Transcript for AIDS Reseach Program Symposium

Speaker 3: Oh Redonna thanks very much. And I want to welcome all of the attendees to this very exciting symposium. Where we're going to be hearing science from some of the sort of age and breakthrough new technologies, new insights into how drugs interact with HIV and ways to use that knowledge, of course, in order to prevent and treat.

Speaker 3: It displays a [00:00:30] group of researchers that have been selected because they were able to get an Avant-Garde Award, which is one of the awards that we give to very promising new investigators that are willing to take risks in the area of science and not follow necessarily the traditional paths. But sometimes the regular [RO1 00:00:53] mechanisms requires us to do in order to get the approval. So it has been very successful [00:01:00] and you will see the exciting sightings that you will be hearing today.

Speaker 3: And before I end, I do want to thank also the leadership of Dr. Redonna Chandler and Dr. Vasundhara Varthakavi who have basically seen the oversight of this program and make it possible. So I hope you enjoy the meeting. Please feel free to ask questions, to give suggestions. This is the way that we can get dialogue in both ways, otherwise it just actually will live [00:01:30] in isolation. And we don't like that. Science actually very much [inaudible 00:01:34] in isolation is for everybody, knowledge is the product of all of us. So having said that, I give it back to Redonna for so we can start that the symposium.

Redonna Chandle...: All right. So let's get right to the science. I'm really excited to see these presentations. Our first presenter is Dr. Ashley Buchanan, and she is from the University of Rhode Island. So take it away.

Ashley Buchanan: [00:02:00] So good morning everyone. And thank you very much for this invitation to speak about our Avenir Project and titled Causal Inference Methods for HIV Prevention Studies Among Networks of People Who Use Drugs. I'd first like to give a few acknowledgements. First to the participant study staff and investigators of the four primary data collection studies, excuse me, used to motivate this work. I'd also like to think the following collaborators for their contributions to this work as well. And [00:02:30] this work is supported by this Avenir Award.

Ashley Buchanan: A brief outline of the talk. We'll first introduce our team members, give an overview of our project, share some research highlights, discuss our accomplishments fueled by the Avenir Award, as well as future directions. And I have no conflicts of interest to report.

Ashley Buchanan: So at URI, our team consists of myself, Dr. Natalia Katenka, who is an Associate Professor of Computer Science and Statistics, [00:03:00] Dr. Hillary Aroke, who's a NIDA Diversity Postdoctoral Fellow, Dr. TingFang Lee, who is an Avenir Postdoctoral Fellow. This award has also supported multiple graduate students,

both in the Masters and PhD programs in Health Outcomes and Statistics at URI. We have some key collaborators, including Dr. Samuel Friedman, Elizabeth Halloran, Brandon Marshall, and William Goedel, and our Project Officer is Dr. Richard Jenkins. [00:03:30] Just an overview of the project. So the project is focused on the population of people who use or inject drugs who face increased HIV risk through both injection and sexual routes and unique barriers on the HIV care continuum. At the same time these individuals are part of communities, risk or social networks where there's an increased risk of HIV transmission. So in this project, recognizing that these individuals are embedded in these risks or social networks, [00:04:00] we aim to develop methods and apply them to understand disseminated or spill-over effects, which is one, when one person's exposure affects another person's outcome.

Ashley Buchanan: So the methodologic problem is the causal inference methods are often challenged by network studies, but at the same time, within these network studies, we want to understand these causal effects. So basically a causal effect is trying to understand if the intervention actually [00:04:30] prevents or causes the outcome to happen. So we want to solve these methodologic challenges to address questions at the intersection of HIV and substance use among network-based studies of HIV treatment and prevention among people who use drugs.

Ashley Buchanan: So this work is motivated through collaboration with different study teams, and we use two network randomized studies. These are similar [00:05:00] to a cluster randomized study, however here, the cluster is actually an egocentric network. So it's a single individual in their context. So we have one site in Philadelphia, an HPTN 037 and another study in Baltimore known as STEP. So in both of these studies, there is a peer education intervention, which showed improvements in reducing HIV-risk behaviors. And in our work, we sought to understand the disseminated or spillover effects of these interventions [00:05:30] in these populations.

Ashley Buchanan: We also have network-base studies, so these are either what are known as sociometric or respondent driven samples. So instead of just the egocentric cluster in these studies, we have more information about how the individuals are connected to each other or the edges shared between the individuals. So one study is the Transmission Reduction Intervention Project. So far we've focused on the Athens Greece site [00:06:00] in collaboration with the Principal Investigator, Sam Friedman. And in this study, they sought to find individuals recently infected with HIV and referred them to treatment as prevention, as well as disseminated community alerts to help individuals realize they may be at an increased risk if there is a recent infection detected in that area of the network. They looked at such outcomes as HIV incidents and risk behavior. The other study we're working with is in [00:06:30] New York City, among young adults who have non-prescription opioid use. And then here, looking at the exposures of such as poly-substance use and the outcome such as injection drug use or HIV, HCV infection.

Ashley Buchanan: So a few research highlights. So first I'll discuss this project in collaboration with Dr. Brandon Marshall and Will Goedel. [00:07:00] So in this project, we are using an agent-based modeling approach, which is essentially a mathematical modeling simulation approach to generate this ideal randomized trial. So it's a two-stage randomized trial. And then we can use this model to simulate that trial and then with the outputs from the model, we can quantify causal effects. So here we're specifically interested in this disseminated effect. So the question we wanted to know is, is there [00:07:30] a disseminated effect or pre-exposure prophylaxis for HIV prevention? And we adopted a model that was among men who have sex with men in Atlanta, Georgia. Well also considering whether or not those individuals, if you will, the simulated individuals or agents were engaging in substance use or not.

Ashley Buchanan: So a little bit more about the two-stage randomized design. So in this design, what happens is in the agent based model, we first [00:08:00] generate the sexual network among the men who have sex with men in Atlanta. And then among that network, we identify what's known as components. So those are individuals that are connected to each other, but not connected to anyone else in the network. So we find those separate components. And then in our agent based model, we can actually randomize those components to an, what's known as an allocation strategy. And so that will determine how many people in that component actually are [00:08:30] individually randomized to the intervention. Now the beauty of this simulated agent-based model approach is that it's no longer ethical or feasible to conduct such a randomized trial, given the known efficacy of prep. However, in this agent-based model setting with our simulation, we can determine if there's a possible disseminated effective prep.

Ashley Buchanan: So a little bit more about the different effects that we can identify in this two-stage randomized trial. So we have our [00:09:00] intervention components and our control components on the left here. And then within the intervention component, a certain percentage of individuals will be randomized to the pre-exposure prophylaxis and then the otherwise randomized to no prep. So if we look in the intervention component and we compare individuals randomized to prep, to individuals randomized to no prep, we get what's known as the direct effect. So that's the additional benefit to being randomized to prep beyond being in an intervention component. [00:09:30] We have the disseminated effect or the spillover effect, which I personally find the most interesting in this problem. So we would then look at the individuals who were not randomized to prep, but in the same component, or at least sharing some path in the component to individuals randomized to prep. And we compare those individuals to individuals in the control component. The composite effect gives us the maximal intervention impact. So that compares those randomized to prep in the intervention [00:10:00] to those randomized to the control.

Ashley Buchanan: And lastly, if we marginalize over the individual level exposure, we compare just everyone in the intervention component to everyone in the control. So an important connection here is that with the two-stage randomized design, these effects are readily identifiable in the data.

Ashley Buchanan: So a brief summary of our results from this agent based model. So on the left, we have the estimated direct [00:10:30] risk difference effects and on the right, we have the disseminated risk difference effects. And this is of the pre-exposure prophylaxis on cumulative incidents of HIV. And then if we move along the X-axis here, this is coverage. So this is the percentage of people or agents, if you will, randomized to prep in a component. So interestingly, we see as the coverage increases, the direct effect attenuates somewhat towards the null. And as the coverage increases, [00:11:00] we see that the disseminated effect becomes stronger or larger in magnitude more protective. So interestingly, what's happening here, is as we increase the coverage level, the additional benefit to an individual, person or agent receiving prep. If for example, 90% of their contacts are randomized to prep, would be slightly smaller or weaker. Whereas the disseminated effect, if you're randomized to no prep and more people around you are randomized to prep, we see that there's a [00:11:30] stronger benefit as that coverage increases.

Ashley Buchanan: So to highlight another project from our group, this one's led by Dr. TingFang Lee. So in this work, we developed methods to estimate causal effects of non-randomized interventions in the presence of interference or dissemination in a network-based study. So we're using causal inference approaches to quantify essentially the effects I discussed on the figure a few slides ago, [00:12:00] among individuals who are embedded in a network, such as people who inject drugs.

Ashley Buchanan: So for this particular study, we're using the transmission reduction intervention project in Athens, Greece as the motivating data. So this visualization on the left, the green dots represent the individuals or the people who inject drugs and the lines between them represent shared drug use and social connections. So here we focused on, if we look here, I've [00:12:30] highlighted a what's known as a single component in this trip study. And then when we zoom in on the component just to define what the neighborhood is, we had this as for example, the individual of interest in red and their neighbors are these individuals in orange. So without sharing formulas or too much detail, just to give a flavor of what the method is, we use this neighborhood to define what's known as the interference or dissemination set so that this person's outcome, [00:13:00] not only it depends on their exposure, but the exposure of the orange nearest neighbors. And then to conduct inference, we identified non-overlapping independent components like the pink component displayed here.

Ashley Buchanan: So to briefly summarize, we wanted to see if there were disseminated or spillover effects of the community alerts and trip. And just to refresh the community alerts were when the team disseminated information about a recently infected individual near that [00:13:30] person in the observed network. So about 11% were exposed to these community alerts from the study staff. And in our analysis, we found there were actually 13 per 100 fear individuals reporting HIV risk behaviors if 75% of their neighbors received alerts. Compared to the scenario where only 25% of the neighbors received alerts. So

evidence of a possible spillover disseminated effect of the community alert information in this particular [00:14:00] study.

Ashley Buchanan: So to summarize some work from Dr. Hilary Aroke, one of his projects is developing causal inference methods to evaluate contamination effects in cluster randomized trials. And he's adapting concepts in mediation to see if there's contamination effects in HPTN 037. So recall HPTN 037 was the peer education intervention among people who inject drugs in Philadelphia. In the study of this intervention [00:14:30] was effective for reducing injection risk behaviors. In a subsequent study, we did find and quantify disseminated effects of the intervention, but still contamination remains a concern. So what was the intervention actually reaching individuals in the control or were those clusters actually interacting with each other? And through this work, we have landed upon contamination in the setting is actually only meaningful if we can demonstrate that the intervention [00:15:00] is actually mediated through the intervention knowledge. So we were going to quantify the contamination to see if the control clusters actually had knowledge of the intervention. And for that to be meaningful, we first have to see if the intervention is in fact mediated by that intervention knowledge.

Ashley Buchanan: So first we're looking at mediation, which is to decompose the total effect of the intervention on self-reported injection risk behavior into that mediated through the recall of intervention knowledge, [00:15:30] as well as the remaining direct causal effect through all other mechanisms.

Ashley Buchanan: So we used the information at six months to assess the patient recall of the intervention knowledge and at 12 months assess their self report of injection risk behavior. And our preliminary findings only about 5% of the total intervention effect was mediated by intervention knowledge, which tells us there could be other pathways. And we suggest that this may be pathway such as behavior modeling. [00:16:00] And just to briefly mention some work in progress. [Tin Yu Sun 00:16:05] is a PhD candidate in our group contributing to this work, to use a multi-state model to investigate the opioid use disorder care cascade in Rhode Island. So there has been ongoing work to try to define the opioid use disorder care cascade, as well as several papers looking at cross-sectional assessments for risk factors. So our group recognized that the cross-sectional assessments are helpful, but there's actually an [00:16:30] underlying longitudinal process by which individuals are moving through this cascade. So we're going to develop and apply a multi-state model to look at factors associated with both transition and barriers along the OUD care cascade. And this is using the Rhode Island, all payers claims database.

Ashley Buchanan: So here's the conceptualization of our model. Essentially, we're focusing on the engagement and retained in care piece as that is [00:17:00] more reliable information from the data source that we have. So we're looking at first engagement, short-term retention, and then long-term retention, and then also how long patients are disengaged.

Ashley Buchanan: So just to highlight some accomplishments of the team so far. I'd like to mention some opportunities provided by the Avenir that without this support, I don't think our team could have made [00:17:30] progress so quickly. So our team is uniquely positioned having a person specializing in network science, as well as causal inference with a focus on substance use research. So I think this uniquely positions our team to be working at both the intersection of HIV and substance use, as well as network science and causal inference. The Avenir has led to invited talks for multiple team members, which resulted in new collaborations. [00:18:00] And including access to important data sets, which is incredibly important for the biostatistics methods work. We like to use the studies and the data to actually motivate the methods development. And then the methods development can then in turn, provide better guidance and information for design of future studies.

Ashley Buchanan: We're also delighted as a team to help support open access to some of the data sources that were given to the URI Avenir team, which I personally find to be a very important scientific endeavor. [00:18:30] The Avenir also has created incredible research experience for the graduate students, working with principal investigators and incredibly rich data sources. It has also provided a mentoring platform for postdocs, with opportunities for training and grant writing and speaking engagements. Our team also gets to meet regularly and collaborate with key researchers in the field, which has greatly improved our work. And it has also opened opportunities for the development of didactic [00:19:00] lectures and materials for the research coming out of this project.

Ashley Buchanan: So just briefly future directions. So as I mentioned, there's a synergy in our work between the methodologic developments and the substantive area of substance use and HIV. And I see the two working with each other to move along. So methodologically we're interested in areas such as missing data [00:19:30] and longitudinal in the context of dissemination and interference. So looking at things like a time-bearing interference set, we're interested in looking at some additional modeling approaches or mark of random fields to work on further methods development in this network-based study.

Ashley Buchanan: And then of course, this is related to substantive questions. Our team's interested in addressing issues related to the improvident of COVID-19, HIV, HCV and the opioid crisis, as [00:20:00] well as addressing racism and health disparities. Specifically they're developing a project to do some COVID-19 modeling that incorporates network structures to help address health disparities. And also of course, continuing to work on the OUD care cascades, synergizing definitions, developing new methods, so we can better improve the inference that we're getting from these routinely collected health data sources. [00:20:30] So in conclusion, in many cases, and so far what we've seen in this project, is dissemination and network structures seem to be substantial and possibly meaningful. And I see this as good news, so that many NIDA funded studies and other interventions could possibly have higher benefits for public health than standard techniques reveal. And I think, you know, our team is very much interested and excited about this and different questions try to

understand [00:21:00] what these disseminated effects are and how we can better use them to help improve the uptake of interventions in this population.

Ashley Buchanan: So a few recent publications from the team and some publications in preparation. So thank you very much for listening. That's all I have for today and we'd be happy to answer some questions.

Redonna Chandle...: So [Kavi 00:21:30] [00:21:30] is going to proctor our questions. If people have questions, you can type them in the chat or in the Q&A and then she'll read the questions for Ashley to answer. We've got about 10 minutes or so to have a robust discussion and some Q&A. So [Kavi 00:21:49] you ready?

Vasundhara Vart...: Any questions here? I'm looking at the chat. [00:22:00] Also we can unmute, if anybody wants to ask questions.

Redonna Chandle...: Kavi I don't think that the participants have the ability to unmute. I think we're going to need to read questions that they type in.

Vasundhara Vart...: Yeah. Don't see any coming in. Okay, there is-

Speaker 1: If you'd like to also ask a question verbally, you can [00:22:30] raise your hand and then we can note to call on you and open your line up momentarily.

Vasundhara Vart...: So there is a question in the chat from Karen Ingersoll. She says, it's very encouraging data. I think the question is how much are we underestimating the ethics?

Ashley Buchanan: Yeah I think that's an excellent question. I think this is somewhat of a vague answer. But I think it somewhat depends on us going back and looking [00:23:00] at, we can look at studies that have already been conducted and see what is the magnitude of that disseminated or spillover effect and how does that compare to the overall. So I think it may depend on the study context, but I think, moving forward, our team is also collaborating. And I think this is very important, particularly for study design, we're working on sample size and power calculations to help inform the design of future studies so that if investigators are interested in quantifying this, [00:23:30] we can make sure you have enough power in your study to do that. So we could be, maybe in the future, we, if there's a possible... And then the important thing though is that there could be a possible disseminated or spillover mechanism and making sure we know what that is first. And then if we suspect that could be there, why not power and try to estimate this.

Vasundhara Vart...: Thanks Ashley. I don't see any more questions here. Oh, there's one [00:24:00] more. This is from Denish Kumar. Has the method or tool to measure contamination have effect over assessment?

