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TRANSCRIPT - GR 11 17 23 "Immune System and Cardiovascular Disease" guest speaker Coleen McNamara, MD from University of Virginia

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- Welcome to medicine, grand rounds. It's my pleasure to introduce our speaker today. Dr. Colleen Mcnamara, Dr. Mcnamara, attended Medical School at the Medical College of Ohio. She then came to Uva for her Residency, Chief Residency and Cardiology Fellowship. And lucky for us she's stayed here since she's currently professor in the division of cardiovascular medicine. She's been incredibly productive and has been busy exploring the role of the immune system in heart and vascular disease.
- Some focuses of her scholarly work include elucidating the influence of lymphocytes on atherosclerosis characterizing the role of immune checkpoint molecules in cardiovascular disease. And using this work to find novel biomarkers and therapeutic targets in humans her lab in the Center for Immunology research at Uva is currently working on translating their innovative discoveries into clinical applications.
- Their work is broad and includes looking at different B-cell subsets in mouse and human atherosclerosis. The correlation between Ig antibodies to alpha-gal and cardiovascular disease, and the role of specific transcription factors in the regulation of atherosclerosis and obesity.
- She's a leader in the field and sits on many national committees and editorial boards. She's also a master collaborator with active collaborations among 16 different institutions in 5 different countries, in addition to the many local collaborations she has here among Uva faculty.
- Please join me in welcoming Dr. Colleen Mcnamara.
- Okay, can everybody hear me? Okay with this, with this microphone? Alright. Great! Well, thanks, John, I mean thanks so much for the invitation to present it medical, grand rounds. I was telling John and Joe that I had their job many moons ago and getting grand round speakers when I was also a chief resident in the Department of Medicine, so I appreciate the invitation. So cause I know there's many people to pick from. So thank you for that. And thanks for the nice introduction.
- As John said, I'm gonna talk about the immune landscape. Excuse me, and coronary artery disease.
- And John picked up on my outline right away. I'm gonna talk about residual risk, a little bit about the clinical aspects of residual risk. But then, also how we're how we and others are approaching it from an immune standpoint. And I'm gonna focus on when we get to the research portion of things. Some of the work that we've done in this in the area of different V cell subsets in atherosclerosis. And then I will touch on our tick troubles. And, as you know, is a is a problem for this area, Virginia and Virginia in general, and I know you recently had. Jeff Wilson here? Speaking about the same topic. So I'm only gonna touch on some of the cardiovascular aspects. And what we're doing in that sector?
- And then I wanna talk some about our interest in precision biomarkers and talk about a project that we're doing in collaboration with Antonio. Abate again just briefly. And then, if time allows, I want to talk about our precision immuno medicine initiative because we had a Town Hall yesterday. In the Department of Medicine, led by Bill Petrie and Susanna Keller, talking about the importance of developing research affinity groups.

- And I think this is a really nice example of a research affinity group, plus, we're, you know, really want you all to know about these resources here at Uva. And if you have any interest in this area, this affinity group, we we really welcome. You know, people to join in.
- Okay, so what's the evidence for residual risk. We know that statins have definitely decreased CAD events, but despite these reduction and reduction in events.
- see if we can whoop might not have my most up to date presentation. But that's okay. So but what I was gonna show you. So let me go back there. Is that when you look at the events not avoided, they're actually greater than the events prevented. So clearly. There's still a lot of work to be done.
- And this slide also really underscores the important role of inflammation and cardiovascular disease. As you can see, plasma level levels of inflammatory bark biomarkers in particular, Hsc Rp, really robustly predict first and recurrent Cv. Events and Jupiter demonstrated Statin benefit even in those with low 8 ldl. So. But as long as they had high. Crp. So again underscoring the importance of inflammation. And then, lastly, more recently, and a paper from Paul Ricker's group really shows that among over 30,000 individuals. Statin treated individuals in a variety of trials. The risk of cardiovascular deaths were high for those with high, so high crps. So these 2 groups here, regardless of whether or not the Ldl levels were elevated. So again, not that Ldl isn't critically important, it certainly is, but there's risk beyond that.
- So how common is this residual inflammatory risk? So these are a couple of statin trials. Prove it and improve it. And you can see in these pie charts, or for both of these trials, are divided into those that still that have residual inflammatory risk defined as Ldl. Less than 70. But the crp is greater than 2. Excuse me. residual cholesterol risk their crps not high, but they still have a high cholesterol, and that's about 13. Both is 14, and neither is 39. So you can see between those that have just inflammatory risk and those that have both an inflammatory and a lipid risk. It's nearly half of the population in these and these clinical trials. So it's pretty common. It's a pretty big issue.
- So that several years back. There was a major trial that you've probably heard quite a bit about from others as well, cause there's been a lot of really important lessons in this trial. And I think the trial was to test the drug. But it was at the same time testing the inflammatory, anti inflammatory hypothesis in humans been a wealth of marine data to really support for that inflammation. And immune cells are very involved in atherosclerosis. Yet you know whether this was really occurred in humans was really poorly understood. So this trial was really critically important.
- They chose as the agent Canicinumap, which is an antibody that blocks IL. One beta proinflammatory Cytokine involved in cardiovascular disease, but also involved in many other diseases.
- Think it'll just there.
- But I'm gonna have to go back to my slide. There we go. So it binds and it activates. I/O one Beta, and has a long half-life so you can get the get dos every 3 months, and the way they organize this trial was that people with stable CAD. But post-mi.
- So a high risk group they'd already had one heart attack, and they were on typical agents that we treat people with after heart attacks. Statin ace inhibitors, beta blockers, aspirin but they had persistent elevation of their crp. So they had inflammation. So that group was randomized to placebo and then 3 different doses of canyonab. And here's the primary endpoints being nonfatal, mi non-fatal stroke and cardiovascular death, secondary and endpoints being total mortality, new onset diabetes, other vascular events, and there were some exploratory endpoints so what cantos actually showed was that there was this 15% reduction in major adverse cardiac events which did achieve statistical significance. But

