each of these antibodies. And for example, the ones that focus on V3 PGT121 and 10-1074, we see enrichment at V3 in those antibodies. And indeed for all the antibodies that we study, we do see the predicted epitopes using this method, but what's highlighted in the ribbon structures to the right of each antibody is I think an interesting finding from this work. And that is that if you look at the green highlights, those are the regions or the amino acids where we identified escape and where previous structural studies had shown this to be a contact residue.

Julie Overbaugh (01:55:08):

But what's highly interesting, I think is looking at the blue amino acids. Those are ones where we identified escape, but we previous studies had not captured those just based on structural interactions. And there's a third interesting group, which is one where we see no evidence of escape, but where the structural studies suggest there's contact. So these kinds of analysis, I think illustrate the much more comprehensive nature of using this approach to map escape for broadly neutralizing antibodies. So in this method what I've shown you is that this mutational antigenic profiling could identify known escape mutations as well as novel escape mutations not detected in structural studies. I want to just point out that this method is not just suited for studying antibody interactions with HIV envelope, but you can actually study other interactions as well.

Julie Overbaugh (01:56:07):

And we studied interactions of HIV envelope with CD4 receptor, and also use this method to identify sites of what you could call escape or resistance from antiretroviral therapies. And we did a proof of

concept study in this domain focus, of course, on an antiretroviral that targets the envelope protein because that's where we had made our library. And this is enfuvirtide, which is a salvage antiviral therapy that blocks fusion. And we did that because we have the Env library, but I will make the point that this could be applied to other proteins as well, like polymerase, et cetera. So this just shows you the data from the selection that we did with the HIV Deep-mutational scanning library in the presence of this antiretroviral drug. And you can see that we again could clearly demonstrate resistance mutations or escape mutations using antiretroviral therapies, not just broadly neutralizing antibodies. And a number of these mutations, again, correlated with known mutations. And in addition, there were novel mutations identified using this approach.

Julie Overbaugh (01:57:26):

So what I hope I left you with from this first part of the study is the power of this approach called mutational antigenic profiling to provide complete mutation level mapping of viral escape. It massively increases experimental throughput compared to structural studies and individual mutagenesis, and importantly, what we showed is that defines a functional epitope, which is different than the structural epitope. It can also aid in evaluating clinical trials in refining vaccine regimens and identifying drug resistance as I showed you. But I will say there are some limitations to this approach. So even though it's high throughput compared to structural studies, it still requires large amounts of virus. And generally you can do one antibody at a time using this method. It also requires as a readout neutralization. So by definition, we're studying neutralizing antibodies in this experimental setup. But there's clearly increasing interest in non neutralizing antibodies and antibodies that kill target cells through effect or mechanisms. And those kinds of antibody interactions would not be captured with the method as it was developed.

Julie Overbaugh (01:58:40):

So this really motivated us to think about how we could take this method and move it forward so that we could interrogate epitopes of both non neutralizing, as well as neutralizing antibodies. And I'll show you that in the next slides, and then also how we extended this method to study SARS-CoV-2. So the approach that we developed, we call Phage-DMS. And basically we take the same Deep-mutational scanning concept. And rather than putting all these mutations into the replicating virus, we put them into peptide sequences, introduce them into phage.

Julie Overbaugh (01:59:19):

And then we use the phage to probe the antibodies, either monoclonal antibodies or antibodies in plasma by looking for interactions through binding. So we capture those bound phage through immuno precipitation. We can then deep sequence the immuno precipitated phage and use those sequences computationally to first of all, identify those enriched in the wild type sequence. In other words, those in the wild type sequence that bound to the antibody where we're using and that defines the epitope. And then within the epitope, we can compare the sequences with mutations to see if they were captured at the same level or whether they were reduced in binding, which would indicate escape.

Julie Overbaugh (02:00:08):

And again, we did a proof of concept study. This was a study led by Meghan Garrett a graduate student in my lab. And this was with a known well-defined antibody targeting the V3 region of HIV envelope. And in the top graph, you see that in fact, what was enriched when we look for wild-type peptides that bound to this antibody in this format was exactly in the V3 region. And then when we looked at the

mutations that disrupted binding, you could see evidence for escape or disruption of binding in what was considered the core motif of this epitope GPGR and that's shown in two ways in the grasp below. One is just sort of an inverse kind of logo plot on the left. And another is a heat map on the right. And the heat map, basically the red shows that amino acid positions and the specific amino acids that disrupt binding.

Julie Overbaugh (02:01:05):

Again, we wanted to make sure that what we measured in this assay would correlate with traditional assay's. So Meghan tested individual peptides that were identified through the Phage-DMS and a competition ELISA, and compared the binding disruption in that ELISA format with the extent of how these were selected in the Phage-DMS. And you can see there's a really strong correlation between these two measures.

Julie Overbaugh (02:01:35):

So just for the last couple of slides, I want to mention that I think as many of us have done taking what we've learned from HIV and applying to SARS-CoV-2 has been instrumental in really tackling this new pandemic. And it's really been a lot of what was done in the HIV field that has moved this needle forward so quickly. So we wanted to use Phage-DMS to profile the epitopes in the Spike protein of SARS-CoV-2. So for that reason, we generated a phage library just like we had with HIV with Deep-mutational scanning. This library was almost 25,000 peptides, and we use that library to interrogate the responses in COVID-19 patients in the convalescent sera from these patients in a collaboration with Helen Chu who leads a respiratory infection study called the HAARVI study team and in collaboration with a computational collaborator, Eric Matsen's group, again led by Meghan Garrett, a student in the lab.

Julie Overbaugh (02:02:41):

This just shows you the enrichment of wild-type peptides across different patients showing you where they are on the structure and the linear sequence of the Spike protein. So let me orient you a little bit here. The first thing is that on top, you can see the structure of the spike protein, the S1 protein and the S2 protein, much like gp120/gp41 of HIV. Two of the major epitopes that we identified as common across different patients were in the fusion peptide and in the linker region in HR2 region. And most of the individuals we studied had a response in this region of the Spike protein in S2. In gray, you can see other regions where we identified epitopes that were more individual specific. And I will note, we did not identify the major responses to the receptor binding domain, the RBD, because our method does not detect confirmational and glycosolated protein responses.

Julie Overbaugh (02:03:45):

So I want to drill down. These are the responses that we saw for 18 of the individuals shown collectively. I want to drill down on the individuals and show you some interesting aspects of these responses. So these are for individuals now where I'm just showing you their individual enrichment plots on the left. And first of all, you can see that again, most but not all of them recognize the fusion peptide. The patient nine did not show a response in this region. And almost all of them showed at least some evidence to a reaction to the HR2 protein, although patient nine had a very strong response to that protein. On the right I'm showing you the logo plots. And because here we're looking for a negative effect on binding, they're shown in the inverse orientation, but the concept is the same. And what I want you to appreciate is if you look at patient one, patient eight, patient 12, they all respond to the fusion peptide, but their escape pathways are all unique.