Ashley Buchanan: I'm not sure I follow that question. So the, I mean and maybe I can make it a little bit clearer. So today I focused primarily on discussing what the mediation was that we found. So the contamination is, it's like we're basically estimating and I'm sorry, an affect that if that's protective [00:24:30] or harmful, it will tell us if there's possible contamination happening in the study.

Ashley Buchanan: And then I guess there's sort of two pieces with that. One piece is, do you first want to know is there contamination or not? And then the second piece would be, do you want to adjust your estimated effect to account for that contamination or not? So there's sort of two separate questions I see in network. So maybe that was what the attendee [00:25:00] was asking.

Vasundhara Vart...: All right. He's trying to explain. Okay effect over results he says. Aaron, can we unmute the [inaudible 00:25:23]? If he can speak, we have time.

Speaker 2: [00:25:30] Thank you for giving me the opportunity to ask question. What my question is, I'm sorry, I was not clear. Does the way we are going to assess [inaudible 00:25:46] the contamination, like asking question from the person or whether it is interview based or the court based, or self-reported. whether it's such kind of an assessment has [00:26:00] an impact or where the results that this has contributed to an direct effect, and this much has contributed to direct effect or direct [inaudible 00:26:10] contamination. So that's my questions. Thank you. Thank you very much.

Ashley Buchanan: Yeah I think that's definitely an important consideration. So for this initial work we're doing in the contamination or contamination effects, we're basically using a study that's already been conducted and this was the information they [00:26:30] collected to see if there was contamination. It seems like it could be very wide open to figure out how can we measure and quantify contamination? What's a valid way to get at that for these different interventions. But in HPTN 037, the recall of specific terms from the intervention was how they measure contamination. And of course that would definitely impact, you know, how we're measuring that and the quality of that would impact our ability to assess [00:27:00] contamination.

Speaker 2: Thank you, thanks for that.

Vasundhara Vart...: Okay. So there is another question from the audience. It says, it's a general question regarding future goals for understanding impact of racial, ethnic, gender bias, social determinants of health, including health care access. Is there data that you wish the community would be collecting?

Ashley Buchanan: Oh, so like the research community?

Vasundhara Vart...: [00:27:30] Yeah. I don't know, maybe the community in general that is [crosstalk 00:27:38].

Ashley Buchanan: Yeah. I mean, I guess one thing I've noticed in my work with routinely collected health data and some of this is administrative claims data, or even the all payers data. I would encourage us as we move towards continuing to work with health disparities and quantifying those that if we have department of health data, for example, if we're able to collect race [00:28:00] and ethnic information and other factors like that to help better understand the disparities in these data sets. So I know the Rhode Island Medicaid does collect race information, but then working with the Rhode Island all payers data, it actually doesn't have the race information. So we're actually not able to even dig further into possible health disparities about the OUD cascade in that dataset. But that's routinely collected data. And then I'm trying to think for, I mean it sort of depends on like moving forward [00:28:30] for primary data collection, what would be of interest in the study and additional information to collect to understand disseminated effects.

Vasundhara Vart...: Thank you. Well, it looks like this all we have. So thank you very much Ashley. That was a fantastic presentation.

Ashley Buchanan: Thank you everyone.

Redonna Chandle...: Okay. So [00:29:00] we will move to our second presentation from Dr. Daniel Lingwood and he is from MIT, Harvard.

Daniel Lingwood: Good morning, everyone. I'd like to start by thanking the organizers for the chance to present some of our work that was, is and has [00:29:30] been supported by this Avenir Award. And how we think it's really been transformative for us in terms of actually introducing us to the HIV field as somewhat of an outsider. So the title of my talk today is called Epitope-shifting to an HIV vaccine target through the acquisition of low affinity: applying the activity of an innate-like immune receptor. And I hope it'll become very clear what all of that means as we play out.

Daniel Lingwood: So just [00:30:00] by a brief background, just to start off with, of course the development of ART has become a transformative lifesaving intervention. However, I think as this audience is well aware, much more so than I am, that the number of individuals, including in this country who are diagnosed with HIV, very many of them are [00:30:30] still left out of the system in terms of being left off ART. And there are socioeconomic behavioral barriers that complicate this adherence behavior. And I think it's well appreciated that this is particularly problematic in substance users. And so this sort of strengthens the notion that an HIV vaccine would particularly be amenable [00:31:00] to improving the life of these individuals. And so one of the fundamental questions that I guess one can consider is, what are the challenges to generating such an HIV vaccine? Why is it for example, that we can't roll out a vaccine as fast as for example, the Corona Virus, Sars-Cov-2 has been done. And the simple answer to that is the diversity challenge. So HIV presents the immune system with [00:31:30] something that is sort of never been seen before in terms of a diversity challenge.

Daniel Lingwood: So if we look at the genetic diversity on the left here from a total global influenza epidemic, we can see that it's actually comparable to the HIV diversity that occurs within a single person. And that that's just orders of magnitude higher when we look at sequence diversity in the HIV space [00:32:00] across the world. So it represents an unprecedented challenge to the immune...

PART 1 OF 6 ENDS [00:32:04]

Daniel Lingwood: ... across the world. So it represents an unprecedented challenge to the immune system. How do we develop a vaccine, a set of molecular instructions, which teach the immune system to keep up and constantly be able to neutralize this incredibly diverse virus. If we look at the problem at the immune response or antibody response level following HIV infection, most of us would develop [00:32:30] what we'll call type specific antibody responses. So the blue antibodies neutralize the blue virus, the green, the green virus, the purple, the purple virus. And if we blow up that type specific neutralization, those antibodies are intrinsically directed towards those hypervariable parts of the virus, which are, as I say, are constantly evolving and contribute to that global diversity problem.

Daniel Lingwood: However, there are rare cases [00:33:00] when certain individuals produce what I've titled here, Genetically Encoded broadly neutralizing antibodies, which under these conditions are able to reproducibly target some of these conserved regions and neutralize much of that global diversity. What do I mean by genetically encoded? Well, for each individual, of course, we're made up of genes on our chromosomes, including part of this package. We come equipped with these so-called antibody [00:33:30] VH and VL genes, antibody V genes, which go into making up the diversity of our antibody repertoire and our ability to respond to different incoming antigens. And so some of our work in the past has looked at how certain V genes, some of these antibody genes, naturally produce certain kinds of antibodies which have this natural affinity for functionally conserved sites of vulnerability, [00:34:00] which viruses like influenza are functionally constrained to preserve. And this endows humans with a reproducible gene encoded antibody response. And so some of our work in the past and ongoing has been focused on trying to harness this principle as a means to develop universal flu vaccines.

Daniel Lingwood: So what the Avenir has done is allowed us to explore this similar [00:34:30] concept in the HIV space. Now, there is this analogous antibody VH gene, and I'm going to refer to it as the HIV gene, I'll specify a little bit more later. Which is also in these rare individuals reproducibly used to create broadly neutralizing antibodies against HIV in particular, that target a functionally conserved feature on the virus, which is called the CD4 binding sites. So CD4 is the receptor HIV uses to engage cells. [00:35:00] This HIV gene endows for antibodies which engages this target in a very precise way that enables neutralization of over 80% of that global HIV diversity that we were talking about.

Daniel Lingwood: So the notion is, potentially, there is this analogous gene endowed antibody response that may be amenable to vaccine activation and amplification akin to some of the work that we've done with influenza. And that was really what the Avenir [00:35:30] allowed us to now explore and define in the HIV space. And so basically the overall topic that we've been working on under this award is the question, how does this so-called HIV antibody VH gene actually work as an immune receptor? And there are two stories, which I would like to focus on during this talk, which I think have been the most exciting discoveries of [00:36:00] our work in this space.

Daniel Lingwood: Number one is, why would humans be equipped with such an HIV gene that reproducibly endows for this broadly neutralizing antibody response and potential template for HIV vaccines to conquer that diversity? And the second question is, how do we actually activate that gene encoded potentially amplification principle in the vaccine [00:36:30] space? So in order to answer these questions starting with the first, why might humans be equipped with this VH gene? The question really is, what is it about that gene that endows, in this case, antigen receptors, what I call germline B-cell receptors, which are the membrane anchored forms of antibody precursors, which are present on the surface of your germline B cells that are in your spleen [00:37:00] and your lymph nodes. These receptors are charged with initially recognizing incoming antigen. So, whether it be on an infecting virus or a new vaccine, defining the geography of that epitope targeting and then that's going to, as I say, define how and where the given antibody response is going to target. And so [00:37:30] the question that we're asking is, what is this HIV gene playing with respect to that?

Daniel Lingwood: So if we blow up this antigen receptor a little bit closer and look at the molecular goings on here, we can think of each B cell receptor as a series of fingers which grab the antigen. So these fingers are made up of V gene encoded loops and also essentially positioned, highly variable, random antigen binding loops. And it's these V gene encoded [00:38:00] loops, which the antibody V genes, including this HIV gene are playing into. So we want to understand what this red gene is doing with respect to these gray loops, which is somehow predisposing for recognition of this vaccine target. Now, in order to experimentally interrogate this, we had to actually build a system where we could do that and I'll just say that I'm going to finally [00:38:30] name this HIV gene. It's called a IGHVH1-2, so I'll be referring it to that throughout.

Daniel Lingwood: But in terms of the experimental system that we built, we had a collaboration with our industry partners at BMS to build a system where we could experimentally ask, what is the contribution of a given antibody VH gene to antigen recognition in the immune response, in the context of an experimental transgenic mouse system? So we built this transgenic [00:39:00] mouse system, which basically recapitulates all of the human antibody loops, in particular this hyper variable CDRH3, but at the same time, systematically restricts antibody responses to a single user defined VH sequence in the animal. So basically, we can perform an experiment. What is an experiment? You fix one variable, vary

something else and assign causality to the variable you fixed. [00:39:30] So essentially, what we're dealing with is a preserving type of variable feature [inaudible 00:39:34]

Vasundhara Vart...: [00:40:00] Sorry, there is some technical glitch here, so we're waiting for Dr. Lingwood to get back online, please be patient for this.

Redonna Chandle...: [00:40:30] While we wait. I want to call everyone's attention to the fact that if you have not applied for the Avenir Award, and you're interested, [Cabi 00:40:48] has posted information in the chat that everyone should be able to see, including the link to the funding opportunity announcement. And please feel free to reach out to us at any point in time where you have ideas.

Daniel Lingwood: [00:41:00] Apologies for that. I'm not sure what happened. Let me just quickly start this again.

Vasundhara Vart...: Yeah.

Redonna Chandle...: It happens to everyone. And always at the most inopportune moment.

Daniel Lingwood: Yeah, let me just go back to where I was. [00:41:30] Can people see that?

Vasundhara Vart...: We're good.

Redonna Chandle...: Yes.

Daniel Lingwood: Okay. Thank you. Again, apologies that my computer froze. So basically, just to quickly summarize, we built an experimental system which basically allows us to fully humanize and mimic that hypervariable [00:42:00] middle finger loop but systematically preserve and control the V gene input, that gray input, to the surrounding antigen binding site and then ask experimentally, what is it doing with respect to antigen recognition, vaccine targeting? What is this so-called HIV V gene actually doing? And so just a bit of characterization of these animals, we can look at that hypervariable middle finger loop and see in terms of what we call the CDRH3. We can see [00:42:30] that it's matches the human repertoire in terms of length distribution and amino acids that go into that loop structure but again, we're constraining the system to use individual, single V genes at a time across the entire animal.

Daniel Lingwood: So, coming to this question, why might humans be equipped with this HIV V gene? And so it's unlikely that we evolve this specificity recently. HIV [00:43:00] is a fairly new virus. We said, what is it doing with respect to antigen recognition, particularly at the level of primordial antigens, things that would have been present in our past? And what we thought was, first, let's look at some bacterial antigen. These are ancient, ancient immune stimuli, which come equipped with a very sensitive, rapid response system [00:43:30] in our blood that's tuned to recognize these targets. So if you put either bacterial sugars or

lipids into your blood, they're very potent septic signals, so you get a very rapid antibody response in your blood to clear those antigens, target those bugs. Of course, bacteria presence in the blood is very dangerous.

Daniel Lingwood: It's very easily measured by what we refer to as Extrafollicular IgM Response. [00:44:00] And so we said, okay, these are some primordial antigens. Here's a response system, which we can use to gauge microbial input to this, what is this HIV gene doing in this context? And so what we do is, we take our transgenic animals, which is using our HIV gene here, VH1-2, control different human VH gene, VH1 69 and we can put in a couple of control antigens, sugar polymers, TMP called [00:44:30] over albumin and we can see that we get this rapid extrafollicular response coming from the spleen in all of our animals subsets. I think that the interesting discovery that we made in this context was that if we put in bacterial lipopolysaccharide, which is the major specific antigen of gram negative bacteria.

Daniel Lingwood: So we put that in, covering diverse strains of bacteria over thousands of years of evolution, over two orders of gram negative bacteria, [00:45:00] what we find is that it's only the animals that are using the HIV gene that are able to accommodate antibody responses to this bacterial antigen. If you're using a different V gene, despite the fact that you've got all the diversification machinery working for you, you're unable to recognize and respond to that. And so to make a long story short, we did some work to actually show that this HIV [00:45:30] gene was tuning the germline B cell repertoire to respond to a conserved part of this antigen, the so-called Liquid A core of these bugs, which doesn't change.

Daniel Lingwood: So what you've got is this HIV gene, which is naturally tuning the immune system to recognize a conserved microbial target. So like an innate immune receptor system, [00:46:00] but for the adaptive immune response. And so we thought that was quite an interesting, at least functional perspective to think about, why it is that we have this VH1-2 in our human repertoire. Why is it useful? It endows the immune system with a broad spectrum antibody response targeting this conserved hydro carbon core of an otherwise highly diversion form of bacterial LPS. So, again, this innate like immune recognition, okay. So we've got a functional idea with our system [00:46:30] as to some specialty of this VH1 one to HIV gene. How do we actually now make it work for us in the HIV vaccine context? We know that it somehow predisposes for this neutralizing activity, how can we now harness that? And so we go back to this transgenic animal system that we've built where we can constraint antibody responses, to use this gene, [00:47:00] but still let them have the appropriate diversity play to explore the antigenic space across a potential HIV vaccine.

Daniel Lingwood: So what we decided to do was to apply a non-conventional vaccine strategy to think about ways of which we can immune focus the attention of the antibody response on this conserve site of vulnerability, the CD4 binding site on HIV, all in this VH1-2 context. So if we think about... Here's a picture of [00:47:30] Edward Jenner, 200 years plus years ago. We have the conventional form of vaccination,

where you have a prime, we call that injection one, a boost. So those of you who are getting your COVID vaccines, you know you get a prime and then three weeks later, you're scheduled for a boost. This is a very classical vaccine paradigm. However, in the context of HIV which of course is immune destructive, the prime will likely give us a lot of antibody responses that are targeting the hypervariable blue part, [00:48:00] right? And then when we boost that, we simply boost the immune memory against the blue, and then we call that homologous to immunization. So that's unlikely to work for us in order to refocus the attention of the immune response on this red conserved part.

Daniel Lingwood: However, if we take a different approach, we started off with a blue immunogen, we'll get a response against the blue, maybe a little bit against the red. But what if we take advantage of the existing HIV diversity that we know, and now hit it [00:48:30] with a different strain of HIV, which is functionally conserved to still hold the red, but changes the green? Maybe we can refocus part of the antibody response where we're basically showing the immune system two different entities, but the only thing that's conserved is the red, so maybe this heterologous immunization concept can help us refocus to the red.

Daniel Lingwood: And so this is what we ended up doing in our vaccine model, where [00:49:00] basically we came up with a number of different strains of HIV envelope strains of the display that conserve target in otherwise varying background and sequentially immunized our transgenic animals. So if we, sequentially immunized with a homologous where it's just, we're doing blue, blue, blue, blue, we can measure the antibody response. And we're looking at IgG titles against either with the wild type form of this [00:49:30] blue immunogen or the D368R form, which basically blocks access to the CD4 binding sites, so there's no Delta between the curves. However, if we now apply a heterologous immunization scheme where we're constantly changing the bearing energy, we can now refocus part of the immune response on this otherwise immunologically invisible, a target. So we are quite excited about that discovery. [00:50:00] And so we said, molecularly, what is going on in terms of how this HIV, VH1-2 immune receptor is working? We noticed something very interesting. If we re-perform these assays in the presence and absence of urea, so urea is a chaotropic agent, which basically destabilizes interaction. What we looked at as resistance to urea in the homologous scenario versus heterologous scenario, what we found is that when we're refocusing to the CD4 binding site, what we find is [00:50:30] a decreased resistance to urea, suggesting that when we're refocusing attention to the immune response to this target, there's an affinity drop that's happening. And this was interesting because conventionally, when you sequentially immunize, there's not supposed to be any affinity drop, you're supposed to gain in affinity.