there was no difference in all cause mortality, and this was thought in part due to an increase in fatal infections. And so the application for approval of this drug for prevention of recurrent mi, despite the fact that the mi's were significantly reduced, was denied.

- But there were other lessons to be learned in this trial, and in particular. This is a an analysis that they did retrospectively, and they looked to see because of the all the data that crp. If you have inflammation as measured by crp, you have increased risk. So it would kind of follow that your Crp level may be important predictor of your response to this therapy. So when they divided the subjects out, based on what their crp was after the first dose of the medication. And here's the different tertiles.
- So there are those, and I'll get back to this in a moment, which is a third of the people getting the drug that actually didn't budge their crp. So in that group, for reasons that are not clear, and I'll circle back to you to kind of. And when I talk about precision medicine, because trying to figure out who's gonna respond and who's not gonna respond is something I think that's really critically important for us.
- But so a third of them getting drug are not really getting their crps down to levels that they need to be at another third are having some reduction. This blue line here, but a reduction that's really only leading to this 17 and just you know. Much more mild statistical significance as compared to those that get into the lower troop tile, which is below 1.2, and then there is significant reduction and cumulative incidence of major adverse cardiac events.
- So again, this analysis really provided further proof of concept of the importance of inflammation in atheroscotic cardiovascular disease, and again highlighted the need for better biomarkers of likelihood, of drug response prior to therapy initiation. Because this was after a single dose of the agent and the these markers prior to therapy were really not very well predictive, and also in highlights, the importance of testing other agents.
- So another one of the agents that were tested around this same time was Methotrexate. So again, in anti inflammatory, used in in autoimmunity, and this study kind of end up telling the same theme, and that is, if you're not bringing down inflammation. So here's what cantos did it did bring down. I/O one beta il 6 crp levels. And you did see this reduction in mace in Cert, where the subjects were, and there were, you know, almost 4,500 were randomized to Methotrexate or Placebo. There was absolutely no difference, but there was also no difference in I/O one beta, I/O, 6 cr. P. And so no reduction in May. So again, really underscoring this importance of inflammation in cardiovascular risk.
- So then investigators turned their attention to culture scene sort of repurposing and old and cheap agents. So there's a lot of rationale for doing that, and this is called the Cold Cut Study. And so they randomized almost 5,000 for so 47, 45 post mi subjects to point 5 milligrams a day of colchicine or placebo, and again followed them for similar amounts of time is the other 2 trials so kind of shy of 4 years and the end points being stroke, urgent hospitalization or angina in this particular graph. But a lot of times. It's other cardiovascular events like MI. And stroke. And you can see they did get a statistically significant reduction and risk with the culture scene. Having said that there's still risk, isn't there? So? And this is in subjects, post I. But what about the bigger population?
- And I'll get to that in a moment. So again, I just want to underscore again with the colchicine. There was a reduction in complement. Reduction in I/O one beta decreased expression of endothelial selections, inflammatory markers decreased leukocytes, fibrosis, neutrophils and decrease hscrp. So and again, and we have a response. So linking inflammation reduction to response in this post. Mi cohort.
- So one of the issues related to Colchesene is that it does have a long half-life and a narrow therapeutic window. So you really have to be careful using it. And people who have

renal dysfunction. And also you have to be careful about drug interactions because of the way it's metabolized so, but really should be thought of as added risk reduction agents in this population.