Julie Overbaugh (02:04:50):

And this is actually really exciting because it suggests that no one mutation that might be selected in nature would be able to escape in all different individuals. The responses are very different and very individual. The other aspect of this that I think is really interesting is where these epitopes lie on the protein. And I'm just showing you a structure here with highlights of the variable regions and the conserved regions. And what I think you're going to appreciate in the purple is that this S2 subunit where we map these dominant responses are in a more conserved region of the Spike protein. And this is exciting because this may mean that if we target this more conserved and functionally constrained epitope, it could limit escape. And in fact, in the plot in the right, which I won't go into details on, we basically don't see evidence that the mutations that we've identified are becoming dominant in those circulating in nature.

Julie Overbaugh (02:05:53):

So that supports the idea that these epitopes might remain more functioning constrained if they're under antibody pressure. So I'd like to conclude by just summarizing and hoping that you take away from this, that this process of Deep-mutational scanning can be applied in a wide variety of ways to define escape pathways and viral resistance and other protein interactions. It is comprehensive, and the results correlate well with other more traditional assays, such as neutralization, Elisa, et cetera. I didn't show you all of the data where we've applied this, but we've also applied this approach to a variety of setting, including being able to map and define the epitopes of new broadly neutralizing antibodies we isolated in the lab and also in collaborative studies, looking at vaccine responses to specific epitopes particularly in non-human primate models. It's also provided insights beyond those afforded by structural studies.

Julie Overbaugh (02:06:58):

And it's much more high throughput, especially the phage Deep-mutational scanning. And of course the work that we've done with COVID-19 infected individuals, we are extending to SARS-CoV-2 vaccine responses so that we can also anticipate any escape pathways outside the receptor binding domain that might be important in the vaccine responses. So I'd like to, again, acknowledge the people who contributed to this work. I think I've mentioned the key players. I'd like to, again, thank the NIDA AIDS Research Program and the avant-garde for supporting some of this work and really getting us started in launching some of this Deep-mutational scanning work, which is, I must say, a very expensive experiment because of the deep sequencing. So this work was really quite essential to that. And again, thank you.

Vasundhara Varthakavi (02:07:55):

Thanks Julie, for this wonderful talk. I'm going to ask you the first question. Do you have plans to looking at people who use drugs? We're just getting the sparse data that people who use opioids may not mount immune responses to the same level as people who do not use drugs. Is there something that can be validated using the assay that you have?

Julie Overbaugh (02:08:30):

[inaudible 02:08:30]. Are you thinking for the SARS-CoV-2 vaccine or infection or?

Vasundhara Varthakavi (02:08:35):

Or even to future HIV vaccine candidates. So, yeah. COVID would be good. Good. Because we really want to understand, are people able to respond at the same level? Did they pathogen our vaccine?

Julie Overbaugh (02:08:51):

I think that would be very doable with this method. And I will say that for the Phage-DMS method, we can interrogate a hundred samples at a time, which we couldn't do with the viral replication assay. So it is something where we could apply this to a population like that if there were samples available. We could readily do that and would be interested.

Vasundhara Varthakavi (02:09:14):

Yeah, there would be of interest in NIDA secondly. Hi Melanie.

Melanie Ott (02:09:22):

Hi, this was a great talk, Julie. I just wanted to ask you a little bit about your general take on how the wonderful work that you and others are doing now with SARS-CoV-2 by going from previously acquired knowledge to SARS-CoV-2 and back. How will that affect the HIV vaccine in the future? And what is your prediction? And because I think it's a unique opportunity and super exciting time.

Julie Overbaugh (02:09:50):

Yeah. That's a such a interesting philosophical question, really Melanie. And I feel like it's been such a good thing to take what we've learned and apply it to Corona viruses. I think obviously we cannot stop working on HIV and those of us who do both now have to figure that out, right? And not completely crush our students in doing that. But I think looking at this, we're also learning a lot from COVID-19 and SARS-CoV-2, that's going to come back.

Julie Overbaugh (02:10:20):

So I think the circle will in the end benefit HIV, even though some of the energy for HIV probably got depleted a little bit this year. But I do believe that it's going to come back that I think the science of what we learn is going to be applicable. And obviously the B mRNA vaccine, the fact that this concept is now clearly so potent already tells us that probably that's where we have to spend a little more of our HIV time. And we didn't know that before. So we've learned a lot right there, I think.

Melanie Ott (02:10:55):

Thank you.

Vasundhara Varthakavi (02:11:01):

Thank you.

Aaron (02:11:02):

Yeah. So it looks like we have an attendee with their hand raised. I'll go ahead and allow you to unmute and move through.

Julie Overbaugh (02:11:17):

Okay. Is there a question... Sorry.

Vasundhara Varthakavi (02:11:23):

Yeah, maybe not. Maybe it's not able to unmute. That's why.

Julie Overbaugh (02:11:31):

If you'd like to ask a question, move through. You should be able to unmute and speak.

Vasundhara Varthakavi (02:11:44):

So just to remind the audience still can ask questions through the Q&A. And the panel members will get back to you with answers as much as they can before the symposium concludes. So with that, there are no questions. I just want to make sure. Julie, that was a wonderful talk. Look forward to hearing more stories from this research in future, and please do come back to NIDA. And if there's anything we could do to help support this research further, to address more questions related to drug abuse, we would be really, really interested in engaging in those discussions with you.

Julie Overbaugh (02:12:29):

Thank you.

Vasundhara Varthakavi (02:12:31):

So with that, I would like to introduce our next speaker. It's Dr. Davey Smith. He's from University of California San Diego. He's going to talk to us about Network Informed Prevention and I'm thinking HIV prevention.

Davey Smith (02:12:56):

Hello. Can you hear me and see my slide?

Vasundhara Varthakavi (02:13:04):

Can you switch it to the slide more? Yeah, there you go. Perfect.

Davey Smith (02:13:12):

Can you see it and can you hear me?

Vasundhara Varthakavi (02:13:16):

Yeah. Good.

Davey Smith (02:13:17):

Perfect. Okay. So today I'm going to talk about Network Informed Prevention. I'm very humbled and honored to be in this group. And I really appreciate NIDA for the avant-garde award. It has changed my career. My name is Davey Smith. My pronouns are he, his and him and I'm at UC San Diego. I'm going to talk a little bit about evolution cause everything makes a lot more sense once we understand that through the lens of evolution and when Darwin established that all species of life are descended over time. For ancestors, that evolution resulted from a process of natural selection. Then this change can be observed in the genetic code. And my project was about understanding how to use that to our advantage. And first off, starting with HIV, HIV among all the RNA viruses. There's low fidelity and then there's high fidelity viruses, RNA and DNA. And HIV has a pretty good mutational rate.

Davey Smith (02:14:12):

Julie talked a little bit about this earlier. Hepatitis C has more, but there's a spectrum, but there's enough there for perhaps we can use it to our advantage. And one of the things we did, first of all, first off looking at Chinese study, we saw that there was differences in mutations, in different risk groups. And here is the percentage of viruses that we found in different populations. And some of these clusters had different mutations with lysines and et cetera, and different groups. And we could saw that people who use injectable drugs had E92, 96 strains while MSM in this population had only K92 in the 96 strains. So this gave us some interest in, could we track what happens over time within these individuals in terms of mutations.