Daniel Lingwood: So just a quick review on that. Step one, when you immunize with an antigen, you come in, germline repertoire engages the target as we discussed, defines the target epitope. There's [00:51:00] a process called somatic hypermutation that takes over, that increases affinity to that target. But what we're seeing is this affinity drop when we refocus the attention of the immune response to this

putative HIV vaccine target in this VH1-2 context and so we want to explore the molecular basis for what might be going on. We developed a way to actually isolate the antigen specific B cells that are underscoring the vaccine response so we can basically define using a set [00:51:30] of fluorescent B cell probes, the on target antigen specific B cell memory that's targeting the CD4 binding site versus the off target responses which are seeing elsewhere on the target epitope. And then we can reconstitute them in terms of BCR antigen interactions, which we developed in our lab.

Daniel Lingwood: And so what you're looking at is a BCR antigen interaction readout, where basically what we're doing is [00:52:00] reconstituting the BCRs that we vaccine expanded and asking which of our vaccine immunogens across the top did they see, and how did they see it? So, for example, as a positive control, we look at one VCR, this is a classical Human HIV, broadly neutralizing antibodies not neutralized 80% of circulating strains. What you can see is that when we look at how these vaccine antigens bind each different [00:52:30] component, we see that it's binding to the wild type, but not the D360 radar of YU2 so we know that it's focused on CD4 binding site, same with the turquoise immunogen, same with the red immunogen, same with the blue immunogen.

Daniel Lingwood: If we look at S1, which is in the lineage we expanded in our vaccine animal system, we can see if you just look at the bottom, the mature response, the mature VCRs that we've expanded, we can see [00:53:00] that we're recapitulating the same CD4 binding sites specificity. We're binding to the wild type of the yellow, the wild type of the turquoise, the wild type of the red and the wild type of the blue. So we've expanded what looks like to be a VRC1 like antigen specificity. The interesting thing is when we look at the germline precursor, so what gave rise to this S1 lineage? What started it all off? [00:53:30] We find that there's only antigen specificity for the blue antigen, so it was the blue antigen here that triggered the response, when we look at the germline response. But unlike the mature, which is binding only wild type and not the mutant form, we find that it binds both forms.

Daniel Lingwood: So it's saying that this process was actually triggered by an immune response that targeted not the CD4 binding sites elsewhere, and that it was only following application of affinity maturation [00:54:00] that this blue went away and we're targeting the right specificity. And so we can actually map out that process by looking at the pattern of somatic hypermutation that went on during this immune response. And basically to make a long story short, we can reconstitute some of those specificities. We can see that we start out from germline, we're binding both immunogens wild type versus D3 CD4, so we're starting off target. And then only after application [00:54:30] of subsequent affinity maturation, do we switch to see the CD4 binding site. And the interesting thing that we see with regard to this switch is that at the mature stage, when we're switching this, if we actually look at the corresponding biochemical affinity between antibody, which is part of the B-cell receptor that is the single Fab binding arm, and the target, what we find is that there is this affinity drop [00:55:00] scenario.

Daniel Lingwood: So basically we measure this using bio layer interferometry, where we look at the Kd. So a high Kd means low affinity, a low Kd means high affinity. So we see if the Kd starts at around 1.5 micromolar comes along. It's only suddenly when we go from off target to on target that we get this huge affinity drop. And again, remember that we have this polyclonal [00:55:30] response pattern where we're, again, seeing this affinity drop in the refocused antibodies across the board. And I should say, it's not just this lineage that we're seeing this and this is just a representative example. We're seeing this affinity drop come time and time again. And so what we think we found using this targeting system is a new way of thinking about how antibody somatic hypermutation participates in the epitope discovery process. [00:56:00] So the conventional view again, is that the germline BCR defines the target and then somatic hypermutation increases the affinity to that target.

Daniel Lingwood: What we've found in terms of understanding how this special VH gene works in terms of being able to see this CD4 binding site, is that it can start off with an irrelevant target that's initially engaged and then it's only in the context of somatic hypermutation [00:56:30] that they don't have to gain affinity as conventionally thought, but somehow the hypermutation can actually act as a further diversifying agent, which laterally explores the antigen and acquire specificity for this target and the system enables this discovery of what we would call this Affinity Constraining Target. And once that is then established, our hypothesis and [00:57:00] new next steps is to define how now we can push that engagement along towards broadly neutralizing activity.

Daniel Lingwood: Very generally, just to summarize, I'm an influenza B cell biologist, who was able, thanks to the Avenir Award to now enter the HIV space and defining what I think are two fundamentally [00:57:30] new contributions to our understanding of immune recognition. We know that this VH gene is reproducibly used in a subset of humans that give these broadly neutralizing antibody responses. We have found that this HIV gene is a natural, innate like immune receptor for microbial antigen, suggesting maybe that microbium didn't actually play a role in priming some of these responses. And with respect to question two, in terms of understanding how we activate [00:58:00] this receptor biology, we've defined a non-conventional mode of antigen complementarity, a new role for antibody somatic hypermutation, which enables these antibody products to engage this affinity constraining target and acquire that specificity. So we're very interested now in thinking about ways in which we can potentially harness this further in the vaccine context.

Daniel Lingwood: So [00:58:30] I'd like to end it there by again, thanking the Avenir Award which continues to support this HIV program in my lab. It's supported, in particular, two post-docs, Ashraf and Larance, grad student Vindus, who has all worked heavily on this area or industry collaborators. Thank you to the Reagan Institute and thank you for your attention.

Vasundhara Vart...: [00:59:00] Thank you, Dr. Lingwood. We have some questions coming in the chat now. So here is one. What are the implications of your findings for Germline Target vaccine strategies?

Daniel Lingwood: Yeah, so I think what we've found in terms of... So [00:59:30] the Germline Targeting notion is to engineer a high affinity immunogen so that you can actively select those correct germline precursors of interest, and then expand them. What we've found is we haven't used a Germline Targeting principle in this case. I think our discovery [01:00:00] is more along the lines of defining a natural way in which the immune system is able to perceive these low affinity BCRs. That there is actually inbuilt flexibility in terms of non homogenizing affinity selection principle at play that enables low affinity BCRs to subsist for long periods of time. And I think this is more informing of the natural way in which [01:00:30] this target is perceived by the immune system.

Vasundhara Vart...: And there's a [inaudible 01:00:38] question here. How problematic do you think existing BCR repertoire will be? Does that constrain how much you can educate the system?

Daniel Lingwood: Does that mean, can I just ask, in terms of preexisting immunity to other things? [01:01:00] Is that the question?

Vasundhara Vart...: [crosstalk 01:01:04]

Speaker 4: Can I use the fact driver panelist to clarify rather than type?

Vasundhara Vart...: Yeah, if you can unmute.

Daniel Lingwood: Yeah.

Speaker 4: Daniel, maybe I'm speaking right to your wheelhouse, right. Because the thought that pops into my mind is original antigenic sin, like tissues and flu vaccine. And now most people haven't seen HIV antigen, but I wonder if that effect is something [01:01:30] you have to overcome?

Daniel Lingwood: I think you're absolutely right in terms of... Number one is, as you point out, that most individuals obviously haven't seen HIV, unlike influenza. So the notion of preexisting potentially destructive immunity in the context of sin would be less of a problem, is my 65,000 foot answer. But [01:02:00] in terms of individuals that, for example, have seen HIV or have been vaccinated with some of these concepts, I think that's probably where the notion of heterologous immunization is potentially to going to help play into this where you can display very antigen and boost unconserved sites. That would be [01:02:30] my generic answer to that problem. But I think like the influenza situation, it's complicated because it's going to be slightly different for each individual depending on the strain of HIV that they've seen and so it does complicate a universal solution to this.

Speaker 4: Okay. So we have a question from [Naida 01:03:00] [01:03:00] , Dr. Chandler and myself. Have you looked at this model in the presence of opiates in that how do they [crosstalk 01:03:08]

Daniel Lingwood: So, we're just basically trying to understand some of the fundamental principles of targeting, but that's certainly a direction that we'd like to go, one of [01:03:30] the, I would say important next step. I think really what we've concerned ourselves with is really making sure at this point, because we found a result that is contrary to what we would predict based on how we understand antibody affinity maturation to work. We've really tried to be comprehensive in assuring ourselves that this mechanism really [01:04:00] is the way that we think it is. And then...

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

Daniel Lingwood: ... It really is the way that we think it is and then saying, "Okay, how can we come in and perturb?" But I think in terms of asking what are the consequences of exogenous parameters because I think we, as I say, we have this now unexpected finding that says at least one way that these natural immune receptors work it's backwards to what B [01:04:30] cell immunology might say. We really wanted to be sure that that's, in fact, the case before we can now add parameters and say, "Okay, well, if we now put these animals on opioids, how does that refocusing now change?" That's certainly something that's on our radar.

Vasundhara Vart...: Absolutely. Thank you. So we have a question also from the audience. This is from [inaudible 01:04:57] proprietary. So is this immune [01:05:00] focus vaccine strategy will be beneficial to HIV infected drug abusers? How and why as compared to non-drug using individuals.

Daniel Lingwood: Yeah, again, I think that that speaks to two of the previous questions. Number one is the potential for antigenic sin, i.e. if you're already infected with [01:05:30] HIV, there is a pa .... you already have immunological memory against HIV. And so one needs to understand what heterologous immunization principle you can apply to that scenario.

Daniel Lingwood: I mean, I think now that we have kind of proof of concept that under certain experimental conditions we actually can get the immune system to see this target, I mean, that was really the first question is, can we actually do this [01:06:00] at all? Because if you just simply immunize with HIV vaccine antigens, you don't get antibody responses against the target. So we had to sort of establish a baseline to say ... really work hard to define a way in which we can actually achieve, number one, bald polyclonal antibody response, refocusing to the target.

Daniel Lingwood: Now that we can do that I think it's amenable now to explore the more [01:06:30] complicated parameters where, okay, you're on ... for a substance

abuser, what is the impact on the antibody response titre, the ability to do this? If you're pre infected with HIV, that will sort of change the B-cell memory landscape upon which you apply the regimen. [01:07:00] We've actually done a fair bit of work sort of asking, what ... if we change the order of how we do the heterologous immunization, we actually see different effects in terms of the ability to refocus the antibody response on the target. So that sort of may play into the kinds of ... or the ability to sort of apply this [01:07:30] in a context of pre-existing immunity to HIV. Yeah.

Vasundhara Vart...: That's great. So we have a question from Dr. Volkow. She's asking if there are known polymorphisms for the HIV gene, and if so, how do they affect responses to HIV?

Daniel Lingwood: Sure. So the answer is yes. There are a number of alleles of, in this case, VH1-2. [01:08:00] The allelic variant that is sort of the so-called HIV gene in this particular case is called VH1-2. And so that's generally ubiquitous across the global population. So I don't know of any reports suggesting that we ... that the humans are not equipped with this particular allele.

Daniel Lingwood: I [01:08:30] think one of the interesting features of these so-called VRC01 class responses that flow through this antibody VH gene, they're all using this very specific allelic variant, VH1-2. And so that is actually the allelic variant that we used to generate our animal model.

Vasundhara Vart...: [01:09:00] Let me see if there are any more questions coming in here.

Redonna Chandle...: I think we have maybe one more quick question [inaudible 01:09:09], and then we need to move on.

Vasundhara Vart...: Okay. Oh. Yeah, I think we'll just move on to the next step. Thank you, Dr. Lingwood. That was a fantastic talk.

Daniel Lingwood: Thank you. Sorry about the freeze.

Vasundhara Vart...: No problem. That's the [01:09:30] world we live in now.

Redonna Chandle...: And so next, we have Dr. Ryan Westergaard who will be giving us a presentation, and he is from the University of Wisconsin. So Dr. Westergaard.

Ryan Westergaar...: Thank you, Dr. Chandler, and for the organizers for putting this together. It's a tremendous opportunity to be able to share this work with you.

Ryan Westergaar...: I'd [01:10:00] like to start by acknowledging a really hardworking team that has done this ... that has shared in this project with me at the University of Wisconsin, Johns Hopkins University, and our community-based collaborators, an organization called Vivent Health, which provides HIV care and prevention services.

Ryan Westergaar...: One of the things that the Avenir program has been really helpful for is building such an interesting and interdisciplinary team. And [01:10:30] it's been fun to work with people who are in industrial engineering and mass communications among other fields I don't get to work with. And in particular, from my perspective as a physician who sees HIV patients, the Avenir Award Program has really been a privilege to ... in that provided an opportunity to focus a major part of my research on a specific and enduring challenge that has caused a lot of frustration for those of us who see patients [01:11:00] in HIV care.

Ryan Westergaar...: And it's the fact of disengagement in HIV care and where and when that happens. And to describe what this challenge is, I made this little cartoon. The main determinants of poor engagement in HIV care are social and behavioral factors. And in every HIV care setting that I've worked, and most are funded by Ryan White care program, we have a robust set of resources that address social determinants of health and behavioral determines. Alcohol and drug use [01:11:30] counseling, housing assistance, medical case management.

Ryan Westergaar...: These things are well-developed and meet a lot of the needs, but they happen over here on the right side of the screen. They're embedded in clinical settings where people essentially have to show up and be engaged in order to benefit from them. The threats to care engagement happen over on the left side here in the community. And we don't have good information about when and where these threats are occurring. So that is the challenge that our work tries to address. [01:12:00] The implications of this challenge for our goals of ending the HIV epidemic or meeting goals like the World Health Organization 90, 90, 90 goals really is highlighted by the data among people living with HIV who have substance use disorder. And this slide shows data which are a little old but still highly relevant to the problem I'm discussing today. The continuum of care among people who inject drugs shows that cross-sectionally, they seem to do pretty well. This [01:12:30] is our data from the live cohort in Baltimore, which shows that of people who are HIV diagnosed greater than 90% are linked to care. Most initiate anti-retroviral therapy promptly, and most of those have an undetectable viral load.

Ryan Westergaar...: But when we look at the issue of sustained viral suppression, which is really what's necessary to prevent onward transmission and the foundation of our treatment as prevention paradigm, it's actually greater than 90% who will fail if you follow them long enough. So only 8% after they become [01:13:00] virally suppressed remain virally suppressed through the follow up in this study. And that is not unique to this population though. There are ongoing threats to medication adherence that are complex.

Ryan Westergaar...: So good news, our ability to predict which patients in our clinical settings are at risk for poor outcomes comes from studies like this. This is a predictive model using data from electronic health records at the two Harvard hospital or Harvard affiliated hospitals HIV care programs [01:13:30] that should using data from EMR with some basic characteristics that are derived at baseline, meaning at entry to care, we can risk stratify people. And so people who are at high risk.

People that had, I think, four or more of these seven counts, seven variables within two years, 50% of them had failed.

Ryan Westergaar...: So it's not a mystery who is at risk. The challenge and mystery is where and when over the course of their illness course is the threat going to be greatest? Because our ability to intervene and to support people when and where they [01:14:00] need it really depends on us understanding that.

Ryan Westergaar...: So that's really one of the research questions that drives the work that's been supported by this award. What are the time varying determinants of lapses in HIV care? And then subsequently, what can we do about them? This is some other background work before this study also from Alive, which addressed this question. The benefit of using the Alive study is that it's a community based cohort of people who inject drugs, who have HIV and who mostly have been linked [01:14:30] to care and have good access to treatment.

Ryan Westergaar...: What we did in this study was look at paired visits, so visits where they were assessed and people had viral suppression or undetectable viral load. And we look retrospectively in the data to subsequent visits when they were not suppressed, meaning they had treatment failure. And we looked at what social and behavioral factors were captured through their battery of questionnaires that could give us insight into what are the main drivers of that. And these were the top five.

Ryan Westergaar...: The number one by a substantial [01:15:00] margin was incarceration. Particularly short stays in jails just had a really disruptive effect on medication adherence and was the strongest predictor of virologic failures. The other words were stimulant use, unemployment, homelessness, or any injection drug use. So these things, by contrast to what we saw in the Robbins paper from the electronic health records data, are typically not contained in patient's charts, and we need to go elsewhere. We need to have a window into the sort of daily lives of patients if we're going to understand when and how [01:15:30] these things are acting.

Ryan Westergaar...: So this is kind of where I'm leading. If our goal to meet our ending the epidemic goals require novel strategies to prevent and mitigate lapses in HIV care. What do those strategies need to address? And I put them here in two categories. The first is that, as I said, threats to HIV care engagement occur out of you from the providers, and providers I mean broadly, who are in [01:16:00] HIV care settings, who are sort of dedicated to help them. They call this information gaps.