- So then, in looking at a broader population in the low doko, too, so low dose colchicine study was patients with chronic coronary disease, and again about the same numbers followed, for you know this time about 5 years, and, as you can see, there actually is a statistically significant reduction in cumulative incidence of events in the colchicine group.
- So this was a positive study as well.
- So one of the caveats is that, despite the positive result, the study did show a trend towards increased risk of death from non cardiovascular causes. And that was just sort of a broad category. And so use really should be individualized decision depending on other clinical factors. But it's in the armamentarium cause you can see in both the colcot and this low doko that it does reduce inflammation and can reduce events, and those that still have residual inflammatory risk.
- So this is from kind of a more recent publication by Paul Richards Group, and is kind of putting forward. So how do you manage people in 2023? So particularly the largest group of patients we have, which is chronic chronic stable atherotic disease.
- So if they've ha! Already had high intensity statin therapy, then and you're worried about their risk, then assess their risk, and if they fall into this category of having elevated lipids still, but not a high crp. Then be thinking about additional cholesterol lowering agents.
- If they have elevated cholesterol and elevated crp, you would think about dual pathway of residual risk and consider, perhaps adding both cholesterol, lowering and anti inflammatory. And in this group over here, if their lipids are low in their crp is high. This is the residual inflammatory risk group, and you really would want to consider adding colchicing at this at this juncture and you can see when they graph it. They're putting up here many studies that you're familiar with using, adding on Xitamide to high intensity, statin therapy, and other agents, and showing the relative risk reduction relative to the relative risk reduction with culture scene, and you can see the culture scene fares quite well so is colchicing the only option. And so this is where I'm going to segue into more sort of future directions, that of things that are in clinical trials, and that you might be seeing coming down the pipeline with time.
- So this is a cartoon from a fairly recent review that was in in Jack talking about this new era of immunotherapy for immunomodulatory therapy for cardiovascular disease. So this is the vessel wall. And we talked about canna map. We talked about Colchescene Anacinra Block, CIO. One receptor so similar in ways to can you map? But you can see there's many other agents that are on the horizon, and these are actually most of the ones that are in currently in active clinical trials.
- So inhibiting Il 6 and other pro-inflammatory Cytokine depleting B cells.
- vaccination, development, low dose. Il 2. So again, we'll we should be hearing results from these trials in the coming years, and they may allow additional therapies that may be more powerful.
- For really limiting again, probably in the group of trying to prevent recurrent mi's, which, again, is the highest risk group and contributes a lot to cardiovascular mortality.
- So I do wanna segue a little bit into just talking about ather sclerosis as a complex chronic, inflammatory disease. Just so we can share some of the work that's going on here at the University of Virginia. From people in my lab and others. And then I'm gonna be touching on in the Red Circle. You can see B-cells, which, as John said, is one of the things we really focus on in my lab and then the other red circle mass cells which is linked to the Ige Alpha-gal, because mass cells mediate a lot of allergic reactions.