Davey Smith (02:15:10):

So to step back in terms of evolution, perhaps we could use evolution to our advantage here. And evolutionary processes leave measurable footprints in the viral gene and the genetic distance between two viral loops and gene sequences reflect their relatedness. So you could look here at envelope or gag or our pol for HIV. And you could see that these trees look quite different and spread out depending on their genetic relationships amongst each other. And each of these represents the different clade. So viruses within clade C look closer together than viruses that are C to A or just within perhaps A. For example, I am genetically close to related to my mother than I am to your mother. And if we were to do sequencing, we could figure it out. But in HIV, that difference is on steroids. And we can really sort this out in the follow genetic way. And we can make maps of this-

PART 4 OF 6 ENDS [02:16:04]

Davey Smith (02:16:03):

And we can make maps of this transmission network, how HIV has gone from one person to another person, to another person. And this is a really good work from Sergei Kosakovsky Pond and Joel Wertheim, looking at how to make an HIV transmission cluster engine turn phylogenetics into an ability to look for clusters. So each person has their own unique virus that's adapted to them. We can then use phylogenetic or genetic distance methods to sort it out, who might be closely related within a transmission chain, to come up with clusters. And then we can use that information to, let's say, investigate outbreaks. So here, we took 288 HIV sequences that were generated between 2004 to 2016. About half of them were men who have sex with men, a third of them are women, 40% reported transactional sex, and 28% reported injection drug use as their risk factors. And 13% of these people were recent infections.

Davey Smith (02:17:01):

And then we analyzed these sequences using distance-based methods to figure out that 37 transmission clusters were within this group, involving 122 sequences. And then while we were working on this, in part of our Avant-Garde work, we saw a great increase in the number of new infections in mid-2016, so we're like, "Okay, what does that look like?" Well, since we could map it out and we can map it out by risk factors, we started making these little clusters. So this group right here had very closely-related sequences, this group here had closely-related sequences. And each of these little connections, we could make based on their genetic distance between their viruses that were sequenced at a certain time. And what we noticed is that there were big groupings of... Here's female sex workers. And then the green were heterosexual, the blue were men have sex with men, and the light blue were the

bisexual. And then people who injected drugs were in the red big squares. We started to see a pattern that there was an increase of people [on 02:18:10] hotspots who were using injectable drugs.

Davey Smith (02:18:17):

So what was going on? Well, this study was conducted in the border region between San Diego and Tijuana. And specifically, we had a population of people who injected drugs in the Tijuana River Canal, El Bordo. And this is a picture of what it looked like before, and then this is what happened in late 2015. They went in and they cleaned it up. We're like, "Okay, what was going on here?" This was associated with a time when we actually saw an increase in the number of infections in that [cohort 02:18:51]. What we saw was that, unfortunately, after moving from the canal region, the people who injected drugs found new galleries which were safe from the police. So the police were the ones that came in and cleared out those galleries, so they just moved out into the suburbs, basically, and found new galleries to shoot. But this was also away from the NGOs that were giving harm reduction materials in terms of, basically, needle exchange at that time. And this, we're pretty sure, increased the risk of infection that we were seeing within those galleries. So that was one really good way of figuring out that we could see when there was an issue. Another one is, can we intervene on the HIV transmission network once we have it mapped out? And [one of the things 02:19:45] we noticed when we map out these clusters for transmission networks is that people have different positions within the cluster that we identify. Some people are connected, basically, to everyone else in the cluster, and then some people are only connected to some people in the cluster, or maybe even only one person in the cluster. And we call this degrees, so if this person is connected to seven other people who have infection, then that's seven degrees. Versus a person who's only connected to one person who is infected, that's one degree versus three degrees. So now, we have a simple but qualitative measure where we can see what the centrality in the network might make a difference.

Davey Smith (02:20:35):

So our hypothesis was that people who were more central in the network, I had more degrees when we mapped it out, were more important to intervene on for prevention. And we did a nice study in the China MSM Acute Infection Cohort that [would have 02:20:56] been collected for a long period of time and had been tested on a regular basis, and all the participants had CD4 counts and HIV viral loads and genotypes that allowed us to look at it.

Davey Smith (02:21:06):

And our question was, "Well, what if we targeted antiretroviral therapy? This was, at a time, in China where they didn't give everyone therapy based on CD4 count and viral load. And so it was all done retrospectively, but we want to see whether or not degrees made a difference. And our strategy was, "If you just treated all the participants, how many people would you have to treat? How many cases would you have prevented? And then what kind of prevention efficiency would you see?" So then we wanted to say, "Well, okay, let's see if you targeted the people who had the really low T-cell counts. That would have been nine people, and we would have prevented one case."

Davey Smith (02:21:47):

And really, any of those CD4 counts didn't give us much prevention efficiency. In fact, it was less than if we just treated everybody. If you look at viral load, which has definitely been a predictor of transmission, if you gave it to people who only had really high viral loads, 10 to the fifth, you would have treated 36 people, but only would have saved three people within our transmission network [on

02:22:13] infection, and that was 8%. Versus greater than 50, it would have been 57 people you would have treated and got to 23%. All of these are less than if you just would've treated everybody, so there was no benefit for using these other measures as surrogates for prevention targeting.

Davey Smith (02:22:31):

Okay, well, do degrees matter? So does the network tell us anything of who might be important for targeting prevention efforts, such as increasing adherence to antiretroviral therapy and other things? And I'll talk about those in a second. So in this group, we have the number of participants on this Y-axis, we have the efficiency on this axis. We have the number of degrees of our participant in 2009 and how many infections we'd have prevented in 2010. If you treated everybody, a 120 people, you would have saved this many number of infections.

Davey Smith (02:23:12):

But if you started treating everybody who had one degree or more, you would have had this efficiency. And as you can see that there's basically a good stepwise progression, that the more degrees that somebody had in the network, then you would have prevented more infections in the future, with a 55% prevention efficiency of targeted antiretroviral therapy for those who had the highest degrees. So even though there was only few people, I don't know, I think 15 people who had these high number of degrees, if you could get them on therapy in this setting or some other prevention, then you would have the biggest impact in future transmissions within that network. So then we came up with an idea, and we basically structured this to where we take HIV pol sequences, which are used in standard clinical practice to look for transmitted drug resistance before somebody goes on therapy. And then we put that into a real time in the background of all sequences. And then this gives us a network for our local community, and we can start to map out what degrees everyone has in real time in this network. We then generate what we call a transmission network score. If the score is high, which means they have a lot of degrees, or if it's low... If it's low, we just follow them, but if it's high, we generate a risk profile and an intervention plan. And that's where we look at the demographics, the risk behaviors, the venues to where they're, might be, at risk. We look at the estimated duration of infection, acute, early, chronic.

Davey Smith (02:24:57):

And then we also add in CD4 and viral load since we have them. And then we propose a ring intervention where an intervention team of a nurse, social worker, test counselor, whatever that intervention looks like, and then target venues such as HIV testing, counseling, STI testing, and needle exchange. Another big point about this is these methods also allow us to know whether or not we can be successful. It's whether or not our intervention is actually successful, looking at the phylogenetic lineages that die out over time. So it's a real iterative process, you can say, "Okay, if I target, let's say, bathhouses or shooting galleries more than I target something else, I can see those lineages die faster, less transmissions going forward than I would expect," or not.