Ryan Westergaar...: The second piece is that the addiction and the disruptive life events that are associated with it, including unstable housing and decompensated mental health conditions and so forth, often compete with HIV care seeking behaviors for priority. So when people are struggling with these other things, they feel less motivated to engage in HIV care because they have other [01:16:30] things that they're struggling with. And we'll consider this a motivation gap.

Ryan Westergaar...: So the tool that we're studying in this research to try to address these two determinants is the CHESS mobile health application. CHESS is the name of both the mobile health app and also the center, which is at the University of Wisconsin. They have a P30 NIDA Center of Excellence grant and David Gustafson, who is the founder and still director of CHESS [01:17:00] has been a collaborator and a wonderful mentor for me through this work.

Ryan Westergaar...: And they have been doing this work since before HIV, frankly, and way before smartphones using home computers, information communication tools to try and get people to feel more engaged in the care that they received and have greater autonomy and self-management of these conditions.

Ryan Westergaar...: And more recently, this has become ... there's developed a commercialized version that has helped in the scalability of [01:17:30] CHESS related tools. And there's now a private company that has served larger systems of addiction treatment, including the state of West Virginia has a contract for this. So it's been used, and this is a mobile, this is an evidence-based strategy for addiction relapse. And the innovative piece of our Avenir Award funded program project is to try to understand what is highly impactful about this and translate this to the HIV care setting.

Ryan Westergaar...: The [01:18:00] framework, the theoretical framework behind CHESS is self-determination theory, which holds that for, when it comes to health-related behaviors, people are more likely to engage with them if they are intrinsically motivated to engage in the behavior much more so than if they have an extrinsic motivation or they're not motivated at all. And intrinsic motivation is supported by three things, perceived competence, perceived autonomy, and the sense of social relatedness or connectedness to [01:18:30] other.

Ryan Westergaar...: So the CHESS app, and it's taken different names here. A-CHESS, it stands for the addiction CHESS platform, incorporates different services to try to support these three things among users. We, to distinguish it from the A-CHESS or addiction CHESS, we called our study or nicknamed our study ART for antiretroviral therapy CHESS, and this is our conceptual model of how this might be beneficial.

Ryan Westergaar...: The CHESS system [01:19:00] use, as I just mentioned, is designed to support intrinsic motivation. And this is really the evidence-based pathway for reducing relapse and relapse is a well-known risk factor for reduced antiretroviral adherence.

Ryan Westergaar...: The piece that we're trying to study and build with this research is this other pathway, is that by using the CHESS system, which can capture social and behavioral data, we might be able to detect people who are at risk through the use of the apps closer to real time. And [01:19:30] this builds on some of the prior work that our group has done with shorter term EMA or ecological momentary assessment trials with people who use drugs. And the theory here is that we can capture this data in real time and make this timely and support

timely access to appropriate services addressing that information chasm that I mentioned in the first slide.

Ryan Westergaar...: So the study is in two phases, and it spanned the two research [01:20:00] groups that I acknowledged at the beginning at HIV, at Johns Hopkins HIV care program, where many of the patients in the United funded Alive cohort received care. We had an initial pilot phase, the target enrollment of 60 individuals, that was intended to be a more intensive data collection period where we got people the app and we monitored them very closely with the goal of building a database that can be used to understand the predictive value of some variables.

Ryan Westergaar...: And the second phase, which is now underway and [01:20:30] soon to wrap up, is more an implementation study for HIV clinical practices here in Wisconsin, which is where I am currently on faculty. The phase one study design recruited patients with HIV who participated in Alive, had a history of opioid use disorder, and a recent history of treatment failure. So this meant a viral of greater than a thousand in the past year.

Ryan Westergaar...: So we were selecting people that we knew had sort of demonstrated them, or had a track [01:21:00] record of poor engagement in care, and were likely, although we try to prevent it, to experience lapses in care during the study. The study design involved every ... evaluations every three, six, nine, and 12 months. Again, using the infrastructure of the community-based study of Alive. We provided a smartphone and provided training for the A-CHESS app for participants. The EMA component was actually limited to weekly surveys, not multiple [01:21:30] daily prompts as we did in some previous studies with an eye toward more sustainability of the engagement with the data collection. And there wasn't formal integration with HIV clinic staff. Again, we wanted to get this off the ground quickly to start building data systems to understand the prediction.

Ryan Westergaar...: And this slide shows some of our data that we collected. The heat map on the right shows what I think is a really interesting feature or deliverable from this [01:22:00] product, which is we have this database with a number of patients who churned rapidly between periods of being virally suppressed and non suppressed. So green means good. So each row in this grid is a patient. If their box is green, that means during that month that they were assessed, they had an undetectable or suppressed viral load. And if they switched to yellow or orange, that's low-level viremia, and red is treatment failure greater than 500.

Ryan Westergaar...: You can see there's a large number, [01:22:30] nearly half of people flipped at least one or more times between green and red. And those are the areas really analyzing the CHESS and EMA data to see what is happening in. Are we able to collect these behavioral risk factors in more real time?

Ryan Westergaar...: And we're starting to analyze those data. And there's complex, there's a lot of different dimensions to the data, but I showed some of the basic findings here. The weekly survey that people use in CHESS [01:23:00] was designed to use ...

to capture some elements that are built around addiction relapse, but then also some things that are just hypothesized red flags or things that we, based on clinical experience and prior literature, know are associated with lapses in care. And it's injected drugs in the past week, did not have a place to sleep, skipped a meal because they didn't have money, criminal justice involvement, and then increases in anxiety and depression symptoms.

Ryan Westergaar...: And just for example, we showed here that the people [01:23:30] who reported two or more miss doses in the past week had a four fold greater odds in having virologic failure, to be in that red box. People who injected drugs were twice as likely to have a missed visit or a no-show visit. So these aren't big headlines. We know that these are important risk factors.

Ryan Westergaar...: But what's exciting about what we've found here is that this relatively high risk group that is poorly engaged in care and not smartphone users, over the course of the year, remained engaged enough to report these data [01:24:00] reliably and they have some predictive value. So that was an exciting thing to sort of see how it works and sets us up I think for some next steps or some next studies.

Ryan Westergaar...: Another aspect that we will use the data that we're collecting through CHESS is, in what ways are individuals who are using the system engaging with the app? I didn't go into detail of what the app does, but there are different ... there's sort of different elements related to network communication, [01:24:30] clarify, code that as exposure or expression. Are people posting things on a discussion board? Are they just reading what other people have shared?

Ryan Westergaar...: There's a motivation sections where people engage in journaling or expressions of gratitude that have been developed through previous studies. And we showed in our smaller study, the degree to which people engaged in these, and these are just clicking a click. This is 27,000. It's a very high number. That's essentially anytime someone touches the screen in one of these sections, it [01:25:00] gets logged. But this data, this table just shows how these things diminished over time. So there was a fair amount of decrease in how highly people were engaged. But it remained high throughout ... remained substantial throughout the whole year. And some of them didn't decrease as many as others. For example, the weekly survey did not decrease very much. People were still engaging in the self-monitoring aspects of the weekly survey.

Ryan Westergaar...: Some of the gains and relaxations the help with cravings features also did not decrease as many as some [01:25:30] of the messaging or the discussion boards. So there's more than to analyze there to understand how does this population interact with this kind of technology.

Ryan Westergaar...: And the second phase of this study is more of a pragmatic clinical trial, where our goal is to evaluate the feasibility and preliminary effectiveness of the CHESS intervention on HIV care outcomes using a pre-post study design. The differences here is that it's over a longer period of two or more years. We are not providing phones. [01:26:00] We're not incentivizing use of phones. It's

more to give people access to the service and see the ways in which they use it to support their own HIV care.

Ryan Westergaar...: The goal of the project were to figure out ways to formally integrate this. And one of the things that we proposed in the application was to grant providers access to CHESS data through a data dashboard. And this is something we actually didn't do. And there was a deliberate decision based on things we learned, which I'll get to in a second, but it learned [01:26:30] some interesting things about how this technology works when we try to bring it further to scale.

Ryan Westergaar...: These are the preliminary findings from the study. That's the phase two study, which is still underway. We had a fair amount of enthusiasm. Most people who were approached and screened were interested. We enrolled 208 people. We've had a modest amount of attrition, but still, but fairly good, as far as this population goes. Retention in the study related aspects. The outcomes, [01:27:00] our frequency of missed visits and viral suppression, these are obtained from the EMR, and the intervention that are being used is moderated A-CHESS and access to the weekly survey.

Ryan Westergaar...: So the framework, again, these are preliminary data. There's more to analyze here, but this is kind of the crude findings. We're planning to look at these outcomes of viral suppression and missed visits as a pre-post. So the top are the first 151 participants. [01:27:30] We look at their viral load outcomes, and the year before they enrolled in the study for the six first months with a ramp up period, and then afterwards. And we're seeing, without any test of statistical significance, a modest increase in the percent of viral load measurements that are undetectable or suppressed from 80% up to 88%.

Ryan Westergaar...: We have a contemporaneous non-treatment cohort of people who were also identified by providers in the participant clinics as having a need for substance abuse treatment. [01:28:00] And that's just, there was also a modest increase over time.

Ryan Westergaar...: So this is the type of framework that we'll use to understand the visit, but we don't have final results to share. And another really important part of this study is thinking about, how do we use this type of technology at a systems level to improve aggregate outcomes? We've had anecdotal evidence that this works really well in the way that we intended. In the second phase of the study, [01:28:30] case managers and providers, if patients were interested, could get notified of these red flags symptoms. Someone said they missed three doses. A study coordinator could send a secure message to the case manager who would then reached out to the patient and get them engaged. So it works. It works in instances, and there's been some very rewarding success stories. But if we want to build this into a way that really supports practice at a larger scale, we need to have a pretty careful implementation research.

Ryan Westergaar...: And that's something that we're starting to [01:29:00] scratch the surface of. We've collected sort of data from providers about how they use information

and communication systems. We've had focus groups with care coordinators to understand how the sources of social support are given and how they work currently. And in the participants part in particular, we're interested in how the system meets their needs. How do they feel about the design elements? And with an eye toward further refinements in improvement.

Ryan Westergaar...: And this is probably the biggest lesson that we learned [01:29:30] talking to providers. Everyone was very enthusiastic about our goal here. It showed that gap, the information chasm, everyone says, yes. That information about drug use, about social determinants of health, is critical. But when we asked them, would you like to get reports of these surveys in real time? They said, no, frankly. They have information overload. They don't know what to do with it in real time.

Ryan Westergaar...: It varied by provider, but it was clear that if we want to harness this information, we have to do a fair amount of work to figure out how this [01:30:00] information flows to support the delivery of care in a coordinated way. And I think that sets us up for the next steps.

Ryan Westergaar...: So we're really enthusiastic and really grateful for the support of this project to show how the system can meet the needs of patients in our care and are excited to take further studies to systems level adoption.

Ryan Westergaar...: And so we have now an R01 grant that's underway that [01:30:30] was just submitted, trying to translate these preliminary findings of this award to a hybrid implementation effectiveness study in four US states. And we'll over the next six to 12 months be digging into this really nuanced and very interesting data from the population that's been collected by the app and some of the surveys.

Ryan Westergaar...: So I will stop there and be happy to take any questions. And thank you again for this fun opportunity to share our work.

Vasundhara Vart...: [01:31:00] Thank you, Ryan.

Redonna Chandle...: So Ryan, I'm going to take prerogative event since I can ask my question. Ask my question, and then we'll go to the ones that are in the chat. So this is great. And I know you've also looked at some provider level and maybe even community level factors that impact interruptions in care and quality of care. And I know some of your work has particularly [01:31:30] focused on the level of stigma that providers have around people who use drugs and people who inject drugs. And I don't know if you have carried on any of that work through your Avenir Award and if you have any more recent data or findings that you could just even just quickly talk about.

Ryan Westergaar...: Yeah. I would say not systematic data collection, but the interviews that we did with providers that I alluded to, stigma might be a role, but I think [01:32:00] it's

more of a sense of helplessness. Essentially, the problems that we're trying to address through this work, a lot of physicians, providers see as social problems that I'm frankly not trained to address. So this needs to be someone else's problem.

Ryan Westergaar...: They're sympathetic with the need to it, but they don't feel like they're empowered or they have time to address them. So I think the way we did this, that I think the silver lining is here, is that we ask similar questions to [01:32:30] large number of people on a care team. And we really got support for this through that role, which I think is going to be key in the future research, is the medical case manager or the patient navigators. They are hungry for this type of data and are very enthusiastic about hearing it.

Ryan Westergaar...: But again, trying to support team-based care is really the work. So I think you're ... We definitely found a sort of a lack of enthusiasm among some providers. I don't know if it's directly caused by stigma, [01:33:00] a target against the population that we're trying to serve as much as just not sort of maybe not my problem kind of sentiment.

Vasundhara Vart...: We have a question that came through chat now. This is from Dr. [Friedman 01:33:22]. We're trying to unmute so he can you.

Dr. Friedman: Yeah, I think I'm unmuted.

Vasundhara Vart...: Okay, great.

Dr. Friedman: Yeah. Wonderful talk. Really [01:33:30] suggests good ideas. When you presented some of your earlier slides, they showed that things like incarceration or becoming homeless or things like that might be risks to engagement. It wasn't fully captured near a theoretical model, but particularly with what you were just presenting about provider overload, would it make sense to develop [01:34:00] contacts with community groups and an easy way to tie the A-CHESS data and what you're finding oriented towards evidence that may be those things are going to happen and then get community groups to provide support or training, or even legal help to help prevent some of those?

Ryan Westergaar...: Yeah. Yeah. Thanks for that question. It's a really important element. [01:34:30] What's unique about our HIV care setting that we're working, it's not unique among all of HIV care settings, but it's the community partner that I mentioned at the beginning, Vivent Health, has really adopted a patient centered medical home type approach.

Ryan Westergaar...: So even some of the things you mentioned, like legal support, they have those types of services in-house or referrals to community based organizations to help with some of these other [01:35:00] things. The conduit to a lot of those things is that role, that care coordinator, that medical case management. So a medical

case manager who sort of sees as the person who coordinates access to these services.

Ryan Westergaar...: So really, I think you really nailed it. The idea that when those things are ... perhaps come to the awareness of the team, there is a fairly well-developed [01:35:30] infrastructure for making those connections to community-based sources of support. I guess the problem that this project is trying to solve is, how do you facilitate those existing connections in a better way? And we're lucky in HIV, people who have similar risk factors and have injection drug use problems who don't have HIV, have a real ... a dramatically different [01:36:00] landscape of services available to them. But in this HIV care ...

PART 3 OF 6 ENDS [01:36:04]

Ryan Westergaar...: Landscape of services available to them. And in this HIV care setting, there's a lot that's already been built to meet some of those needs. And they're often unmet because of the sort of lack of awareness. And that's that's kind of what we're trying to target.

Sunil Suhas Sol...: In the Ukraine, we've experimented with having essentially community based health managers. And if they were connected to something like the HS system, they could be proactive.

Ryan Westergaar...: [01:36:30] Yeah. That's a great suggestion.

Vasundhara Vart...: All right. Still looking for any questions in the chat. I did not miss anything from Q&A. All right. I don't see anything coming in, but [01:37:00] I guess we'll just move on to the next talk, in that case. Ryan, thank you so much. Wonderful talk and Redonna, you have something you want to ask?

Redonna Chandle...: Kavi, it looks like we have a break now. We have a short break. Is that correct? Am I reading the agenda correctly? Can Aaron or Jeremy let us know? Do you want people just to stay on, but mute their phones for the breaks, so that they don't have to sign back in? Is that your [01:37:30] preference?

Speaker 5: Yes. Yeah. If folks want to just stay on, we'll put on some break slides and we will return here at 11 o'clock.

Redonna Chandle...: And at 11, Dr. Sunil Solomon will give his presentation and he is from Johns Hopkins University. So we'll just move right into his talk so we have more time for the [01:38:00] science and for the discussion. Thank you all so much for attending. We have had as many as 85 participants, so I think we could consider this to be a great success. We'll see you back and start at 11 promptly. Enjoy your break. Okay. Are we ready to get started? Okay. You're ready. Dr. Solomon take it away.

Sunil Suhas Sol...: [01:38:30] Okay. So, good morning, everyone. I want to thank the NIDA leadership for inviting us here today to share some of our data related to the Avenir grant. So my presentation is going to be a little bit different from the others, because I want to give you a little bit of how I got the idea for the Avenir grant, what we have done on the Avenir, as well as what we're currently doing based on things we learned from the Avenir itself. [01:39:00] These are my disclosures.