- So and the idea would be to drill down and understand immune mechanisms better than giving broad based. You know, modulators like colchicine, because some cells are actually pro inflammatory, and other cells are actually anti inflammatory. So you don't really want to be inhibiting them all. You would like it to be more of a targeted
- therapy.
- So, as I said in in my lab, you know, we do a lot of discovery and marine systems, and the mice has taught us a lot. I'm not going to go through all of this here. I'm just going to draw your attention to the fact that these are all, except for the T cells here, and the mono sites down here are all B cell subtypes.
- and even beyond one immune cell, could be pro inflammatory and another one add inflam, anti inflammatory even within a subset. So even within the B cell subset some of these B cells are actually pro inflammatory, much like A B cell making an Igg that would worsen autoimmune disease right? So B cells that make Igg can worse than cardiovascular disease. But other B-cells are actually anti inflammatory, and we've done a lot with these cells in mice and shown that. And more recently, we've moved to human studies. And I'm gonna show you some of that, because to look for this protective cell in humans that we've found in the mouse with the idea that we could bolster protection that way. And really, what we found is, it's really probably more that circulating marginal zone B cells. And I'll and I'll show you that data.
- So how would a B cell actually contribute or protect you? From atherosclerosis. And like, I said, since we're gonna be talking about the protective cells, I'm gonna focus on that mechanism for the sake of time.
- And these, and I showed you in the prior cartoon that those B cells make Igm predominantly make Igm overwhelmingly, predominantly make Igm and unlike b 2 cells in autoimmunity that are making Igg and can be pro inflammatory. These B one and marginal zone cells make Igm and Igm can be very protective. It can bind up what's called danger associated molecular patterns. So these are patterns that can activate inflammation through toll-like receptors. They can actually even get into macrophages through scavenger receptors and cause lipid accumulation in the wall that leads to cell death and having a big necrotic core which we know, puts you at risk for heart attack but and the and these danger-associated molecular patterns actually originate with normal self.
- So when normal Ldl gets into the artery wall it gets oxidized, and when it gets oxidized I find this actually quite an intriguing from a teleological standpoint, but when it gets oxidized, it creates a neo-antigent. And if you sequence that it's actually identical to the sequence of PC. Which is on the cell wall of strep pneumo so it converts Ldl into looking like it's a bacteria. And so, of course, you get an immune response to that right? Your artery wall is sensing a bacteria.
- The same is actually true for this Mda, which stands for Melinda Aldehyde. It's another product of oxidation. When Ldl gets in the wall. If you sequence this, the neo-anagen that's produced is identical to group a strep.
- So again, another example. And so that's why I say, sort of teleologically, it's really kind of fascinating, because otherwise you would say, Well, why do you have these cells to make these antibodies to oxidize? Ldl? Why did you? Why did those ever even exist. They didn't really come about to protect from Athro. Well, they really came about to save your life when you get infected with strep pneumo before your cells can go to the spleen and mature and have T cell help and give you highly specific Igg antibodies.
- So that's why they're there. But good news for us is they're also here to protect from atherosclerotic disease. also viable cells in the artery wall. Once they take up all this lipid. They're really unhealthy, and they can apoptos, and they can also then release their

contents into the wall, creating a necrotic core which is very thrombogenic these igms, these same epitopes. That's why they're all pointing to these oxidation specific epitopes. These are the same epitopes. They're on bacteria. They're on apoptotic cells, and they're on oxld. So these igms block all of that. So this is the mechanism whereby they're ather protective.

- So but what about humans? And again, a lot of that data was was really discovered in terms of mechanism. As much mechanism needs to be done in pre-clinical models, because we can't knock things out in humans. So, but what about humans? And I only have one example. Cause I'm gonna show you our own data of some work by our collaborator, Sam Tamika, said Ucsd.
- But there are no fewer than 10 papers out there showing the same finding, and that is as your Igm to that Malindi Aldehyde, Ldl goes up. And so this is increasing tighters your likelihood of having greater than a 50% stenosis and your coronary arteries goes down, whether your lipids are high or not.
- And again, that's these levels of these antibodies have also been in shown to be inversely proportionate to, to cardiovascular death, to mi, to many other endpoints. So it occurs in humans as well. And it occurs in humans that present to the University of Virginia to have coronary and geography as well. So we have been actively in in collaboration with Angela Taylor.
- recruiting patients that come in, and it looks from the coronary angiography that it's safe we're putting in ultrasound capital into their coronary, and we're able to pull it back along the length axial length of the vessel, and get these cross sections, and be able to not only quantitate how much plaque is there, but say what it's made up. So is it necrotic? Is it calcified?
- Because, as you know, the necrotic core is been associated with increased risk of mi. So we've done that in did lead while we've done that. Now in 250 patients. But in this particular study we just looked at 50 patients. We measured these igms and Igg's, and you can see here's the Igm to Mda Ldl, and here's the Igg and the Igm even this small cohort. The amount of Igm is inversely so. Again, it's protective. So it's inversely associated with stenosis.
- With burden of plaque that's in the wall with how much is necrotic and how much is calcified. So even in our small cohort, we continue to see sort of the same thing. So we've been using this cohort. Then to kind of better understand? Because one key question we have is, you know, we're interested in Mba. Ldl, because of all of these clinical studies showing its importance. But it's really not the only neo-anagent. So we're really more interested in developing cellular approaches and so finding out what cell is making these antibodies that are protective from atherosclerosis. So a really talented MD. Ph. D. Student who got her Ph. D. In my lab, and now is an MD. Candidate will graduate from Uva's Med school this spring.
- Really did a fabulous body of work within the laboratory, and in this, in this human sector to try to answer this question, what human cell is making protective antibodies so that we can maybe understand more about it and use it for prevention. So she used high dimensional analysis, and in vivo functional assays, using humanized models
- to dissect out and obviously with the with the human imaging that we're doing. All of this is going to be association. But the association allowed her then to have candidates that she was able to put into a humanized mouse and show actually function in terms of making these. So these humanized mice are designed so they don't have B-b cells. They don't have T cells, and they have dysfunctional and K cells so they won't reject the human cells

you put in. So if you don't do that, and you put human cells into a mouse, they'll just kill them all.