Davey Smith (02:25:56):

And this data, I'm proud to say, is part of the respond pillar for ending the HIV epidemic that the CDC now has adopted a few years ago. And this respond is to respond quickly to potential HIV outbreaks, to get needed prevention and treatment services to people who need them. And through this Avant-Garde, we worked for multiple different groups, including the CDC for establishing the pillar. Joel Wertheim who got a K award, with me as his mentor along these lines, is really ingrained in this and has done amazing work. We also worked with the China CDC for MSM and substance use. We've worked

with India, for their NACCHO, for substance use and controlling HIV outbreaks. The Mexico Center for Infectious Disease Research is ongoing right now, with big collaborations on establishing their respond for molecular epi. South Korea CDC has similar plans, as does the Mozambique Ministry of Health and Cape Town HIV control program. All are up and running.

Davey Smith (02:27:05):

So another one, since we are in the time of COVID, we've adopted a lot of the methods and stuff that we learned early on in sequence analysis and molecular epidemiology and brought in new techniques. And one of those big techniques is called phylogeography. And here, I'm just going to talk a little bit about determining the dynamics of the SARS-CoV-2 across the border. I talked about HIV across the border earlier. Can we do the same thing with SARS-CoV-2, which actually has a higher fidelity [level 02:27:37] than HIV for its mutational rate? And we were very interested in the impact of human mobility on the viral dynamics that we saw.

Davey Smith (02:27:48):

And here, we looked at between January of last year to January of this year, we had almost 12,000 SARS-CoV-2 complete genome sequences downloaded from this public database that has been absolutely amazing. It's a worldwide effort to get these sequences out into the public domain, so that researchers like myself and Antoine Chaillon who did a lot of this work can access them. And here, we're interested in California and Mexico, and you can see that there is a big lopsided amount of sequences that came from both. But the nice thing is that we have developed techniques and ways to account for that. But in these sequences, we combine them with a representative set of non-California and Mexico sequences, so it was a very large database. And we identified clades, i.e, basically, you can think of clusters with only mixtures of California's and Mexico's sequences. And then we did another analysis to come up with, [at the time 02:28:50], the most recent common ancestor lineages for each of those clades that had two or more sequences that were attached to it. And we came up with a date of when that occurred. And then we looked at clades that were greater than or equal to three sequences originating from two different locations. So if there were three sequences in San Diego, they didn't do [them any 02:29:12] good. But if I had two sequences in San Diego and one in Baja California, or one in Los Angeles, then I could include that on my further analysis. Decreasing the amount of data that we have to analyze, that's informative, greatly gives us a chance to actually get it completed since [it's such a 02:29:33] large computationally-intensive datasets. But then we went on to do phylogeographic inference for these clades using BEAST, so a Bayesian sequence analysis.

Davey Smith (02:29:49):

So here, we had 96 clades of greater than or equal to three sequences, including both California and Mexico that were identified. The ranges were from three to 48 of cluster size, and then we did a discrete phylogeographic analysis, looking at the viral migration network across the border. And we also looked at the migration patterns so we knew, in general terms, how much human migration was going back and forth from across the border. And basically, what we found, we could draw these... Well, I call these intercontinental ballistic missiles, but we can see which of these places were connected. And so we had a few sequences in San Diego and we have a few sequences in Mexico City, and then we would draw a line. Or if we had some in San Diego and Los Angeles, we would draw a line. And these are the transitions that we saw.

Davey Smith (02:30:44):

And we can add, since we're using Bayesian analysis, we can use what we call Bayes factor analysis to come up with statistics, "Which of these are actually supported transitions," which has greatly increased our ability to have confidence in what connections that we actually see. And basically, what we saw was that most of the transmissions that we observed were coming from Baja California, which is in Mexico, over into San Diego and a little bit above into San Francisco or [in Chula 02:31:24] But also within Baja to other parts of Mexico, we can also see. What's interesting is there were tons of flights that went back and forth, but the land border seemed to be really important for a lot of the observations that we saw. And it didn't really matter how many sequences we had from each of these, it was all about the clades and the connections between those two. Because there are a lot more sequences that were used in San Francisco versus San Diego but, still, the migration was mostly from Baja California to San Diego. So that's SARS-CoV-2. And that work is directly related to all the great stuff that we did within the Avant-Garde, but another component of this is along the same lines. So you can think of big things happening between places as phylogeography, but there's also little things that happen within a person that has HIV. And this is back to HIV. And this is a project that we have here in San Diego that's part of our Last Gift cohort. So these are people who are terminally ill. They have HIV and they're terminally ill, or something else such as cancer or ALS or cardiovascular disease. And we follow them perimortem, and then when they pass away, we do a rapid autopsy and collect specimens all across their bodies. So we get blood before they die, we get lymph nodes, [all the gut 02:32:51], et cetera, you can see. And then each of those tissues, we can sequence it.

Davey Smith (02:32:55):

And since we followed them antemortem, we have blood beforehand, and we can also get genital secretions and rectal biopsies, et cetera. And some of these people actually stop their therapy, they're passing away and they don't want to take their therapy anymore, so they oftentimes... They sometimes stop their therapy, so we'll actually be able to observe rebound as well. But independently, we can sequence all the HIV from all these tissues, and then we can use very similar techniques to figure out, "Aha, what tissues are most closely genetically related within the same person to see where HIV might migrate between all these different places." So it's a different ICBM map within a person. And then we can actually look at genetic flow with a statistical support called the Bayes factor. And here, you can see that within the brain. It goes to other places, maybe the prostate or the rectum is where we see it next door, or the big flow almost always is PBMC lymph nodes.

Davey Smith (02:34:03):

And we can drill down into very specific things looking at, let's say, the blood-brain barrier. How does the basal ganglia attract to the rest of the basal ganglia, or the blood clot going to the hippocampus, or within the esophagus going to other parts of the gut which is very interesting? And then the genital tract is another barrier. What are the ones that are connected? And as you can see, there's a lot less connections in the genital tract.

Davey Smith (02:34:32):

And now, we're doing experiments underway to evaluate this same HIV migration in relation to people who take opioid. During the last parts of people's life, they're often under considerable pain. And there's lots of opioids that are being used in that setting, and that gives us an opportunity to look at those concentrations, in relation to what we might see when somebody stops their therapy or even when they continue their therapy, to look at migration. We do see quite a bit of provirus that looks like to be

moved within the blood, and then seeding within tissues even though someone is completely suppressed with antiretroviral therapy.

Davey Smith (02:35:16):

So I just want to acknowledge my collaborators in South Korea, in China, in India, and South Africa who really adopted a lot of these techniques. And we published quite often with them, but really worked closely with their ministries of health to get their molecular epidemiology programs together. And a lot of that work with Joel Wertheim and getting his HIV trace out into the world has really greatly increased its utility, [and in here 02:35:45] , developing new methods along the Sergei and Sara and Antoine. And of course, Susan Little, with a lot of big programs here in San Diego, still going on with our health department and trying to really use these tools for the maximal prevention efforts as possible. And then I'll stop there and take questions.