Sunil Suhas Sol...: Just to set the stage because I know we had been talking a lot about HIV and PWID in the US, but to set the stage and to bring everybody to the same page about HIV in India. India is home to about 2.1 million people living with HIV. It is a largely heterosexual driven epidemic, but we do see concentrated epidemics among key populations. And as you can see from the latest Sentinel surveillance data, PWID with the prevalence of 6.3% is [01:39:30] the key population with the highest prevalence.

Sunil Suhas Sol...: For those of you who are also aware about India's unique position, we are located right between the Golden Triangle and the Golden Crescent, which are the two largest heroin producing regions in the world. And we have drug trafficking routes going through India from both sides, from the East and the West. And as you might also expect, India with 6 million opioid users, is home to the largest number of opioid users globally, which should not be surprising because with the population [01:40:00] of 1.3 billion people, we're home to the largest number of a lot of things globally. The most recent estimate for people who inject drugs in India is about 850,000 PWID who live across the country.

Sunil Suhas Sol...: I want to start by acknowledging the Indo-US Joint working group, which was really spearheaded by Jack Whitescarver and Jacques Normand from NIDA. Everything that I'm presenting here today is a result of two R01 supplements for $100,000 that [01:40:30] really has transformed our work in India. The one thing we do know in India is how to stretch a little money and make it go a long way, so using these two $100,000 supplements, we were able to establish a longitudinal cohort of people who inject drugs in Chennai. We recruited 1,100 people, categorized HIV Hep-C incidents, one of the first longitudinal cohorts in India. And simultaneously, we also demonstrated via a randomized clinical trial that more non-monetary [01:41:00] vouchered incentives do improve linkage to care and ART retention among people who inject drugs in India. So, these two grants really spearheaded our work in India, but most of this work was focused in Chennai.

Sunil Suhas Sol...: And we very soon realized that people across India faced similar challenges with access to HIV services. We follow that up with another NIDA funded cluster randomized trial, and the objective of this trial really was to look at the impact of integrated HIV service [01:41:30] delivery, integrating HIV prevention and treatment services into integrated venues, which would be PWID friendly. And as you can see from this map, we were literally all over the map. All the orange spots indicate sites where we work with people who inject drugs. So I'm not going to go into this trial or its findings, but the baseline assessment of this trial

is really what gave me the idea for my Avenir application. As part of the baseline assessment, we recruited 1,000 [01:42:00] PWID per city across 15 cities in India, so we recruited a total of 15,000 PWID over a year.

Sunil Suhas Sol...: Just to give you an idea of how respondent driven sampling works, this is the city of Kanpur in central India, where we really had no information on PWID before we started our work there. RDS generally starts off by identifying two individuals who are considered seeds, who are highly influential. We give them two coupons, have them give it at random to any other two PWID they know in the city, [01:42:30] have them come into the study. When they come into the study, the recruiter gets an incentive and the recruit also gets an incentive. It's a dual incentive mechanism. And when that first person comes in, we repeat the process, give them two coupons. And in this process, the PWID population recruit each other in a city. What I wanted you to look at this figure and really highlight, is one, in most cities across India, we only used two seeds to recruit our entire [01:43:00] sample of a thousand people.

Sunil Suhas Sol...: The second thing I want to highlight is just the extremely high burden of HIV and Hep-C in this population, almost 65% of the population were infected with Hepatitis-C. Virus. But at the same time, I think what was most striking to us as researchers that we're conducting a baseline study was only 3% of the 350 HIV infected PWID in this population were actually aware that they were HIV infected [01:43:30] and none of the 650 PWID were Hepatitis-C virus infected actually knew they were infected with Hepatitis-C. This really prompted the question of, can we leverage networks like the RDS networks to improve HIV case identification among people who inject drugs? In my Avenir, we tried to adapt respondent driven sampling from what it's traditionally used for. Traditionally, it's used for surveillance to try and assess disease burden [01:44:00] or drug use patterns or something else.

Sunil Suhas Sol...: And we said, can we adapt it from using being purely a surveillance tool to actually start delivering services? This is not the first group to do it, but I think there are other groups that have also been doing it, especially there's a starter group in Greece. We were looking at three different strategies. The first one was time-based RDS. The way RDS traditionally works is you set a sample size of 500 or 1,000, or you have process measures. And when you meet that, you stop. What we said [01:44:30] is, why don't we just let it run for a fixed period of time? And we decided this period was going to be one year. We implemented this in the city of Varanasi, in Uttar Pradesh, which has one of the fastest growing PWID epidemics in India. It was estimated there are approximately 600 PWID who live in Varanasi.

Sunil Suhas Sol...: Over the one year period that we ran in Varanasi, we recruited 1,300 PWID, which is more than double the number of estimated PWID. Everyone was provided with HIV testing and all the positives were linked to care. It really was [01:45:00] a very efficient strategy of finding PWID and finding people who are unknown and linking them to the actual HIV program itself. The second approach we took was precision RDS. Data from this contributed to the PhD

dissertation for Alison McFall, who continues to work with us. The hypothesis with precision RDS really was, there are recruiter characteristics that can predict the ability to find undiagnosed cases. As in, there are some [01:45:30] people who, by default, were going to be much better at finding people who are undiagnosed compared to other people. The approach we took was we built a prediction model using Random Forest and Logistic Regression.

Sunil Suhas Sol...: And then we randomized participants to either the standard coupon where everybody got the same number of coupons or the precision RDS system in which people who are considered good recruiters by the algorithm got more coupons and people who were considered bad recruiters got fewer coupons. [01:46:00] We implemented this in the city of Morinda, in Punjab, which is again, one of those really high prevalence PWID populations across India. We used two characteristics to look at whether this was an efficient strategy. The first one was a number needed to recruit. This is the number of people you needed to recruit to find one undiagnosed infection. The lower the bar, the closer the bar is to zero, the better. And as you can see, the precision RDS did better throughout, it wasn't statistically significant. [01:46:30] The difference is about two and a half, three coupons, fewer, but I think one thing that you need to understand this from an implementation standpoint, three coupons fewer translates to about $10 less.

Sunil Suhas Sol...: And we're looking at a program which is trying to reach 1,000 drug users, 10,000 drug users. That translates $10,000 or $100,000. It really does impact program efficiency and program yield. The second indicator we used was the identification rate, which was a number of [01:47:00] undiagnosed HIV infected PWID diagnosed per week. In this, what we want to be is we want to be the higher bar. And so, as you can see, the precision audience approach did significantly better over a standard approach in terms of the identification rate. Based on these two, it is safe to say that the precision RDS does appear to be more efficient than standard RDS approaches [01:47:30] as a case identification strategy. But one thing we always recognize through RDS is what 50% of the coupons that go out and never come back.

Sunil Suhas Sol...: And so we always wondered where these coupons went to, whether particular pockets of particular parts of the city where the heavy injectors were living, where you couldn't really bring them back into the venue. Could we actually supplement the RDS with field-based testing by going to these different places? Our hypothesis really was prevalence of HIV and prevalence of Hep-C is [01:48:00] not going to be evenly distributed across the city. There are going to be hotspots with a high burden and some hotspots with a much lower burden, and targeting high burden hotspots will be a more cost efficient strategy. So, our focus really has been from an implementation standpoint. We implemented this strategy in the city of Gorakhpur, which is also an [inaudible 01:48:20] at the foothills of the Himalayas. We recruited 500 PWID and we brought it out the prevalence on the map. And as you can see, just as we expected, there were about four hotspots where the [01:48:30] prevalence of HIV was significantly higher than all the other hotspots.

Sunil Suhas Sol...: We were about to start processes when COVID hit. And so we have stopped, and now we are currently redesigning the RDS+ to make it RDS++, which also integrates COVID restrictions, COVID precautions. We've just got permission from the IRB to restart our activities. This data is definitely coming in the next couple of months, I hope, I think the one thing about the pandemic is none of us know exactly where the pandemic [01:49:00] is going. Avenir really did provide us a lot of answers about service delivery, but it brings a lot more questions to us about transmission itself. For example, we were reaching all these people, getting them tested, but why was incidents so high? I haven't presented this data, but in some of these cities, the incidence was highest 10 or 12 per 100 person [inaudible 01:49:21].

Sunil Suhas Sol...: And as I said, RDS doesn't really recruit transmission networks. It's built on the principle that you recruit randomly [01:49:30] from your networks. It's not necessarily a transmission networks, and it also doesn't really connect people across different networks. And while I showed you that the prevalence is different by different spaces, it also doesn't incorporate the role of spaces in the transmission. We felt that if you really wanted to prevent onward transmission of HIV and Hep-C, we really need to understand underlying network structure, it's overlap at space and time. Which really prompted us to start another NIDA funded grant, which is called the spatial [01:50:00] network. This was a longitudinal cohort of PWID in the city of New Delhi. The objective of this trial really was to understand the role of egocentric, social metric, and social spatial networks on HIV and Hepatitis-C transmission.

Sunil Suhas Sol...: I'll explain what we mean by these different networks for those of you who are not very familiar with network recruitment strategies. The way we built this cohort was we started off with 10 index participants, the index participants were asked to [inaudible 01:50:29] the people [01:50:30] whom they injected with in the prior one month. And they were also asked to give us descriptive characteristics, unique identifying features of these people. And only those specific individuals were allowed to come into the study. It really was that specific injection network within the prior one month. And when those network participants came in, they served as the index of the next wave of recruits. And we kept doing it until the entire network was saturated, then we added a new index.

Sunil Suhas Sol...: The one thing [01:51:00] I want to point out here, which is different from RDS was in RDS, they would say, give it to any two people whom you wanted, versus in this study in Delhi, we actually had them give us specifics about the name, the description, and a unique identifier or appearance of how this person actually looked like when they would come in. The other challenge of working in India, as you may imagine was the unique identifier of the description for most people, was he's short and with a mustache. It was getting really [01:51:30] hard for us in India to try and distinguish one short person with a mustache, with another short person with the mustache. And so we actually incorporated something called a fun fact where we allowed them to tell us something about this person, such as he is originally from Bihar, his wife's name is Jodi, he has

two children, or something along those lines. The other thing we also did was by design, we allowed duplicate participants to come in for the trial, but we [01:52:00] didn't just establish the cross network linkages.

Sunil Suhas Sol...: To give you a sense of what the recruitment looked like, when an index participant came in, we asked them to name the people whom they immediately injected with. That would form the egocentric network. Then we ask those participants, did they inject with anybody else? And if they didn't inject with someone else, that would also be the social metric network for that particular index. And this is another example where the egocentric network is essentially the same, but when we asked them if they inject with someone else, [01:52:30] it's a much larger network. And so the social metric network ends up being a little bit different. And then we also asked people to give us specific locations where they injected and we considered everybody injecting within a particular location to be one spatial network. And similarly, there are other spacial networks in Delhi. And over time we asked them, did you inject with the same people? Did you inject with different people? Did you inject in the same location? Did you inject in different locations? And as you can see over time, people move, people [01:53:00] in-migrate, people out-migrate, and the network structures also change accordingly.

Sunil Suhas Sol...: This is essentially how we categorize the different networks. To give you a quick snapshot, those 10 indexes recruited 2,512 people who inject drugs in New Delhi, about 75% of the coupons came back in. But what is really striking was the HIV prevalence at baseline was 37% [01:53:30] and only 8% of them were virologically suppressed. The Hepatitis-C antibody prevalence was 65%, and only 20% of them had cleared the virus. In terms of HIV incidents, though, the study really was looking at transmission. I think what was striking was the extremely high incidence, especially given the baseline prevalence. We were only talking about 63% of the cohort that was not infected at baseline. And in that cohort, we had 159 new infections [01:54:00] during longitudinal followup. And with a similar thing, we also saw with Hepatitis-C. The HIV incidence was 22.3 per 100 person [inaudible 01:54:09]. And Hepatitis-C, there was antibody zero conversion.

Sunil Suhas Sol...: If you include [inaudible 01:54:13] RME clearance and reinfection probably is going to be a lot higher. Because the focus really was social spatial networks, this is what the social spatial network look like. This is part of the dissertation of Steven Clipman, another doctoral student from Hopkins. As I mentioned, we did [01:54:30] start with 10 indexes, but five of these indexes merged into one large network in the middle, which seemed really hard for us to understand, so we actually went out to the site and what we found was there was this one particular location in New Delhi, where on any given day, at least 1,000 PWID cycle through that location. There were a lot of migrant workers, a lot of migrant laborers, who all lived here.

Sunil Suhas Sol...: I know this is a very beautiful figure and it's very complicated, but I want to take you through two specific [01:55:00] situations of what exactly how complicated

this is. If you look at this one red incident infection, this is like the classic incident infections you would expect when it's connected directly to another incident infection, three prevalent infections, and one person who is HIV negative. But if you look at the second case, this person has four connections and it's egocentric network, none of whom were HIV infected at their prior visit, but he was only two steps away from several people who were HIV [01:55:30] infected. In terms of the predictors of HIV incidence, we looked at the standard individual level predictors, as you would expect, age, recent injection, an injection frequency rest from the associated with, but what we really were interested in red network level predictors.

Sunil Suhas Sol...: The first thing we looked at was your egocentric networks. This is the people who are in your immediate circle. For every person in your immediate circle who was HIV infected with detectable virus, your risk of HIV [01:56:00] seroconversion went up by 31%. And as I showed you in that second example, there was no one in your immediate circle who was HIV infected, but you could still be connected through someone who was HIV infected, either one step or two steps or three steps away, which is what we try to assess with your social metric or the path distance.

Sunil Suhas Sol...: What we found there was for every additional step between you and someone who was HIV infected with the detectable virus, your individual risk of HIV seroconversion [01:56:30] dropped by 40%. These definitely do show you some signal that HIV viremia does play a role in transmission, even among people who inject drugs. But I think what was really striking was that injection. We ran a machine model, and we found that this one particular location was associated with a 3.4 times higher likelihood of seroconversion, and this association held good even after adjusting for egocentric and social metric factors. [01:57:00] What I want to show you in this slide is what this network actually looks like and how it evolves over time. These purple notes reflect the hotspots and these different nodes that these different colors reflect different participants. What I really want you to focus on are these red nodes and these red edges of these lines, the edges connect nodes with each other and nodes with hotspots. And what you can really see in this figure is over time, this network, just the walls and that big purple spot in the middle, [01:57:30] that's pretty much where spatial network hotspot number 40 is. And as you would expect, you see a lot of clustering of infections close to that spatial hotspot number 40. And what we found was 113, 70% of all these incident infections reported injecting at one particular hotspot. And when you're looking for socio spacial distance from that particular hotspots, even if you were not injecting in that hotspot, if you were injecting with someone who was injecting in that hotspot, there was a definite risk. [01:58:00] The further you were from that hotspot, your risk of HIV seroconversion kept going down. Our next steps, we have completed whole genome sequencing. We are trying to look at this to look at, does U is equal to U also hold good in people who inject drugs? And the other thing we also want to do is want to recreate these networks using purely phylogenetic data, without any of the self-reported data. We have been able to resume our visits post-COVID, because we have this rich network data, they want to look at a

transmission of SARS-CoV-2, and for those of you [01:58:30] who are following, there was a lot of migration lockdowns in India, which sort of serves as a natural experiment in India itself on terms of network structure.

Sunil Suhas Sol...: But so far the spatial network study is only in one city in India. How does this fit into the larger elimination agenda? The last two slides, I just want to show you something that we were able to do with funding from Abbott, where we sequenced the core five UTR region from Amritsar, Delhi, Kanpur, and Imphal to look at clustering of infections. Here you can see there's a lot of intercity [01:59:00] clustering. For example, the cluster in Delhi had infections from Kanpur, Delhi, and Amritsar, whereas the cluster in Amritsar had infections from Kanpur, Amritsar, and in Imphal. We were also able to conduct a basion phylogeographic approach and reconstruct the geospatial dynamics of HIV. The temporal information was obtained using time resolved phylogenies.

Sunil Suhas Sol...: As you would expect, the oldest mean common ancestor in India was in [01:59:30] Imphal. It seems to have moved from Imphal to Kanpur all the way to Amritsar. The size of the circular polygons really are your mark [inaudible 01:59:37]. So essentially showing you that most of the local epidemics are in Imphal and Amritsar, and Delhi and Kanpur serve more as transitory points, which is consistent because Imphal is much older epidemic, closer to the Golden Triangle, and Amritsar is a much newer epidemic closer to the Golden Crescent. So what does this all mean? From the Avenir and the earlier work that I've showed you, networks can definitely be leveraged to improve access to services, but we are just beginning to scratch the surface of networks, because the networks are a lot more complicated than just interpersonal connections, and to really interrupt transmission to achieve HIV/AIDS epidemic control or the [inaudible 02:00:20] [02:00:00] elimination, we really need to take a step back and look at these epidemics from much larger lens.