- Okay, so, but these are special mice. That's why they're white. So most of the black 6 mice that we all use. Have a black coat. So I'll show you a little bit about what she found. So she started with this unbiased discovery. So she started with getting into our cohort, and again at the end. I'm hoping to really sort of highlight for you all that we have a really nice valuable cohort of patients here at Uva that could be mined for a whole host of different questions. But so she went in and said, Okay, well, give me a cohort. Let me find a cohort where their risk factors, their age, their hypertension, their lipids, are all the same. But one of the things that's dramatically different is how much Igm they have to this. Mda, ldl, so, and you can see this is very statistically significant. So the high groups close to 9,000, the low groups less than 2,000.
- And in that group she used a novel technique we have here at Uva. So that's the other thing to kind of advertise is, we have some really nice technology for single cell analytics here at Uva in our flow core. This particular one is called mass cytometry, that used to address this question, and it allowed her to do single cell. So analysis of each individual cell on a million cells from these patients. And so it gives you a lot of power. Even if the numbers that are in your cohort are low because you're looking at so many cells. And because you get so much data, also, it really requires advanced bioinformatics support to kind of figure out how to analyze it. And one of the ways we do that is with this Luvane clustering and this clustering diagram here is just showing you that there's 11 different types of B-cells and human circulation.
- Okay? Because each color is, it is based on some one of combination of these different 20, these 24 different markers to tell us it's this kind of B cell, or it's that kind of B cell.
- And you can see if you're close together. You're similar. So I've got circled here. Cluster one and 8. So they're similar and in terms of their characteristics. And they were similar in the fact that both cluster one and cluster 8 showed that in those with high Igm to Mda. Ldl they had a higher frequency. So again, guilt by association so not causal, but it was really kind of, we thought, fairly compelling data.
- So who went on then? To say, Well, what is it about those cells? So one by their markers. They're defined as Igm memory, which is good because they're correlating with igm so and so again, that was certainly supportive and but you know what marker on these Igm memory cells. Show. This was most important for this association, and she found that it was a marker called, which, interestingly enough, is in different clinical trials for covid for cancer. So it's an important molecule, that regular cellular behavior. But again, this is just an association. So we don't know. Still, don't know that that's causal. Which and so what boom did is use these humanized mice that I told you about that. Don't have bees don't have t's, and they have dysfunctional and Nk. Cells. And she took the blood from people, and she sort purified 3 groups. So one group that does not have this memory. Marker CD. 27. The other 2 do. They're the Igm memories, and one is 24 low, and the other one is 24 high, and she injected them into these mice that would accept them.
- And then, a week later, she bled the mice and did an Eliza assay looking for this? Igm, so because the question is, do these cells are associated? But do they make this antibody? And, as you can see, if you're not an Igm memory, you do not. If you are an Igm memory, you do. And the ones that were 24 high. Consistent with this association were the ones that were making the most antibody she then went on to say, Well, I wonder if we can boost it in an antigen specific way. So kind of a form of immunization, right? So or mimicking immunization.