Vasundhara Varthakavi (02:36:08):

Thank you very much. Excellent presentation. We have some questions coming in the Q and A. So Davey, also, we are very proud that we've been able to award a DP2 recently to another one of your team members who's working on the Last Gift cohort.

Davey Smith (02:36:34):

Yeah, we're really thankful for that. Sara's going to do great work with that DP2.

Vasundhara Varthakavi (02:36:38):

We're looking forward to her progress as well. So here's a question. So you mentioned treating individuals with high degree centrality. Did you look at this by any other centrality measures such as betweenness or eigenvector centrality?

Davey Smith (02:36:55):

Yeah, I-

Vasundhara Varthakavi (02:36:56):

There's a second component to the question. What are your thoughts about treating all individuals at popular injection venues and whether that might further impact network level of prevention?

Davey Smith (02:37:12):

Yeah. So first off, I think I am for treatment of everybody as quickly and as soon as possible, with someone who's diagnosed. To get them therapy as quickly as possible, that's first of all. And then the second one, we looked at a whole bunch of measures, and we still are looking at betweenness and [inaudible 02:37:29] and various centrality metrics. If it turns out that just degrees is the simplest and probably works just as well. However, I have to say that there is some issues around [homophily 02:37:44] with different groups, such as... You can think of... The classic one is a man who has sex with men and with women, and that different bridging effect seems to be very important for prevention efforts.

Davey Smith (02:37:59):

And in every single model that we do it, it definitely comes up. But in real-world practice, we see a signal there, but it's not as strong. And I think that the reason for that is we're not as good at identifying everybody in the network that might be affected. But the other thing about the... I used antiretroviral therapy as the intervention, but there's a whole bunch of things such as needle exchange or other harm reduction services. [It was 02:38:30] just cleaner in that Chinese cohort to use antiretroviral therapy, but we're working with lots of people here in San Diego with other harm reduction techniques intervening on the network.

Vasundhara Varthakavi (02:38:41):

Maybe, perhaps, linking it to medications for opioid use disorder? So there is a question from Sandy Springer. She says, "Did you look at adding PrEP availability intervention model, as well as now for those with OUD intervention model, including those without it?" [inaudible 02:39:03] Yeah.

Davey Smith (02:39:06):

Yeah. So PrEP is really, really good. So in the network that you see using molecular epi, you're only looking at people who have the virus. But with Susan Little's work and with Joel Wortheims's work, they're looking at people who are connected to the network but are not infected. And you can come up with other degrees of connectedness along those lines, so looking at risk networks. And then where does PrEP attach to those people who are uninfected, but perhaps exposed to your high-risk network? And that work is fascinating and is underway.

Vasundhara Varthakavi (02:39:48):

Great. There's another question here about which portion of the basal ganglia that you talked about.

Davey Smith (02:39:59):

I could probably spend way too much time talking about different parts of the brain, but we added multiple different pieces of the basal ganglia. We did not find a particular part of the basal ganglia that made the biggest difference, it is quite variable. The basal ganglia HIV concentrations is quite variable across people.

Vasundhara Varthakavi (02:40:24):

I think that's pretty much everything, just make sure that I'm not missing anything that came through chat. Well, congratulations, Davey, excellent work. And I'll keep in touch.

Davey Smith (02:40:42):

Thank you. Yeah, of course.

Vasundhara Varthakavi (02:40:42):

And we look forward to getting more from your group in future.

Davey Smith (02:40:47):

Yeah. Thank you.

Vasundhara Varthakavi (02:40:50):

So that leaves us to the last presenter. I'm really waiting for her talk. She has some cool story that she is going to present or talk to us about. This is Dr. Melanie Ott. She is from University of California, San Francisco. She is going to talk to us about HIV and aging, from molecules to tissues.

Melanie Ott (02:41:16):

Well, hello, everybody. Let me just make this big. Do you see everything?

Vasundhara Varthakavi (02:41:24):

Yes, it's good.

Melanie Ott (02:41:26):

[Okay. Cool 02:41:26] Thank you, [Kavi 02:41:27]. Thank you very much for the invitation and the kind introduction. Thank you everybody for staying until the last talk. I'm a virologist from San Francisco, and I really want to thank NIDA for the Avant-Garde Award because it has been transformational in two ways. First, it has really helped us to really focus on the molecular biology of aging and seeing how HIV and drugs of abuse would interfere with this. And then secondly, it has also allowed us not only to focus on the immune system but, eventually, to branch out into tissues. And I will show you more about this during the talk here.

Melanie Ott (02:42:17):

[Now 02:42:17] just to step back at the beginning, to review our amazing progress that we have done over the last 2,000 years in terms of enhancing our lifespan and also our health span, but particularly in the last century where we made a huge [jump 02:42:34] in terms of life expectancy, and that is mainly due to water cleanliness and vaccination. However, we know that one group has been left out from this or has actually regressed in this enormous progress that we have done, and these are people living with HIV.

Melanie Ott (02:42:56):

And we all know that this is not because of AIDS, this is just by... People who are living chronically with the virus are experiencing an accelerated rate of certain diseases of aging that basically curtails their life expectancy. And it has been estimated that it's at least five years of lifespan that is actually taken from people living with HIV. And this is increased even further if it's HIV- positive drug users who are, on average, losing more than 10 to 15 years of their life span. So our goal was to really understand what is this, what are the mechanisms of this decrease in life expectancy, and how could we find ways to target these molecular mechanism to potentially reverse it? And as I said, we wanted to focus on the molecular biology of aging because we know from a [, really, burst 02:44:10] of research activity in the past 10 to 20 years that there is a program in us and in ourselves, and every organism that controls aging and lifespan. And there have been many molecules and proteins being identified that play a role in this process and could be potentially targeted therapeutically. One of those are the so-called sirtuins. These are group of protein, deacetylases, that are highly conserved between bacteria and mammals. And specifically, yeast and C. elegans have shown that they increase lifespan if you increase the copy number of the Sir2 protein. Now, there are seven homologs of this Sir2 protein in humans or mammals. The first one, SIRT1, is the best studied and probably the closest in function to the Sir2 protein.

Melanie Ott (02:45:18):

It is, as I said, a deacetylase, and it's a very unique deacetylase because it's directly connected to the metabolism of the cell by consuming NAD for the deacetylation process. And it has many downstream targets that link it to many biological processes. We have worked on this protein extensively and have contribute multiple unique aspects to its function. One of them is that we found that it actually directly deacetylates the [HIV-type 02:45:51] protein and regulates its function. But we also have worked extensively on its role in NF-kappa B and inflammation, and the connection with HIV infection. During this talk, I want to draw your attention to the FOXO proteins, which are very important SIRT1 downstream targets that we have discovered through the course of our Avant-Garde Award to really play an important effect in the biology of SIRT1 in the immune system. And this is very satisfying because the FOXO protein's already the closest link to human aging, in the way that [different 02:46:34] studies have shown that variants in the sirtuins or in SIRT1 are found in higher incidences in centenarians.

Melanie Ott (02:46:47):

Now for us, what was really striking was that we found that that interacted with SIRT1, it was also a target of SIRT1. But it also had a very unique inhibitory function on the SIRT1 deacetylase activity because it binds directly into the deacetylase domain of SIRT1 and is, in here, in a biochemical assay, a very potent inhibitor of its deacetylase activity. In fact, it's even more potent than its natural inhibitor, nicotinamide, in the cell. And it was very striking to pharmacologists when we showed this [view 02:47:23] to them at the time.