Sunil Suhas Sol...: I'd like to thank a lot of people, and I think especially NIDA for all their funding support [02:00:30] over the years. And I think one thing I do want to highlight is while we are doing research, a lot of our research really is service delivery and its implementation research. Over the years, we've provided services to over 50,000 people who inject drugs in India, purely through NIDA research funding. I want to end by acknowledging and thanking one of my site teams, which we have many off in India. Thank you.

Vasundhara Vart...: Thank you very much, Sunil, excellent talk. [02:01:00] We know we have to address gaps in HIV care cascade to be able to address the HIV epidemic, end the HIV epidemic. And so the more ways we can reach out to these people who are falling through the cracks, not able to reach the services, it's better for us to know this and to address and thank you for all this important work you're doing in India. And now let's [02:01:30] look at the questions. Oh, there's Dr. Friedman. He asked that a lot of similarities between your and Dr. Friedman's research and intervention, so we should communicate on it, he says, I guess there is your collaboration, for future. In particular, his Ukraine colleagues have done good work with artificial intelligence [02:02:00] to improve case detection

for HIV. I think it's just an opportunity for collaboration. There is a question from Jason Blackard, is HIV incidence associated with having HCV? Is HCV incidence associated with having HIV? Are baseline untreated wider loads higher for HIV if HCV is also present?

Sunil Suhas Sol...: Thanks [02:02:30] Jason, for those questions. We have not looked at the wider loads untreated in HIV mono infected with coinfected, but we can definitely take a look at that. In terms of the association between HCV and HIV, HIV incidence does not predict HCV incidence. As I mentioned for us, most of the HIV seroconversions if they were HCV antibody negative at baseline, they're seroconverted [02:03:00] for both. I don't know if that's the question you're asking if it's mono infection seroconversion versus dual infection. If that is the question, then yes, people who are antibody negative for Hep-C and when we see them as an incident, HIV infection, 90 to 95% of them have seroconverted for both Hepatitis-C and for HIV. It's very rare that we see HIV seroconversion without Hepatitis-C seroconversion. I'm not sure if that answered the question.

Vasundhara Vart...: [02:03:30] I think there's part of the question, Sunil, I think he also is asking, did you notice any differences in wider loads when there is a dual infection with HCV?

Sunil Suhas Sol...: Right. [inaudible 02:03:43] we haven't really looked at it, but I'm happy to definitely look at it and I can get back to him on that. And I also want to thank Dr. Friedman for his, I think I did see one of his Dean's presentations and they just started last year where they used artificial intelligence, so I think there really is definitely [02:04:00] a role of collaboration.

Vasundhara Vart...: So Dr. [inaudible 02:04:05] is asking, are there distinct position of women versus men in the networks? Do they have less [inaudible 02:04:17]?

Sunil Suhas Sol...: So, there definitely is a role. The epidemic in India, generally, in most of India is completely driven by men. For example, in this cohort, we only had about 25 women and most of them are [02:04:30] in the better free. Whereas if you go to the Northeast, we haven't done this work yet, but I would expect distinct networks of female people who inject drugs that would exist by themselves in the Northeastern States of India.

Vasundhara Vart...: Great. Thank you. That is Steve Shakta, he has a question about opiod overdoses. Steve, are you unmuted? You can ask if you want. So [02:05:00] he's asking, do you have thoughts as to whether opiod overdoses map onto HIV network incidence cases in India?

Steve: Yeah. And related to this question, I am now unmuted, in US, we have syndemics, so we have these intertwining epidemics between opioids, HCV, and other things. Can you talk a little bit, as well as overdoses, about things like mental health disorders, STDs, [02:05:30] maybe the need for primary care within this group, as you think about intervention?

Sunil Suhas Sol...: Sure. Thanks, Steve. We have collected information on opioid overdose, but we haven't looked at it specifically in connection with incidence and prevalence, but I agree with you. I think the way we've been designing most of our programs, India really has been around healthcare needs of people who inject drugs. I think it's very hard to run [02:06:00] a program just for HIV or just for Hepatitis-C, because most times you really have to figure out, what do they need? And the goal of the program really is improving health. We do have these models right now in India, which again, thanks to NIDA funding, we are running these integrated centers, which do primary healthcare, harm reduction HIV, and we have just started a trial where we're integrating Hepatitis-C services. I think really the model is building a one-stop shop, which is not disease specific, but person specific [02:06:30] and really building in social support, building in economic opportunities, homelessness support, food, nutrition.

Sunil Suhas Sol...: I think those really are the drivers of PWID communities globally, not just in India, but I think that's like a uniform theme globally. And we generally tend to look at people with a disease lens. We need to step back and look at it from a personal lens and be like, if I was a PWID, what would I want? And really build our programs around that. I think that's where we are sort of lacking. I think once we switch our focus and realize that [02:07:00] once we put on this people lens, it really will make a much bigger difference.

Redonna Chandle...: So we're running out of time, Kavi, and I see there's some other questions. Maybe people with specific questions or comments can reach out to Dr. Solomon directly, because I want us to be able to go to the next talk since it's 11:30. So, Michael [02:07:30] Newcomb is scheduled to present now and he is from Northwestern University. So, take it away.

Michael Newcomb: All right. All right, well, thank you very much for inviting me to give this presentation today on my Avenir award, which I received in...

Redonna Chandle...: [02:08:00] Hang on just one second. Can you go over to the...

PART 4 OF 6 ENDS [02:08:04]

Michael Newcomb: Ooh.

Redonna Chandle...: Hang on just one second.

Michael Newcomb: Yes.

Redonna Chandle...: Can you go over to display settings and flip it? Because we see your-

Michael Newcomb: Oh, I'm sorry. Yes.

Redonna Chandle...: That's okay.

Michael Newcomb: Actually, I've got aa solution. Please hold.

Redonna Chandle...: Yeah. Just flip it around so we get your slides big.

Michael Newcomb: Oh, technology these days. It's killing me. [02:08:30] Better?

Redonna Chandle...: Perfect.

Michael Newcomb: All right. Thank you. I apologize. So, as I was saying, thank you for inviting me to give this presentation today on my Avenir Award, which I received in 2016. So I'm currently in my final year of this award. And the title of my talk today is using relationship education as a platform to reduce HIV risk in young [02:09:00] male couples.

Michael Newcomb: I first just want to thank and acknowledge my team, including my co-investigators, my incredibly talented staff, and NIDA for the receipt of this award, as well as some other funding that I've received to support this program of research, including an RO1 from NIAAA and the Sexualities Projects at Northwestern University. [02:09:30] So just by way of brief background, why focus on couples and HIV prevention among young men who have sex with men in the United States, in the late 2000s, a bunch of data emerged indicating that a large proportion of new HIV infections in the United States were occurring in the context of primary partnerships or serious romantic relationships. And that this was especially pronounced amongst young men who have sex with men. One [02:10:00] estimate that up to 79% of new HIV infections were occurring in the context of primary partnerships.

Michael Newcomb: Intuitively, this makes sense as you start to think about it. In serious relationships or primary partnerships, people tend to use condoms less frequently. There's a higher likelihood of engaging in receptive anal sex, which carries higher risk for HIV transmission. And quite simply, you have sex with the same person over and over again. So if that person [02:10:30] is a person living with HIV, there's a higher risk of HIV transmission if viral load is not controlled.

Michael Newcomb: More recent data has suggested that it's not just condom use that tends to decline upon relationship entry, but one of the primary predictors of PrEP discontinuation is entry into a relationship. On top of that, a large proportion of HIV positive YMSM under [02:11:00] the age of 25 are not aware that they are living with HIV. So this creates a bit of a perfect storm in terms of HIV transmission, that if young people who are unaware they're living with HIV, enter into relationships, they then reduce their use of protective behaviors and make it highly likely that they would transmit HIV to their partner.

Michael Newcomb: So interestingly, right about the same time [02:11:30] that I started in on this line of research, this is a photo of my now husband and me, I met my now husband. So relationships and relationship skills were something that were very much on my mind at the time, as I was thinking about how to address HIV risk in

young male couples. And then digging into the relationship literature, one of the most robust effects from that literature is what's called the marriage effect, which is that upon entry into a serious, committed relationship or a marriage, [02:12:00] multiple aspects of health and wellbeing start to improve.

Michael Newcomb: That includes mental health, substance use, various other aspects of cardiovascular health, on and on and on. However, one of the factors that can impede the health promotive effects of being in a relationship is stress. So if individuals in relationships or couples are experiencing stress, then that reduces the positive influence of being in a relationship. [02:12:30] And for same sex couples, there are a whole host of other structures that we must face, both as individuals and as couples that compound the effects, the negative effects of stress on our day-to-day lives.

Michael Newcomb: So that just means that it's all the more important that we help young people to build relationship skills in order to optimize the health-promoting benefits of being in a relationship. So that [inaudible 02:12:59] the combination [02:13:00] of my work as well as my personal life lead to the premise that optimizing relationship functioning as a strong platform through which we can promote both relationship health and other aspects of individual health, including reducing HIV transmission risk. And that led to the current project, integrating relationship education with primary and secondary HIV prevention for young male couples, or the 2GETHER [02:13:30] Intervention.

Michael Newcomb: In brief, 2GETHER is a four-session hybrid intervention. It's a hybrid group and individual level intervention for both HIV negative and HIV positive coupled YMSM. The intervention consists of first two group sessions that are attended by anywhere from two to eight couples, and live facilitated by two facilitators, that focus primarily on didactic skills with regards to relationship skills and sexual health [02:14:00] promotion. And then those two group sessions are followed by two individualized skills coaching sessions in which each couple works with one facilitator to apply the didactic skills to the unique experiences in their relationships.

Michael Newcomb: Broadly speaking, the skills that we promote in 2GETHER, first, the relationship functioning skills, we heavily emphasize building effective communication skills, as well as coping skills that couples can use as a dyad [02:14:30] in order to navigate stressors that they encounter either as individuals in their individual lives, or together as couples. And then the sexual health intervention, which actually comes at the very end of this intervention, primarily means this intervention actually focuses on relationship functioning, but the sexual health component is three prongs.

Michael Newcomb: So first, we focus on building and maintaining sexual satisfaction and pleasure within the dyad. Next, we focus on building [02:15:00] and maintaining relationship sexual agreements, which are another word for monogamy or non-monogamy arrangements, which vary according to the couples and the preferences to couples in our intervention. And then finally, layering on top of

the unique needs of each couple's sexual agreement, primary and secondary HIV prevention strategies. So, with regards to the current project, we first conducted a very small non- [02:15:30] randomized pilot trial, which served as the preliminary data for the submission of this DP2. And based on that, I was awarded this grant to evaluate 2GETHER in a comparative effectiveness trial of substance-using young male couples, 200 couples in total or 400 individuals, with 12 month follow-up.

Michael Newcomb: That was randomized to either the active condition, the 2GETHER Intervention or the control condition, [02:16:00] which is based on existing public health practice for HIV prevention and care for couples. So that would mean either couples-based HIV testing and counseling for those who are HIV negative, or the Life-Steps medication adherence and risk reduction protocol for folks who are living with HIV. And then serodiscordant couples would receive both of these interventions at the same time.

Michael Newcomb: In terms of eligibility criteria, so you have a little bit of a sense of who [02:16:30] these couples are, in order to enroll in the trial, couples need to be both assigned male at birth and currently identify as men, both aged 18 years or older, and one partner needs to be aged 18 to 29. We allow partner two's age to be 30 or older in recognition of the fact that having an older sexual partner or romantic partner is a unique risk factor for HIV transmission amongst YMSM, [02:17:00] both individuals need to identify one another as primary partners. They had to have had anal sex with one another within the three months prior to baseline. And at least one of the partners needs to report condomless anal sex with a serodiscordant or unknown status partner within the last three months. And that partner can be either their primary partner, if they are a serodiscordant with their primary partner, or an outside sexual partner. [02:17:30] With regard to substance use criteria, at least one of the partners has to have reported binge drinking or drug abuse episode within the last 30 days. So right off the bat, we experienced our first challenge with this grant, which took the project in a very different direction than what I anticipated. It was actually a good problem, which is that I received another grant just a few months after I received the [CP2 02:17:57] award, that was also planning [02:18:00] to recruit 200 young male dyads in Chicago with nearly identical inclusion criteria.

Michael Newcomb: So I immediately freaked out, freaked out in a good way, but still freaked out nonetheless, and realized that it was not going to be feasible to recruit 400 couples from Chicago in this exact same time period. So it started getting me thinking, could this intervention be done entirely online via telehealth? So not changing it into an automated E-health [02:18:30] intervention, like an app or something like that, but could we just simply do the same intervention with couples across the United States over a platform like Zoom? So I was able to work with my program officer, [Rich 02:18:44] Jenkins, to come up with a plan to do this.

Michael Newcomb: First, we conducted a brief pilot study with 15 dyads in January of 2017, which was very successful and only resulted in us having to make a couple of minor changes to the implementation of the intervention. [02:19:00] So first, the group sessions, which can last up to two hours long, can be very difficult for somebody to sit through online as opposed to in person. So we split those two group sessions into three group sessions, which means that we now have a five-session intervention. And in order to reduce the length of those group sessions even further, we prerecorded a lot of the didactic content that's reviewed in those sessions, sent it to our participants a week ahead of time for pre review, so [02:19:30] that then the group sessions could focus entirely on discussion and activities amongst the couples.

Michael Newcomb: In terms of the enrollment process that we used for this study, it's quite complicated in doing this with couples. It starts off by having each member of the dyad complete an eligibility screener. Once the couple has deemed to be preliminarily eligible, we complete a couple of conversation or excuse me, a couple of confirmation call with each member [02:20:00] of the dyad separately, which essentially is asking specific questions about their relationship and partnership in order to identify fake couples. After that, couples complete the baseline process, which includes a baseline survey, a self-video recorded communication task, and then remote completion of STI testing for urethral and rectal chlamydia and gonorrhea. After [02:20:30] that, couples are randomized to either the active or control condition.

Michael Newcomb: The second challenge we faced fairly quickly with recruitment and enrollment of couples, as you can imagine, we learned a hard lesson very quickly, which is that recruiting couples is really difficult. But if done correctly, it actually has very high payoff, which I'll demonstrate in some of the subsequent slides. And we have indeed completed recruitment, [02:21:00] randomized all 200 dyads. However, one thing that we observed is that there's fairly large loss to follow-up between initial eligibility screening and the actual randomization. And larger loss to up than we see in other types of behavioral trials. This is for a number of reasons.

Michael Newcomb: First, it can be difficult to engage both partners in the study. So often, one partner would be interested and the other person would not. It's very difficult scheduling multiple couples into [02:21:30] group sessions. And so sometimes, in trying to work with people's schedules, couples would be lost to follow-up because we just couldn't place them into a cohort. Also, we found that there were a number of folks who were in partnerships with transgender and non-binary folks, which according to our eligibility criteria were not eligible for the study.

Michael Newcomb: We also found changing eligibility over time, given the fact that there was an extended period [02:22:00] between initial eligibility screening and actually taking the baseline survey. More specifically, HIV risk behavior would sometimes change from eligibility screening to the full baseline survey, which would then make them ineligible for the study. We also observed a fair amount of couples dissolving their relationships between the eligibility screening and

randomization. So for some of those couples who were entering into this and in some amount of distress. [02:22:30] And we currently have a grant pending with dyad, that I'll talk about at the very end, that aims to address all five of these issues.

Michael Newcomb: In terms of study progress to date, as I mentioned, we have enrolled and randomized all 200 dyads or 400 participants. The enrollment period was March 2017 through December 2020, with a bit of a pause on it, the enrollments during the initial stages of the COVID-19 pandemic. [02:23:00] All participants were recruited online predominantly through social media advertisements, and as well as referrals from other colleagues who were recruiting participants online for different types of studies. And here's where the payoff, I think is, really most highly illustrated is that although it's very difficult to get couples to the randomization point, once you do, there's incredibly high retention at follow-up.

Michael Newcomb: So although [02:23:30] we're continuing to follow-up with our couples across the 12 month follow-up period, we currently have a 96% retention of our participants at six months and a 91% retention at 12 months. Also, in terms of other indicators of progress, we've published four manuscripts with three more under review, all use a baseline data from this fairly large sample of dyads. In terms of demographics in the sample, there [02:24:00] is a slight majority in the sample of our participants being non-Hispanic white. We also have fairly large representation of Latinx participants, and somewhat lower representation than we had hoped for black and African-American participants, which is the third challenge that I'll note here.