- So she again bled. Some humans got this got those cells, sorted them into the groups, 24, high and low, and she's simulating them with that mda antigens to see, can I actually increase the amount of Igm and specific Igm that's being made and put them in to put that into the humanized mouse, did the Eliza's. And you can see that the total igms are higher in the 24 high group. Here's the legend over here. So not only are the total igms higher, but the specific Igm to mda, so again, this Antigen and lots of human studies that have shown to shown to be really linked to cardiovascular disease, she could actually boost it by stimulating the cell sex vivo. So perhaps this has some kind of implications for cell therapy going forward. But obviously a lot more work needs to be done.
- So but then, again circling back to the human, now that we know this is causal, is mechanistically leading to increase in this specific antibody do these 24 high, they and they again, they seem more like marginal zone cells to us, have anything to do with human CAD.
- So in addition to doing intravascular ultrasound on people that don't have much CAD, we also wanna be looking at people who do have a lot of CAD. So again, in the ultrasound, you can see a lot in the wall, even if the woman's not encroached on. But these are people who have so much that they have significant encroachment.
- So we enroll these individuals as well to take their blood. We calculate their coronary severity score based on something that was developed probably nearly 40 or 50 years ago, called the Ginsini Score, which takes into account how blocked the artery is!
- How many arteries are blocked, and in what location? So obviously, as you know, approximal blockade is much more important than the distal blockade. So, and you get a Jenseny score for that for that, and you can see in the population. Now, this is a population of 60 individuals, and you can see the Jenseny scores are very different.
- The ones that we're calling high have a score of 61 and one's low, have a score of 2 and very significant p-value. There was no difference in this group in any of these other risk. Factors like age, Bmi, hypertension, statin, statin, use, or lipids. But the one thing that is significantly different is the frequency of the 24 high Igm memory.
- So again, circling back these cells that clearly make this this antibody are, you know, are linked associatively with human coronary disease
- and just budge published this paper a couple weeks ago, and nature cardiovascular research if you want to look and see anymore.
- So where do we go from here? And some of the things we're interested in is, one would be cellular or vaccine therapy. So we aren't actively working on. We're somewhat helping. But it's really our collaborators in Vienna. We're actually working to make An Mrna vaccine for cardiovascular disease.
- So which will be really intriguing. So we'll see how that goes. We're really more interested in cellular therapy, because again, I think that the cells they're making these igms. Mda, but they're making igms to other things. I think there's other antigens and cardiovascular disease that we just don't fully appreciate yet. And so we're really interested in doing more using Crispr engineering to really kind of modify this B cell behavior.
- So we'll move off of the B cells. And that's probably as much as you wanted to learn about B-cells today and but let's go back to the tick cause everybody likes this story right? It's a local story. And as you know, it started with Tom Plattsmills and way back in 2,008, when it was discovered that ige specific to Alpha-gal was the cause of anaphylaxis map and he subsequently then linked it. The Ige antibody also to delayed anaphylaxis to red meat, or which is now called the Alpha-gal syndrome, and in the United States this is caused by the Lone Star Ticker.

- So we had, some years back, collaborated with Jeff and Tom and as I told you before, we have this cohort. And again, this is another example. If you have a good idea, we have this cohort. There was just hanging out talking to him in the hall, and we said, Why don't we measure Ig. Delphigal?
- And so we did, and lo and behold, I will show you the results. Yeah, lo and behold, those that have are positive. So here's a positive group have significantly higher atheroma burden, atheroma, volume, and maximum stenosis by ibis.
- And this was not true in this cohort for other allergens. So we measured total. Ige Ig Delphigalon and ige other allergens like dust peanut things like that. And the association wasn't there. We really just saw it with the Alpha-gal. So here she is again, and you can see that is really quite good at single cell analytics. She likes to take millions of cells and sort of discover new things. So cause the question is, what cell is responsible for switching you know, and what mechanism is switch you switches you to Ige so that you now have allergy to Alpha-gal. So once again, you know, she took a population, and I'm not gonna share. The patients are very similar to the 30 and 30 that had similar risks that I showed down the prior table, so that in that same population she used a B cell panel, and this heat map is just showing you the markers she looked at. Not that you're gonna really wanna learn about them in detail. But once again, you get so much data, you have to have bioinformatic approaches that allow you to cluster these cells. And what we found once again is that we're 10 different clusters of B cells in these patients. Circulation, and you can annotate them as to what each cluster is based on this heat map. But before we did that we said any clusters associated with Ige to Alpha-gal, and sure enough, Cluster 7 came up. So we're like what is cluster 7. And so what we found is that not again really reassuring to us? It's not a huge surprise, except for one piece of it's the switched memory cells. So switch means, you know, most cells have Igm on their surface through development. And that's what they have. If you're switching, you're going from M to either G or E. So of course, it's going to be switched because it went to E, and so but one of the things that was really interesting to us is that there's 2 populations of switch memory cells.
- Cluster 4 and cluster 7. And what's different about them is 7 has a lot of Ccr 6 which is a chemokind receptor. So I'm not gonna drill down into the novel finding that about Ccr 6. But it's something we're now pursuing.
- And we actually think this may be very, very important in regulating who switches to E when they get exposed to Alpha-gal, because we all have Igm to Alpha-gal. It's not. It's harmless because I told you what a good guy Igm is.
- But Ige is bad. So we think Ccr 6 is involved in the switching, and I won't go into that because this is published as well last year, so you can. I'll pull that up if you want to read more.
- So with those findings, we now have an Mpi, RO. One grant where we're going to try to answer some of these questions. This is super busy. So don't look at the details. But just to show you we're doing the basically what the heck is up with getting bit by a tick in your skin. And now you get more heart disease.
- You know. How does that work? So Lauren's really, you know, working hard to try to understand what's going on in the skin. We're trying to see. How does it really? Is it really causal? Is it really making those plaques bigger? And how? And then we're also continuing to increase the number of subjects that we have in our in our cohort so that we can look at other immune cells. And we want to do a therapeutic trial in these humanized mice because one of the things that found in her single cell analytics is that the Il 4 receptor was really important also, besides CR. 6, in regulating the or being associated with Ige. And so we're going to try inhibiting the IL. 4 receptor with Dupilimat, which is what a lot of allergy

patients get. Right? Okay? Okay, so just quickly. Some precision biomarker work that we're doing as part of it. I prime, but also in particular in collaboration with Antonio Abate and some others.