Melanie Ott (02:47:24):

And the cell translates now in increased acetylation of some of these downstream targets, and acetylation is usually associated with increased function. And so when we look at NF-kappa B p65 here, which is acetylated at lysine 310, we can mimic this by overexpressing the acetyltransferase p300. You'll see nice acetylation if you look within acetylation-specific antibodies. If you overexpress SIRT1, this is decreased, but then if you co-expressed increasing amounts of Tat, you see that this completely neutralizes the deacetylase activity of SIRT1, really showing the potency of the Tat effect in these cellular and in vitro assays.

Melanie Ott (02:48:11):

We have, since then, focused a lot on this so-called Tat-SIRT1 tango, that [inaudible 02:48:17] called it at one point, and have published many papers showing that it is really relevant for the biology of immune aging but also HIV infection, and I want to give you some vignettes of this work in this talk.

Melanie Ott (02:48:38):

So we have focused, as I said, primarily on the immune system and the fact that our immune system ages over time, and we know from many studies that this is accelerated with HIV. And for example, methamphetamine use, that transforms, actually, the immune system of a young person more into the phenotype of an old person. And this is mainly shown here, schematically, by trying to illustrate that what we lose over time is, of course, our pool of naive T cells because we lose our thymus. But we also lose our repertoire in terms of the memory T cells that we have because they become more and more focused on very specific clones, or they're very much determined by clonal expansion of certain memory T cells that, over time, become more and more terminally differentiated. That means they do not respond any more properly to antigen exposure and they, instead, have more of an innate cytotoxic phenotype.

Melanie Ott (02:49:47):

Now, when we wanted to see whether this HIV, this SIRT1 connection is in any way relevant outside of an HIV-infected cell, we'll look now, in general, into the distribution of these different...

PART 5 OF 6 ENDS [02:50:04]

Melanie Ott (02:50:02):

I'm into, the distribution of these different memory T-cell phenotypes, the naive, the young ones, the CD28 positive ones, which is the marker that I use to differentiate them or the CD28 negative ones, which are the ones that are terminally differentiated. And just when, by looking very natively at what, whether SIRT1 is in these cells, we found that actually, SIRT1 is very lowly expressed in these terminally differentiated T cells. There's actually a gradient of expression at its highest expression, than the naive ones. Then it goes down in the memory T-cells, but then terminally differentiated ones, they have very low levels. And this is shown here in the, in this analysis of protein levels.

Melanie Ott (02:50:50):

We have recently solved the mechanism, what is going on here together with Shelly Berger, who is a close colleague at U Penn, who has looked at SIRT1 decrease over time and in different tissues and has elucidated the mechanism here and finds that it's mainly degraded by micro-autophagy. So in terms of overtime, autophagy somehow increasing and SIRT1 being basically degraded. But if we block micro-autophagy here with a specific inhibitor in the CD28 negative cells, we can recover the almost normal levels of SIRT1 in these cells.

Melanie Ott (02:51:43):

Now, when we look at, in CD28 cells now, downstream and wanted to see what is the effect? What is the downstream pop-up targets that are mostly affected by the loss of SIRT1 in these cells? We found that FOXO1 one was really a very obvious target because you can see very easily it has exactly the same distribution as the SIRT1 protein. It is basically also lost in these cells and it's very easy to understand because SIRT1 usually deacetylates FOXO1 in the nucleus, and that keeps FOXO1 in the nucleus. However, when SIRT1 is gone, FOXO gets exported. It gets, it's hyper-acetylated, it gets phosphorylated and then it is basically in the, in the cytoplasm degraded by the proteasome.

Melanie Ott (02:52:37):

So to just make sure, that this is really the way how we, how this is working here. We down-regulate it. Or we, we edit it SIRT1 with CRISPR in primary T-cells here. And that led immediately to the loss of FOXO1, as we had predicted. Really establishing that this is sort of the course of events that is taking place. And in contrast, when we use CD28 negative T-cells and overexpress SIRT1 here, we nucleofected a recombinant protein into the cells to avoid some cytotoxicity we could recover and stabilize FOXO1 in these cells. Really showing that there's a SIRT1 - FOXO1 axis, that is very, very prominent in determining biology in T-cells.

Melanie Ott (02:53:29):

Now, we, I spare you all of the detailed experiment or most of the detailed experiments here, because this is published. But what we found is that a very important function of this SIRT1 - FOXO1 axis is that FOXO1 suppresses the glycolytic capacity of these cells. So, and thus also suppresses cytotoxicity under

the, in the resting state of the cells. So that really, that is really what we believe is the break in a resting T-cell. That is, basically preventing these cells from becoming glycolytic, but instead using oxidative phosphorylation, however we know over time, or as I showed you in HIV, through inactivation. And we believe also through drug use, you actually accelerate the loss of SIRT1 in these cells. And that leads to the, term that leads to permanently terminal differentiation in the way that it also leads to a loss of FOXO1. So, one, it unleashes the glycolytic capacity of these cells, and the cells become very strongly innately cytotoxic.

Melanie Ott (02:54:43):

So we, have shown in that paper here, that we can actually manipulate that access here, and the way that we can go into CD28 negative cells, we treat them with Resveratrol, which is a enhancer of SIRT1 function, and that somehow, and partially restores the activity. We can do this also with NAD mimetics when we replenish or enhance NAD concentrations. And that, that also increases SIRT1 activity. And that also really puts back the break onto this glycolytic activity and the cytoxicity of the cells. But one of the things that we also discovered was that there is a very, very specific inhibitor of FOXO1. One that actually mimics this accelerated course of action that I'm showing you here, blocks FOXO1, and thus unleashes this glycolytic capacity and cytotoxicity. It's a very specific inhibitor.

Melanie Ott (02:55:41):

We were very worried that it would cross-react with other FOXO's. However, in this cellular thermal shift essay, you can see that only FOXO1, is reacting to the drug. And it's a very potent inhibitor because can immediately, just by the addition of the drug to a primary T-cells that resting, induce these glycolytic activity, or unleash this glycolytic capacity here shown by this sea horse analysis, where when you treat the cells with a mitochondrial poison, you increase basically glycolysis while under untreated conditions, the cell basically crashes. And at the same time, you can see that this also leads to a significant increase in granzyme B production, transcription, and production indicating that indeed the cytotoxicity of these cells and the resting conditions is, is greatly enhanced. So we have, looked at this and now made back the loop to HIV because we wanted to know, is this only something that we observed in CD8 cells because CD8 cells were the easiest to analyze.

Melanie Ott (02:56:58):

We basically do all these experiments and in human CD28 positive or negative cells because mouse and human immune aging is significantly different. And so the question now was, is this also applying to CD4 cells? And, and I can tell you, we see exactly the same thing in, CD4 cells would just have less quantities in cells that we can recover from PBMCs. And we also wanted to see what is the effect now on HIV. If we manipulate this axis, is this access in any way benefiting or, or not HIV infection in these cells and specifically latency? So we have, we have, you know, just simply what we, what we did is we used, a cell line, a J-Lat cell line that was established here at the Gladstone Institute that gives us a very easy readout.