Michael Newcomb: This is for a couple of reasons. First, during the final months of our anticipated enrollment, the COVID pandemic hit. And this was when we were really [02:24:30] hoping to focus all of our enrollments on people of color. The other thing that's important to note is that our other study of couples that's focused just in Chicago has a much higher representation of black participants. And I think one of the main reasons for this is because with us being based in Chicago, we have a lot closer ties to community-based organizations in the Chicago land area that serve to legitimize our research [02:25:00] enterprise in Chicago. And so we hope in the future to be able to build stronger partnerships with national organizations, working with people of color in order to improve enrollment.

Michael Newcomb: Other aspects of demographics important to note is that just about 20% of our dyads had at least one partner who is known HIV positive, and it was a majority gay identified sample. Also, interesting to note here, that more than 10% of our sample [02:25:30] was never tested for HIV in their lifetime, which is interesting given that our mean age, even when you take out partners who are age 30 and above, is about 25 years of age. We also have really good distribution across the United States, except for the Plain States. And so this shows where our couples are coming from, and strong representation across the index of relative rurality, which includes both rural and suburban MSM, which are highly [02:26:00] underrepresented in research, men who have sex with men.

Michael Newcomb: The largest proportion of our sample came from the South, followed by the West. You might anticipate we would have a much larger proportion from Illinois, but as I mentioned before, we also have a study ongoing with couples in Chicago. So all Chicago-based couples of which there are currently 127 are shunted into that study. I'd be remiss if I didn't talk briefly about [02:26:30] substance use at baseline. So we have a fair amount of heavy substance use. About a quarter of our sample in the moderate to high range for risk of alcohol use disorder, the same for risk of cannabis use disorder, and about a quarter reporting use of some other illicit drug in the last 30 days. The primary substances of use here are cocaine, ecstasy and methamphetamine.

Michael Newcomb: Substance use is also a very common theme that came up in sessions as impacting both communication [02:27:00] within partnerships and sexual health. And the research questions, once we gather all of our data around substance use, are what is the impact of this intervention on alcohol and drug use in general? As well as, how does substance use impact the efficacy of the intervention HIV risk reduction? In the interest of time, I'm going to skip over the impact of COVID on this project. But as you can imagine, it did delay us a bit. [02:27:30] And I want to focus on our interim trial analysis.

Michael Newcomb: So we conducted an interim analysis of the active condition only, about nine months ago in order to support a grant submission. And the findings are very promising. So we see in 12 months, there's a significant reduction in STI infection, that's chlamydia and gonorrhea infection, a significant reduction in the number of condomless anal sex partners, a significant increase in PrEP [02:28:00] use, as well as amongst PrEP users, 100% PrEP persistence at 12 month follow-up. And we also see that certain aspects of relationship health also significantly improve here, specifically the use of positive communication skills.

Michael Newcomb: And the next steps for 2GETHER, I mentioned that we currently have a grant pending with NIDA. We submitted the grant for the D-LITE initiative, or the Digital Limited Interaction Trials and Epidemiology [02:28:30] initiative that are large scale digital clinical trials testing the effects of digitally delivered HIV prevention interventions on HIV incidence. Now, we propose a hybrid type one implementation effectiveness trial of 2GETHER, received an impact score of 30. And we're cautiously optimistic about funding of this award in 2021.

Michael Newcomb: And as I mentioned, this proposal was specifically informed by the DP2, as specifically designed [02:29:00] to address the various barriers that I noted throughout this presentation. So in this project, we aim to eliminate group sessions and harden the didactic content into an automated E-health program in order to get more couples in the door and reduce loss to follow-up, to enroll both dyads and individuals, so allowing individuals who learn relationships to enroll as well as single people to enroll in anticipation of future relationships, [02:29:30] enrolling dyads or enrolled dyad people who do come in as a couple will still receive skills coaching sessions in order to evaluate their additive benefit over and above the automated program.

Michael Newcomb: We're adapting the interventions to the needs of transgender MSM, and non-binary folks assigned male at birth, who partner with cis-gender men. And we're more comprehensively engaging with organizations across the US to better reach MSM of color and black MSM in particular. [02:30:00] Thank you very much for your time. And I will end there.

Vasundhara Vart...: Thank you, Michael. Great talk. And congratulations on your R01 in the queue, I guess.

Michael Newcomb: Hopefully. Yeah, yeah. Still, it's pending. Pending U01. So yes, I'm cautiously [02:30:30] optimistic. But can't say anything for certain.

Vasundhara Vart...: But we keep our fingers crossed. So let's see if we have any questions come through here in the chat.

Redonna Chandle...: So while you're waiting for questions, I have one comment. So if you become a part of that NIAD cooperative agreement, make sure that you continue to have your collaborative partners look at substance use, and remind them of the important role that substance [02:31:00] use plays in achieving virological suppression as well as in prevention.

Michael Newcomb: Yeah. Yeah. It's absolutely been a central theme that we've encountered throughout this intervention. And I didn't mention, we're also doing HIV testing as part of the interventions. So during the intervention, they receive HIV testing and also a 12-month follow-up. Actually, our last couple, both of them tested positive [02:31:30] for the first time in the study. And just as a reminder of the fact that these data on transmission relationships are not outdated. And I think almost every case in which somebody tested positive, substance use or alcohol use was a major theme that those folks were struggling with. So very relevant to NIDA.

Redonna Chandle...: [02:32:00] And also as, we remind NIAID, very relevant to NIAID.

Michael Newcomb: Yes. That's also yes.

Redonna Chandle...: I'm sure Nora is laughing at me by now.

Vasundhara Vart...: They also co-fund several initiatives with NIAID. This is for general audience also, Michael. So we are partnering on the LITE initiative.

Michael Newcomb: Yeah. [crosstalk 02:32:28] I'm excited. [02:32:30] The scale of the D-LITE projects are larger than most behavioral interventions are, and that's designed purposely to be able to detect effects on HIV incidents. But it also has a lot of implications for substance use, that sometimes we're limited in our behavioral trials in the ability to detect effects on the use of certain substances because of power issues. And in this case, with such a large sample, there's a lot of opportunity [02:33:00] to look at moderation effects of specific substances of

use on intervention effects, to look at the ability to reduce the use of those substances, both in individuals and dyads. So it's exciting to be able to collect data at that level.

Vasundhara Vart...: Yeah. Well, if you have any questions for Dr. Newcomb, please reach out to him. So I think we [02:33:30] will just now move on to the final talk in the series. Thank you again, Dr. Newcomb.

Michael Newcomb: Thank you.

Vasundhara Vart...: Redonna?

Redonna Chandle...: And the final presenter is Dr. Zachary Klase from Drexel University. So, take it away.

Zachary Klase: Hello, and thank you. All right. So, how about we just move some stuff out of the way. So what I wanted to start with today [02:34:00] really is a thank you to the grant, sort of an overview of some of the things we've gotten from this. Publications as a measure of output, I think is pretty important. Five publications came directly from this project. I'm going to tell you about pieces of about three of them here. And then of course, the number of collaborative efforts that might not be directly related to the grant, but the capabilities we built for the grant lent themselves to helping others. And of course, the stability [02:34:30] of longterm funding. It makes it a little easier to say, yes, I'll help you with that project.

Zachary Klase: The most important thing for me, in my mindset though, is training. And the grant here got us to train two post-doctoral fellows who went off and got the careers they wanted to do. We minted three PhDs, an MS student. I have two wonderful graduate students still in my lab. And because of my previous position, where I started this grant, it was a primarily undergraduate institution, we got to bring 12 undergraduates into [02:35:00] the lab and show them a lot of molecular biology of HIV. And then also, grew a number of collaborations with people around Philadelphia and around the country.

Zachary Klase: Most maybe pressing to me, got a promotion out of this from assistant professor to associate professor because of the work done here. And then something I'll give you a little bit more info about later is we actually moved the research lab from University of the Sciences to the Drexel University College of Medicine. I'll tell you more about that later. [02:35:30] For now, I'll just say that if you've never moved a research group in the middle of a worldwide pandemic, you are missing an experience.

Zachary Klase: So, what was great about this mechanism is you come in and you don't have aims. You just present to the study section. Here are the big problems that I think I see in the world, and here's how I think I can deal with them. Excuse me. So this is actually a figure that I showed to the study session when I sought the

grant in the first place, [02:36:00] and it's talking about the idea that we have HIV is a disease and all the things that come with that. We have substance use, and we understand that a large number of HIV positive individuals abuse substances, so that worsens the disease.

Zachary Klase: But that in my view, we also need to think about attempts at therapy and HIV cure as another overlap in the terms of problems that we're having here. And the red places were things that I was particularly interested in, and the keys were places that my lab was already making inroads into these problems. [02:36:30] What I'm going to talk to you about today, because obviously this is a very big problem still, is the issue of benzodiazepines as a substance of abuse and how they might fit into HIV cure. And that actually relates to the mechanisms of latency and persistence of HIV.

Zachary Klase: So when we started this project, what we knew was from a paper that I published just as I founded my lab, six, almost seven years ago now, in which we had identified this RUNX1 protein, [02:37:00] which is an important T cell specific transcription factor that actually inhibits transcription of HIV. And therefore, we thought might have a role in latency and persistence of the virus. And we were able to identify both the HIV promoter as the blue bars here, are responsive to when you put more RUNX into the system. You're going to see a suppression of transcription.

Zachary Klase: And we were able to map one particular site, site two up here on sort of the end of the LTR, as a real RUNX binding site [02:37:30] because when we mutated it, the effect goes away. That in itself is just nitty gritty and molecular. What was most interesting is we got a drug out of it. Our collaborator, Dr. Liu was interested in RUNX inhibitors because they're useful, possibly for treating AML leukemia. And he had identified this compound out of [ROS 02:37:48] library Ro53335. And Ro5 on its own wasn't too exciting in terms of activating the HIV promoter, but we saw when we mixed it with SAHA or vorinostat, which is another sort of leading [02:38:00] compound in the quest to reactivate and clear HIV infection, we got this synergistic activation. And that was very exciting.

Zachary Klase: So the Avenir then really started with work from my first PhD student, Weam. And she looked at the structure of R05, and she says, "Well, that's a benzodiazepine." This is a class of compound that might be some of the most well-studied compounds in the chemical library. Benzodiazepines have been in the clinic for 60, 70 years now. They're pretty [02:38:30] well-studied. Why don't we go and look at some of these clinically used benzodiazepines, things like Xanax, which is Alprazolam, and Valium, which is Diazepam, and see what they do in the system? And so she went back to the original assay and whether or not we could activate the HIV promoter and kick cells out of the sleep state using all of these different benzodiazepines.

Zachary Klase: The orange bars here are the synergies with SAHA. SAHA alone being here, so maybe the surprising thing was that every single one of the benzos [02:39:00] that we tested appeared to have some functionality in increasing SAHA activity,

which in itself was exciting. Like okay, maybe we can improve this other drug. But then when you look at Alprazolam and Diazepam here, the blue line is the drug alone. So they're having this great effect on the HIV transcription without SAHAs. They're doing it all by themselves, which is really exciting as a possible intervention and therapy, because these things are already in the clinic. And this works across multiple [02:39:30] dose ranges, even down into the lower end of the clinically used range with the drug, which is very exciting to us.

Zachary Klase: So at this point I should catch us up, because I think this talk is completely different than what we've been hearing so far, and remind everyone that HIV is really dependent and has a real importance in the chromatin state, in the epigenetics of the cell. And what I mean by that is we've got DNA [02:40:00] in our cell, and that DNA is packaged in an organized fashion into new

PART 5 OF 6 ENDS [02:40:04]

Zachary Klase: ... AR cell, and the DNA is packaged in an organized fashion into nucleosomes. That's DNA wrapped around a set of histone proteins. That's the basic structure that makes up the chromosome and helps regulate function in the nucleus. And when you look at a nucleosome, I have one over here on the right, access to this DNA and transcriptional activity of that DNA is controlled by how tight this interaction is between the histone proteins and the DNA, and by what you recruit to these histone tails [02:40:30] sticking out the side. That's what's really the basis of controlling all gene expression in the cell. And because HIV integrates itself into our genome, it's at the mercy of this process as well. So we wanted to know, in the latent state, this virus that we're trying to clear out, what is it that alprazolam and these benzos are doing to the epigenetics and how does that figure into transcriptional control?

Zachary Klase: And then the next star of our presentation becomes Angel, one of my current PhD students. [02:41:00] I think she's actually got a benzodiazepine on her shirt here. She used chromatin immunoprecipitation assay's to look at these epigenetic interactions. She started with this model of, here we have DNA wrapped around nucleosomes, and in a transcriptionally repressed state, like when HIV is off, there should be transcriptional repressors and there should be methylation of the core histones. And then RUNX figures into this because we think it sits up at the end of the HIV promoter and helps drive all of this. [02:41:30] The prediction being that when we inhibit RUNX function, that we should switch to an active state, we should see acetylation of histones and recruitment of transcriptional activators.

Zachary Klase: So Angel started by looking at acetylation of histones. And maybe not surprisingly the three benzos we looked at, our original Ro5 compound, alprazolam and clonazepam, they don't do anything to acetylation. That's really, probably not too strange. That's not what they're supposed to be doing. The histone deacetylase inhibitor, SAHA, that does increase acetylation, but it gets interesting. [02:42:00] And then alprazolam, our winning drug so far, increases that acetylation to a greater degree. So why is that?

Zachary Klase: Well, the literature told us that there's a connection between the RUNX1 protein and the STAT proteins, especially STAT5, which has been shown to be critical for HIV transcription. So we wanted to look at STAT5, with the idea that when RUNX isn't there that STAT5 recruitment would be increased. And sure enough, now we start to understand what it is that alprazolam does and why it's different than these other two benzos that we tested. Alprazolam alone [02:42:30] can help recruit STAT5 to the HIV promoter, presumably through kicking RUNX out and allowing STAT5 to come in, and then that's even better in the presence of the histone deacetylase inhibitor. STAT5 then, and you can tell this as sort of a complex layer of Russian dolls as it will, then recruits CBP/P300, this is a histone acetyltransferase driving some of these assimilation events down here. We see that alprazolam also increases that, so we think alprazolam [02:43:00] is inhibiting RUNX, which allows the recruitment of STAT5 which brings in CBP/P300, and that explains the increase in acetylation that we see up here.

Zachary Klase: Now, Angel then asked a really good question. She says, "Okay, we've been looking at HIV, the promoter specifically, but we've just shown that this prescribed medication also has an effect on STAT5, is that just specific to the HIV promoter or is this something that's more broadly happening in cells [02:43:30] that are exposed to alprazolam?" So she took cells that don't have the benzodiazepine receptor. I think that's key because that means the effect we're looking at here is something other than the classic effect of the drugs. She treated them with our three benzos. She did a western blot looking at STAT5 levels and they don't change. But then she looked at the levels of phosphorylated STAT5, which is the activated form of the protein, and she sees that alprazolam but not the two other benzos we tried increases phosphorylation. [02:44:00] She then did a follow-up experiment where we could track phosphorylation of STAT5 using a phospho-tag, which retards movement with protein through the gel. So as you get movement of this protein further up the gel into these sort of smears on the right, that's increasing phosphorylation. And we see a nice dose dependent effect of alprazolam on phosphorylation of STAT5. So it does seem that alprazolam's having a broad effect outside of what we see with the HIV promoter, sort of suggesting [02:44:30] that it could be doing other things in the body even outside of HIV patients. So maybe this is a good point to remind everyone what that is, right?

Zachary Klase: So JAK STAT signaling, signaling through Janus Kinases and phosphorylation of STATs is the signaling step in response to nearly all of the cytokines and interleukins. And in case of many of the really important ones, STAT5 is the major signaling protein after you get receptor engagement. Which means that [02:45:00] STAT5 is helping to control the immune response because we're signaling to the immune cells into the body using cytokines that signals through STAT5. STAT5 engagement and activation then leads STAT5 to go to the nucleus and turn on a lot of these things as well. There's sort of a feed forward loop on things like IL-2. That is because STAT5 is pictured down here at the bottom. STAT5 is an adapter that's actually helping to recruit a number of epigenetic modifying proteins. I talked about [02:45:30] CBP/P300 as it relates to HIV and

driving acetylation of things, but it also recruits histone deacetylases and methyltransferases, meaning that STAT5 itself is something that's altering the epigenetic landscape. And if we're turning on STAT5, it's going to affect, potentially, the expression of a lot of genes, and sort of near and dear to my heart, the whole inflammatory process as well.

Zachary Klase: So to look at this directly, we found a cell line reporter system that's used for measuring IL- [02:46:00] 2 expression. It does that because it has an IL-2 receptor on the surface, which activates the JAK Kinases, which activate STAT5 and turns on the reporter. So we wanted to use this to see if we could just sort of skip the IL-2 step and directly activate STAT5, and really prove that alprazolam is having an effect on STAT5 directly. So we did that. Our positive control is IL-2, but you can see a nice dose dependent response to alprazolam, really driving home the point that [02:46:30] we're acting on STAT5.