- So this this kind of work it all comes down to, you know, when you have a clinic or a hospitalized patient that has residual risk or residual symptoms. Now remember the red box I showed you, and I showed you that. You know it's a good third of people that were on drug didn't bunch their crp. So how could we figure out that was going to be the case for those individuals, and so that they don't let's say the trial was positive, and the FDA approved the which drug now we give it to everybody who meets inclusion, criteria right? And yet now we know, and looking at that trial at least a third, if not more. People didn't budge their crp and probably didn't get benefit. So we wanna find ways that we can figure out who's going to benefit, who's going to get harmed, and you know, and be able to target therapy that way. So again, we use a million cells. And we do single-cell analytics on them because it gives a lot of power, and with the idea that it would allow us to stratify patients into these groups.
- So this is just one slide about what we're doing with Antonio quickly to show you and Jesse Cochrane and Miles, who are here in the room or part of my lab, and then also part of I prime. But we're taking these patients. Antonio and his colleagues have a anachiner study for heart failure, and they've enrolled over a hundred people although I think the total is gonna be 102, and there's 4 more to enroll. So he's enrolled almost 100 people. And we've gotten we've been working with him for about 5 years. So we have cells from before those people got the drug and then they got the drug and then they were followed, for whether they had improvement or not, and we've been getting blood all along the way. So we wanna be discovering markers that once the studies on blinded that we can go back and say, if you if you get this at baseline, and it could be tested in a randomized trial. Then, if you get this at Baseline, you're tenfold likely more likely to benefit from the drug.
- So that's what Jesse is doing within a pilot study with Miles looking at one of the cells we know are involved in heart failure, inflammation are TH. 17 cells.
- And so right now, we don't know who got what drug, what we do know, who improved and who didn't improve. And we're looking at the phosphorylation of and Nfcappa B, which is an inflammatory transcription factor. And it's higher in people that that baseline and people that improved.
- And this is when you look at change that the relative change in peak vo. 2, which is the marker of improvement, and you can see it is significantly correlated with the amount of phosphorylation of NF. Kappa B. So this is our lead biomarker for our hypothesis going into this study, and then we'll see if that's true.
- So again, I showed you this before that. There's lots of things on the horizon in terms of different types of immunomodulatory therapy. The this is just to put in here, and I'm going to flip through it quick to just tell you the flip side is also true and that is immunotherapy that's targeted at autoimmunity and cancer could also have either beneficial or deleterious effects for cardiovascular disease. So this needs to be another reason why it's important to understand how immune cells affect cardiovascular disease.
- And then, lastly, just to tell you a bit about what I've been alluding to. With our precision immunomason initiative. So we've been enrolling. And we have cohorts of subjects that we've been collecting for probably close to 20 years now. And so we really feel like it's a nice platform, not only for us, but for us to sort of share and help other people be able to ask questions about risks, and in particular, immune, you know, immune risks for cardiovascular disease. So we were able to compare successfully for what the provost called a which is a prominence to preeminence, so something that Uva's prominent, in

which is cardiovascular and immunology, and imaging and informatics and then pull together some type of an ecosystem that can help us move to preeminence. So, together with several centers, several departments, again, we're putting all of our all of our data as well as helping other people generate data. For sharing across Uva for people that are interested in asking questions in this sector of immune mechanisms and cardiovascular disease.