Melanie Ott (02:57:54):

These cells have basically a latent provirus with GFP in them, and then you can activate it. It's a deficient provirus, you can activate and see what the transcription is activated just by looking at GFP. And you can see if we give increasing amounts of the FOXO1 inhibitor, and after 24 hours, we don't see anything, but after 48 and 72 hours, we see a very, very strong activation, a reactivation of the provirus and the cells even better than TNF alpha, which we usually use as an activator. And, and we have shown that this is

done in many different cell lines, and I'll show you in a minute, also in primary cells, but there's also a very strong synergy actually, between a TNF alpha and the Foxo1 inhibitor, where you see that, that small amounts of both actually synergize incredibly well indicating that they might actually work through different pathways in the cell.

Melanie Ott (02:58:49):

And I come back to this in a minute. Just here to show you that also ex-vivo infected CD primary, CD4 T cells respond to this, and in patient cells, we can also see, significant reactivation. However, we see again, the best reactivation when we give small doses of the PKC agonist here, in order to, to pre-activate the cells together with the FOXO inhibitor. Now to cut a long story short, we had thought that this is all metabolically driven, and that there's a direct connection between HIV and the glycolytic capacity that I showed you on these cells. However, it was very, very striking that this was not the case. It was, it's actually all going through a different pathway. And all, especially after we did RNAC all, direction, everything pointed into the direction of ER stress as the mediator of the reactivation of the virus.

Melanie Ott (02:59:46):

We could see that the FOXO inhibitors strongly activated ATF4, which is one of the proteins that during ER stress is still translated and regulates downstream ER stress countermeasures. And we could see that, and I'm not showing you the data here, but lots of studies have shown that it's actually going exclusively to the PERK arm of the ER stress pathway, not through other viral infection or amino acid deprivation stimuli here. So this was very surprising to us because we had really thought completely in a different direction, but this is what the data showed us. And we found that ATF1 directly can bind to the provirus in chip essays here. This is something that has been previously reported, and it was completely in the absence of NF-kappa B activation. But in, this, in the NF-kappa B activation was intact if we treated with TNF alpha, but it was in the presence of a very strong NFAT activation.

Melanie Ott (03:00:52):

So these are two very strong drivers of HIV transcription as transcription factors, ATF4 is something that has not been known to be a primary driver. However, these are very important regulators, and it was very striking to us that there was an either or either no NF-kappa B, but they're very strong NFAT activation here. So in summary, I think what we found in this study is that this SIRT1 - FOXO axis here is very important to diminish the ER stress also in cells. And that leads basically to no activation of the PERK and ATF4 pathway or calcium release from the ER and NFAT activation. So HIV transcription is kept low and latency is big. However, if we remove this access again, either through a natural or here at therapeutic intervention, we see that this unleashes ER stress and that leads to activation of PERK, production of ATF4 and direct activation of HIV transcription through both AT4 and NFAT and basically latency is overcome in these cells.

Melanie Ott (03:02:02):

But one of the things that we were really excited about and that I just want to point out here is that the connection here between FOXO and ER stress lies in the regulation of, autophagy. So there is, basically a feedback mechanism, potentially to SIRT1 production and stability, but there's also a very strong phenotype here where we, when we look at direct protein aggregates in the ER, off these resting, T-cells, we find that they do have that they do have these aggregates, but when we take FOXO away here with the inhibitor, or if we, if we knock it down and we see that these aggregates accumulate, and we

think that, that induces the ER stress and is a very strong, so far under studied pathway that regulates transcriptional activity and biology in these cells.

Melanie Ott (03:02:57):

We think that, ER stress calcium is really important, mimicked here with the FOXO inhibitor is really important to transition from quiescent cell into a, which harbours latent HIV, into a cell that is basically reactivated without activating the cell per se. But we're really interested in this role of ER stress or the UPR and the connection with SIRT1 in the question, how this, is a new player in the immune aging biology, in the fact that we predicted in memory T-cells and young memory T-cells ER stress is very low, but in the CD28 terminally differentiated T-cells, ER stresses unleash because SIRT1 and FOXO1 is gone. And we are very interested in seeing how modulators of ER, stress or this access that I've shown you above could now in any way, manipulate this pathway and potentially rejuvenate some of these terminally differentiated cells.

Melanie Ott (03:04:12):

We're very interested in the role of methamphetamine in this process. There's many studies that have linked this drug with ER stress. And we also have started a new collaboration with Feroz Papa here at UCF. He's a, an ER stress expert with which has started a very interesting drug development program by trying to really surgically interfere into the different arms of the ER stress to see what it does. So we're very excited to, to combine these two new aspects of this biology to see how we can move, potentially revert damage that is, that is caused by drug use HIV or natural age on these people.

Melanie Ott (03:05:04):

But I'm telling you, we have been sort of focusing on these T-cells painfully and in the past few years, to do all our studies, but aging is not only in the immune system we think that it's an important driver, but we also think that it is in many other tissues. And so what the avant-garde award has allowed us to do is to establish the tools and the lab to really look into these different tissues. And we have heavily invested into different human organoid systems that we, that allows us now out of stem cells to reconstruct many organs in the dish. We do this for the lung, the liver, and the gut in the lab, but we also collaborate with our IPC experts here at Gladstone for other PA, for example, neuronal progenitor cells, or we collaborate with other colleagues here to, to use these, these organized now for infections, but also very importantly for co-cultures with HIV infected T-cells. And we think that this is a good way to directly test what effect drugs of abuse, for example, have on direct tissues.

Speaker 3 (03:06:17):

Dr Ott, just a few minutes before the Q&A.

Melanie Ott (03:06:20):

Okay, perfect. I'm I'm almost at the end. I just wanted to say that this has allowed us to be potentially significantly contributed to SARS-CoV-2, also in the last year, because we have used many of our models, lung, gut, and heart, to be infected here in green. You see, SARS-CoV-2 replication centers, but we have particularly focused on co-infections of the liver, hepatitis C infected liver and HIV. And just to say that, of course, hepatitis C is a very important aspect, especially for intravenous drug users, because there's a, the very high infection rate, co-infection rate. And I also have to say that I have waited 20 years for the moment now that I can actually study HCV and HIV to two viruses that I have a long-term investment in on the molecular side, that I have a way to really meaningfully study this in the dish.

Melanie Ott (03:07:19):

So we have developed organoids from HCV positive and negative donors. These are liver organoids that I'm showing you here, either in the proliferative or in the differentiated state. There's not much of a difference. But the interesting part is that we saw that some of these organoids were actually retaining the virus in the tissues and when we build them in the dish. So we had a chance to actually start to study HCV infection in these different organoids while others who came HIV positive patients did not have this, did not carry forward the virus. However, we could use virus from the same donor and infect these cells very effectively in vitro both in the stem cell and the hepatocyte like state. We did single cell RNA-Seq. I might skip over this. So very interesting changes in terms of differentiation, but we're mostly interested in finding out whether these cells actually are antigen presenting cells by looking at HLA expression here, and also co-culturing them with non-HLA matched T-cells and seeing that immediately and used proliferation and activation of these T-cells.