Zachary Klase: Now, at this point, you might start to go, "Wait, you're telling me that alprazolam could be a stand in for IL-2, do we need to worry about this therapeutically?" I'll point out that these low bars down here are what are in the sort of therapeutic range of the drug. So, no, I don't think we're going to get replacement of IL-2 effect in a human being taking alprazolam, but we wanted to check that of course because T-cell activation is something that's, both a bad outcome [02:47:00] for a patient, and important for knowing the activation state of things like HIV.

Zachary Klase: I won't walk you through this whole figure, but we did pretty extensive testing looking at T-cell activation response to all the agents we'd been using to mess with HIV transcription. And the answer is that as you would expect from something that's been used for a long time and is pretty safe, alprazolam isn't activating T-cells. But the idea that it could be helping STAT5 activity really led to [02:47:30] the question of whether or not this might be potentiating immune response in a proper setting. Like, could alprazolam be immuno protective, could it be something that would ... when the T-cell was going to respond anyway, could it actually help and give a better outcome?

Zachary Klase: So we wanted to look at that. One of the ways we have been looking at that is by looking at intracellular cytokine expression in CD8 cells. This is the cytotoxic T lymphocyte response, which is critical to clearing viral infection, and we think would be one of the [02:48:00] things that we would need to actually clear an HIV infection if we were going to do this reactivation strategy. So we took white cells, immune cells from HIV patients who had been suppressed on therapy for at least six months. We exposed them to antigens. So Gag peptides, the major structural protein of HIV, and treated them with drugs to block release of cytokines so we could measure their intercellular levels. This is just showing you an example flow plot of how we get this out [02:48:30] and what we expect to see in terms of interferon response within CD8 T-cells.

Zachary Klase: Now, it's still early days. We're working on increasing our N up to another five or six patients on top of this. But I'd say that the results from our first three in our

pilot are pretty good. We compared alprazolam to the histone deacetylase inhibitor, SAHA, to vorinostat. The reason there is this is a drug that people have been wanting to use to reactivate HIV, but it hasn't been successful yet. [02:49:00] And going back probably a decade, worked for Bruce Walker's group, showed that that's because histone deacetylase inhibitors actually suppress the cytokine response in immune cells and that would be bad as a curative strategy.

Zachary Klase: The statistics aren't there yet, but the trend certainly is going in the right direction. Supporting this idea that SAHA is decreasing the level of interferon expression in response to HIV antigen. What's great is alprazolam is actually higher than that. It looks like it might actually be sort of trending towards higher [02:49:30] than the control as well, which would support that idea that maybe this would be a way to improve immune response when you need that to happen. The great thing, if we could ever get there in terms of statistical power, would be to show that you could use these two together and then alprazolam might reverse some of the bad effects of SAHA.

Zachary Klase: We also looked at IL-2 expression, because IL-2 dysregulation is another part of the problem with histone deacetylase inhibitors, and as have been reported by others, we see this increase of IL-2 expression [02:50:00] when we use SAHA, and we don't see that with alprazolam. So again, I think things are coming up roses. I'm hoping this will be interesting as we recruit more patients and find more samples to work with.

Zachary Klase: Now, in the last couple minutes remaining here, I want to give you some insight into why I think this is important in a bigger sense. Benzodiazepines are ... I mean, they're a therapy. We prescribe them to people. [02:50:30] We prescribe them to something like 75 million people in the US every year, which is a fairly large number of people. Depending on the survey and data you look at, about 10% of the US population has abused a benzodiazepine, and maybe 1% actually qualified for dependence. So this is an abused substance. And all the problems that go along with abused substances are seen with the benzos as well, including increased risk of HIV seroconversion. And I believe something like 30% of opioid overdoses actually have benzos involved in [02:51:00] them as well. So it is a problematic chemical and an abuse issue.

Zachary Klase: The data I just showed you would suggest that benzodiazepines, in addition to their effect on the CNS, are actually driving epigenetic change, which, especially if you think about it in the terms of the CNS, means they're driving pretty permanent changes in the cells that are exposed to them. We wanted to come up with a way to look at that, both for benzos and other abused substances. So a former postdoc of mine, while he was with me, Luca, who's [02:51:30] now a senior scientist in the HIV discovery program at Merck, came up with this assay wherein we take the nucleus out of a whole cell. We strip away the nuclear membrane, and that leaves us with something that in my mind looks like those wicker balls you see in hotel lobbies. In that we have this cage that contains the nuclear components within it, but now has large openings in it. What that allows us to do is we can then just use a fluorochrome labeled antibodies

[02:52:00] to do live staining. So we can look at nuclear architecture without the need for fixation and actually follow changes over time.

Zachary Klase: This has allowed us to take what I think are some actually pretty stunning sets of images showing nuclear architecture in the cell, and allow us to compare that to what has been shown and known before in the literature, and identify where areas of active transcription are and look at actual changes in structure and not just amounts [02:52:30] or a location like you might see with ChIP or a western blot.

Zachary Klase: It's interesting also because we can use this to do live imaging. I told you that this nucleus isn't fixed, so we can actually watch changes over time in response to things we do to this nucleus. And as an example of that, here is a nucleus that we've stained for histone acetylation. So all these bright spots you see are presumably areas of active transcription. And I'm going to show you four hours of time compressed down to [02:53:00] 30, 40 seconds. One hour in we're going to add the histone deacetylase inhibitor, SAHA, and you can actually watch what this does to acetylation in this nucleus.

Zachary Klase: So here we are. We add the SAHA to it. You can see that it gets brighter as the level of acetylation goes up. And then there's actually a reorganization of the acetylation. So it actually moves to the periphery of the nucleus. This fits with ideas on transcription, with the idea of where you would see the most active transcription. [02:53:30] Maybe the easiest way to think about that is if you transcribe something, you want to pass it out at the nucleus, so you do it sort of close to the edge. But we can see these changes happening over time.

Zachary Klase: We've started to apply this to the issues of substance abuse. What I'm showing you here are astrocytes that we've exposed either to SAHA or to morphine as our standard opiate, or the combination of the two. Maybe the real interesting part of this is, if you just look down at the bottom, the green staining is showing you histone [02:54:00] acetylation, marker gene activation. When you treat with the histone deacetylase inhibitor, the level of acetylation goes up. That's what SAHA does. Morphine, you don't get a huge change, but you do see some sort of active areas of transcription at the edge of the nucleus, suggesting that, yes, morphine is turning on gene expression. Most interesting to this, relevant to HIV cure strategies, is it seems like morphine treated cells are resistant to the SAHA effect, which really gives us something [02:54:30] to think about. If we're saying that we're going to use histone deacetylase inhibitors as part of a cure strategy, they might not work in the high percentage of people that are currently using opioids.

Zachary Klase: We've been following up on this opioid issue in both looking ... trying to model over time, we'll get chronic exposure to morphine, and also look across the doses. What we've seen is that it's actually the smallest amount of morphine that drives the largest amount of change. So I've got five nanomole over here on the right, as opposed to half micromolar [02:55:00] here. That's where we see the greatest increase in acetylation. And we see there's a really unique

architectural change as well, where the acetylation goes from being sort of diffused throughout the nucleus, to being lined up at the edge and also very perinucleolar. Which, for those of you in the crowd that love epigenetics in the way I do, you're going to realize that that's strange. Perinucleolar chromatin is thought to be the stuff that should be off. That should be heterochromatin that's kept in the middle. [02:55:30] So not only do we see aberrant acetylation of histones, we see an aberrant placement of those acetylated histones, really suggesting that something strange is good going on here.

Zachary Klase: So, putting this all together, for HIV cure I think we should be looking at RUNX as a way to improve HDAC inhibitors. For the substance abuse side and the interesting in what we're doing to patients as we prescribe things, we need to realize that alprazolam is probably [02:56:00] a RUNX inhibitor and is modulating things like STAT5 and possibly immune response as a result of that. Some things that I didn't show you is that this also actually moves the placement of the nucleosomes on the DNA, which is great. And then the big takeaway being ... that I think we all appreciate the drugs of abuse are a confounding factor in HIV as a disease, but they're also going to be a confounding factor in cure and we should be paying attention.

Zachary Klase: The last thing, just to thank the program, is [02:56:30] what's great about this is the flexibility of not having to chase down the aims you promise to do. So here's what we're going to do in our final year. This is just a scheme showing that we can isolate useful CNS cells from flash frozen CNS tissue. So we have a request in with the NMTC to get human brain tissue from HIV patients, either with or without opiate abuse. We're going to use microscopy to come up with a big atlas of epigenetic architecture. We still have a pie in [02:57:00] the sky thing ongoing, where we're trying to come up with a way to visualize specific genes, i.e., the integrated HIV. I think our winner right now is to use a CRISPR-Cas based system. We're kind of putting these together in a piece-wise fashion. That would allow us to map where HIV is on top of our nuclear imaging. And then the newest collaboration coming out of some work with Peter Gaskill is to move this transcriptional control work, the most of which is done in T-cells, into myeloid cells that really are important for [02:57:30] CNS infection.

Zachary Klase: That collaboration with Dr. Gaskill is one of the things that came out of my opportunity of moving the lab to the Drexel University College of Medicine. Because the Pharm/Phys department at Drexel really has this, I'd say, small but mighty core of NEDA funded researchers and a really large amount of expertise coming out of our chair, Dr. Meucci, and PJ, who I just mentioned. Not to downplay their expertise and their strengths, but I want to highlight Dr. Jackie Barker down here as [02:58:00] a recent DP2 recipient, who hopefully we'll be hearing from in this format in years to come. Also, want to, of course, thank my lab, the large number of people we've trained from this, and you for listening to me. I'd be happy to answer whatever questions may have come up.

Vasundhara Vart...: Thank you very much, Dr. Klase. That was really interesting talk. [02:58:30] I think it will be cool to do some [inaudible 02:58:34] work that you are planning

to do, to get some contextual data in terms of what types of cell types that are ... Do you see effects that are in a cell type specific manner? Because the data you showed is with astrocytes. So I would be really curious to see what happens with microbia and monocytes.

Zachary Klase: So [02:59:00] would I. We've been slowly building up to make sure that we're going to do this right, sort of because of the preciousness of the samples that we're asking for. So overcoming the hurdle of getting the correct cell types out was a big one. We've been working with some of the computational people that I have upstairs at Drexel. And we now have a way that we think ... One of the limitations with the microscopy, of course, is you're looking at one cell at a time. We've taken test sets of images and fed them to [02:59:30] neural nets. The computer can identify the same differences that we thought were there and confirm them across 800 nuclei or something.

Zachary Klase: So the idea is going to be to scan in huge amounts of data, and then in a hands-off way, allow the computer to tell us what the differences in shape are. I think that's really the only way we're going to be able to accomplish this. Because, we need to have a high end to account for human variability, all the different cell types, the various conditions we want to get, and then [03:00:00] to really get to the data. I'm very excited and I'm glad we have a year left to get it done.

Vasundhara Vart...: Yeah. I mean, please do look at other night opportunities. It's interesting work. We want to keep you going. Congratulations on that. And then let me look at what questions we have coming in. There's John. Can we unmute John? Maybe he can ask the question. [03:00:30] So he's asking, "What do you think is going on with the perinucleolar staining? Because it is ..."

Zachary Klase: I don't know. I'm trying not to get too excited about it because that's acute exposure in tissue culture and I think the images there are U87s, so they're sort of a much maligned astrocyte model. We haven't done that yet in the primary astrocytes we've been working with. So I'm [03:01:00] waiting to see if it holds up in real samples before I get too excited. That being said, I don't know. What I can glean from the mapping we've done so far is that there's these established regions of the nucleus where you expect to see certain modifications, and we see that. I think, naively, based on how we do ChIP assays, you sort of expect, "Okay, when I see acetylation, I expect to see the [03:01:30] acetyltransferase with it, because that's what put it there."

Zachary Klase: In the architecture, that's not necessarily true. It seems like the enzymes that are responsible for the modifications are actually held in the other compartment. So you have an inactive compartment, the acetyltransferases are there so they can acetylate the histones, and then they get shuttled out to the other active compartment. So, perhaps what's happening there is a deficit in the transit of the genes and the rearrangement after you get the [03:02:00] change in the modification. But that is an extreme guess.

Vasundhara Vart...: Also have a question from Dr. Volkow. She's asking if ... Okay, I hope I can pronounce this right. If [inaudible 03:02:16] effect not mediated by the benzodiazapine receptor, have you identified the target?

Zachary Klase: Right. Yes. And then I see a follow-up question about the naloxone sensitivity. We haven't [03:02:30] done naloxone yet. What we have done is all the epigenetic work has just been done in cells that shouldn't be responsive to benzos in the classical way. So I'm fairly certain that the effect we're seeing has nothing to do with the classical anti-anxiety effects and signaling through receptors. That implies then that you're getting entry into the cell. That's been established for Ro5 I think fairly well. We have started to do some mapping with [03:03:00] our wonderful chemistry department downstairs. I didn't show them, I didn't have the time and they're a little bit beyond my ability to explain quickly. But we've done computational mapping and there is a binding pocket on the RUNX1 protein that accepts the benzodiazepines.

Zachary Klase: One of the things that's probably setting alprazolam and diazepam apart from all the other ones as a class, is there's a second binding site on the other face of the molecule that appears to accept only them. I think we'll find out ... We have [03:03:30] to actually map out the residues, but I think we'll find out that that's the face of RUNX that's important for the STAT5 interaction, and that the drug is acting sort of directly on that complex.

Vasundhara Vart...: We have one more question from Dr. Valentino. Are morphine affects naloxone sensitive?

Zachary Klase: Oh, the morphine effects on acetylation. That we have not done yet. I [03:04:00] think you're going to find that they are ... There's some hint that SAHA and morphine both have an effect on the same drug transporter. I know some of the early work on SAHA, they had to reformulate it to get it to cross the CNS because it was shooting itself in the foot in terms of drug pumps that were getting it in. I think that's probably what's happening. We haven't done the test to see if naloxone would change it.

Vasundhara Vart...: I think [03:04:30] Rita also has a subsequent question. Can we unmute? Maybe Rita can ask. Go ahead, Rita.

Rita: I'm unmuted. Okay. I think you answered that question. It was, if alprazolam isn't producing its effects on STAT through the benzodiazepine receptor, because you did it in the cells that didn't have the receptor, what protein [03:05:00] is it interacting with? I think you addressed that question.

Zachary Klase: Yeah. I will say, I think it's still a useful experiment. I mean, the T-cells likely have the peripheral receptors for benzos, right?

Rita: Yeah.

Zachary Klase: So it could be an additive effect.

Rita: Right. Thank you. A great talk. Thanks.

Redonna Chandle...: So thank you all. Thank everyone for all of your wonderful presentations. The strength [03:05:30] of your science is demonstrated in the fact that while Dr. Volkow had a very busy schedule, she stayed for the entire half a day and was participating in most of these discussion. So Nora, let me let you close out the meeting. I'm thrilled that you were able to be here. It just speaks to the quality of your all's presentations, and the quality and innovation of your science.

Nora Volkow: [03:06:00] Yeah. No, no, I was supposed to be in two meetings today, apart from this one. I didn't exaggerate when I say that they had triple scheduled me, but I just couldn't stop watching the science. I think that's really the excitement where we want to generate the research that's so unexpected, and yet that gives us clues of how things work with one another. So I really was mesmerized by the talks [03:06:30] that I heard today. I want to congratulate all of the presenters. I also was fascinated by the diversity of the topics and the creativity and innovation in each one of them. So, thanks to also for you and Kavi for overseeing these really amazing program on the Avenir. The Avenir, we chose the title Avenir because these are the [03:07:00] stars of the future. And I would sort of say, they are already the stars of the present. So, it's wonderful to see.

Vasundhara Vart...: Thank you, Nora.

Redonna Chandle...: I was actually in the room, many years ago, when the lights went on in your eyes. We were talking about something and you said, "Let's do an Avenir to go with Avant-Garde." And spoke about the title and what it would mean. So it's a real privilege to be able [03:07:30] to be here now at this juncture and work with Kavi on this program and see it come to fruition. We will be doing a similar half day for our Avant-Garde awardees that will be taking place in late April. We'll be sending out a lot of mass communications around that. So we invite you all. Everyone who's participated today to participate in that presentation as well. And again, if you have any questions about applying [03:08:00] for future funding, please reach out to program or reach out to us. We want to maintain all of your really important high priority work. You're just doing amazing things. So thank you, everyone.

Vasundhara Vart...: Thank you.

Nora Volkow: Bye-bye.

PART 6 OF 6 ENDS [03:08:17]