Melanie Ott (03:08:31):

But eventually we used HCV specific peptides to pulse these liver organoids, and then co-cultured them with a peptide specific clone, we use the specific microfluidics chips that, that hosts the organoids in the middle and the T-cells here. Cytotracker green labeled in the outside channels. And you see that these are then migrating into the channel to surround the organoids. And we could see that there's great co-existence of T-cells, and organize in the absence of peptide. However, when we pulsed it with the peptide, you could see that there was immediate cytotoxicity and destruction of these organoids that really validated the system as a as a good system to study. Now, what is the effect of an infected liver on the generation of, on immune aging and the generation of CD28 negative T cells, but also vice versa? What is the effect of an infected CD4 positive T-cell on the HPV positive liver? And the same thing for HIV if we infect these cells.

Melanie Ott (03:09:48):

So just my last slide, just to summarize, I think it was a very interesting and an exciting journey. And the, in the past seven years now, where we were really focusing exclusively on the question, how molecular pathways of aging would contribute to, the reduced lifespan of HIV infected individuals or in HIV infected drug users. And I think we have identified it as a series of targets that we're following up in terms of what would be a good, what would be a good reversal strategy to prevent some of these, these diseases of aging that are occurring at accelerated rate in, in HIV infected drug users? So with this, I want to stop.

Melanie Ott (03:10:39):

I had a great group of people in the lab over the years who have worked on that and carried the baton with great collaborations with colleagues here at the Gladstone, in the bay area with clinical colleagues who gave us, the liver tissues to start the organoids with Eric Verdin at the Buck Institute as a preeminent aging institution, but also with the Jen Zuckerburg up here in San Francisco. And again, many thanks to [inaudible 03:11:11] for the generous support, because without this, none of this would have been possible. Thank you very much.

Vasundhara Varthakavi (03:11:20):

Excellent talk Melanie, Thank you. I'm going to ask you a real quick question and then go through the questions that are coming in Q&A. Have you looked at elite controllers are people who have long-term, have been under Ott and strong virus suppression. How does the SIRT1 and FOXO activity look like?

Melanie Ott (03:11:42):

That's a very good question. So what we have done is we did look at them and we looked at different HIV, groups of people. What we find, is that, if we look at their CD28 negative cells, we see exactly the same. We see that they all lose basically FOXO and SIRT1. There's no difference qualitatively in these cells. However, what we are currently doing is we're looking in the precursors of these cells, all the different, immune subtypes, and many of the, much of the research that was also presented today is extremely interesting because there's still a great need for better granularity to see how these cells actually arise. And what are the precursors. So we are, we are predicting that we see changes either in the numbers or in the, in the quality of SIRT1 and FOXO1 expression in these precursor cells. And that leads to basically that end stage, which is the same for all of them.

Vasundhara Varthakavi (03:12:44):

That's great. Would love to know the answer to that. And you are going to love this question. Does TAT work better acetylated or unacetylated in inhibiting SIRT1?

Melanie Ott (03:12:57):

That's a very good question. I think what we used in this study, what I showed you in vitro is a non-acetylated protein because it binds both , also the, TAT mutant that is mutated in the acetylated form, also binds to SIRT1 and blocks it. However, we think like you, that the acetylated form might find more avidly and might eventually be a better blocker.

Vasundhara Varthakavi (03:13:27):

Okay. I have a few more questions here. So any links between mitochondrial sirtuins, autophagy and ER stress that you have found in your research?

Melanie Ott (03:13:41):

We have? So we have very much looked at the mitochondrial sirtuins. I think we see also a downregulation of SIRT3 in these cells, less pronounced than with SIRT1. However, we think that this is because SIRT1 controls SIRT3 expression, and that there might be a link through this. So we are still, looking into this. I think it's an, a very important question. We love the mitochondrial sirtuins. So if there's a link, it would be great. However, we think it's an indirect link so far.

Vasundhara Varthakavi (03:14:18):

All right. I think I'm going to give you one more question here, because we're coming close to the clock. Have you looked at the role of any other sirtuins in [crosstalk 03:14:28].

Melanie Ott (03:14:30):

Similar question I think we have focused a little bit on SIRT3, we looked at sort of the expression of all of them. They are all the same. They are unchanged except for SIRT3. And we have been sort of looking into the question. What are the downstream effects is SIRT3 or what potential downstream effects of SIRT3 could be important. We think it's mainly in the metabolism, but we will, as before we will be very open because I would have bet my money on metabolism before, and it was not metabolism. So I, I'm really looking forward to finding out whether there is a role of SIRT3 and what this role is.

Vasundhara Varthakavi (03:15:08):

Excellent. Thank you so much, Melanie, wonderful talk, look forward to hear, hearing more findings from this line of research in future. Now I want to thank all the speakers and I want to hand it over to Dr. Compton to give us some closing remarks for this well attended Avant-garde symposium event.

Wilson Compton (03:15:32):

Well, thank you Kavi. I really want to thank all the speakers for a wonderful afternoon for those of us on the east coast and for a wonderful morning and early afternoon, for those of you on the west coast. That's one of the advantages of this zoom life is that we can join forces all across the globe in a single occasion. It really has been a, [inaudible 03:15:57] set of presentations covering everything from remarkable advances in basic cell physiology, through prevention intervention development, population sciences, immunology a and finally, Dr. Ott, I'm really struck by I'm old enough that I'm still so happy that we get to focus on aging for our AIDS and HIV patients. That that was not an issue, not that meant, while it is quite a few years ago now, but it's just a wonderful change for those of us that have been in clinical medicine for a long time.

Wilson Compton (03:16:33):

And I appreciate your fascinating work, looking at the comorbidity of HIV and HCV. That's a major theme. And as we see continued outbreaks of HCV around the country frequently overlapping with HIV. This is a really important topic for NIDA and for NIH overall. I'm part of a group that's looking at eliminating HCV as a complement to the elimination of HIV that's now an international goal. So really appreciated learning about your exciting program. This is really a fantastic set of presentations and a wonderful example of what the Avant-garde program has meant in terms of support to science, through supporting the individual scientists that are represented here. And you were all representing your large number of colleagues throughout this wonderful program. And with that, I'll turn it back to Kavi and Redonna for any final words before we wrap up for the day.

Vasundhara Varthakavi (03:17:37):

You go ahead Redonna.

Wilson Compton (03:17:39):

You get to unmute.

Wilson Compton (03:17:41):

Just thank you so much, it's been a wonderful afternoon. And I want to give a special thank you to Kavi because she has really been the driving force and pushing to have the symposium for both the Avenir and the Avant-garde recipients. So I really appreciate all of her dedication and commitment and the way in which she continues to want to showcase all of your all's work.

Vasundhara Varthakavi (03:18:13):

Thank you everyone. It's been a privilege learning your work, and we look forward to hosting you again soon. And please do keep us informed in if there is any exciting finding or a manuscript in the pipeline, let us know. And we would be very proud to advertise the work and take all the credit we can, and way we can support you too.

Melanie Ott (03:18:41):

Thank you so much. Really cool. Cool, cool symposium. Thank you. [crosstalk 03:18:45]

PART 6 OF 6 ENDS [03:18:48]