- These are kind of a few of the things, just to give you a rough example of what you could do. And again. These are this is to depict people coming to the Caf lab and meeting all the masked and gown people doing the procedure. And we have over a thousand people that we've recruited in this way. So we really have a rich data source and our cores here at Uva. Really are cutting edge in terms of being able to allow you to do single cell analytics for discovery and our bioinformatics. We have the nation's first school of data science and so linking into that for analysis as well as potential future. AI, I think, could be pretty exciting. So there's about a thousand people in this cohort this could be. Again, we're looking at how cytokines induce intracellular markers and immune cells could be covid. Could be cardiovascular disease could be any disease in which cytokines are involved. I talked to you already about the alpha-gal story and then we also have several others. So I think I'll just kind of well, real quickly. We're actually working with Ken Billcheck to find biomarkers of who responds to CRT so and have published a paper and have some other preliminary interesting data. This is with Antonio. And this is what I alluded to with cancer subjects. Actually, people that get PD. One. Inhibitors by Fdgupet have increased inflammation in their vascular.
- So I think that's why, again, you'll see them having cardiovascular consequences like strokes and heart attacks at young ages. And so understanding who is at risk for that will be obviously critically important.
- So and the most important slide. So these are the people who really have done the work. And these are. This is a group in my laboratory. Everybody here is in my laboratory. These guys are in yellow because they're both in my laboratory and have moved over to help with the I prime initiative that I mentioned to you. So I prime is. We have a new program manager, Jessica Allen, who's in the back of the room in Orange, and is really the point person for for for people who are saying, you know, could you help me with my studies? What do you guys offer? You know this this type of thing but hey, Mokko Thari, who now is in industry and Anne is now at Duke. We're really important in getting the V. Sal. Some of the B cell work off the ground, and I showed you Oms work in particular, Jesse and Miles work with Antonio Abadi, and here are some other of our really talented graduate students, our collaborators at Uva, including Tom and Plus Mills and Jeff and the Elf Miguel and Lauren Ericsson, and both Angela and Todd blinds with the with the Imaging and Antonio. We have a lot of outside collaborators as John talked about, and I didn't get them all on here, but these are the collaborators that have helped us with the projects I showed you at Ucsd. Medical College of Georgia, Harvard Medical University of Vienna.
- I guess I got Sam twice.
- And then here is our I prime staff. So we have a nice group again. Jessica's the manager. Mike's the administrator. Christine and Charlotte recruit our patients as well as work. Develop the redcap database. So we have actually a lot of data on these subjects very well organized. And we're getting it into the research data Commons, which is university and statewide, actually and mica and Chantelle and Vicki do a lot of the processing. Miles Corey and Maria are bioinformaticians. So it's a nice team that can really help with immune translational studies in the cardiovascular system. So touch base, if you're interested so thank you for your attention.

- I can give you mine.
- Thank you. Alright. Thank you for a great talk. We'll have the mic on up here, if there's any questions and I will read aloud questions in the chat. We have more of a comment than a question from Dr. Robatte. But he says, Thanks, colleen, for highlighting the collaborative studies I would like to underscore. How precious is the work you do, bringing bridging between molecules and patient outcomes, integrating several different disciplines?
- Thank you, Antonio, and then I'll ask a question with the caveat that it's been many years since I took basic immunology and haven't thought to that depth. But it's interesting hearing that the Igm is decreasing sort of that inflammatory spont response and endothelial cells.
- For some reason I have it in my head that Igm binding antigens activates the complement system and leaves some more inflammation. So how would you answer that? Yeah. So that's a great question. And that's actually something that we're beginning to work on. Now, we're we're developing with a collaborator, Pittsburgh and Igm that can't find complement.
- So that is one of the features that can cause red cells to lice can cause some sort of bad damage, but it's also a feature of Igm that allows them to be engulfed by when they bind to the apoptotic cells and recruit complement.
- That that's part of the phagocytes coming and engulfing that apoptotic cell which is positive. So it's kind of there are negative aspects to it. And then there's positive aspects as well. Most of what we see in cardiovascular disease is not through the complement pathway although some of that occurs when they're taking up the apoptotic cells. But a lot of it is just blocking the Antigen from activating inflammatory receptors, and that was shown by one of our colleagues in a Nature paper, where he made a single chain variant, so didn't have the Pentamur so couldn't bind compliment, couldn't do anything like that, and over, expressed it, and he improved cardio cardiovascular disease hepatitis, you know other forms of inflammation. So if he can block the antigen without creating too much inflammation from the and maybe the igms that find complement and fuel. More inflammation are different. Have different idotes, meaning recognizing different neo antigens. That could be a possibility. That's it. So it's a great point. And it's a great question. And it needs a lot more research in that space for the talk. I was wondering. I know, the B cell landscape changes with aging and the type of antibodies that they secrete also changes. And obviously, Athos Scrooces. This is an aging related disease as well. I was wondering if you all have looked whether there's a correlation between your productive Igm class and aging versus in younger folks.
- Yeah. So that's a great question. I know you have a mask on. But, Ashley, yes, yeah. So that's a great question. Ashley is another graduate of the Md. Phd. Program here, as you all know, I was on the Executive Committee, I think, during the course of her training.
- So it's a great question. And we are looking at that and a couple of things. One is as you get older, and we've shown this, Sam Tamika says, shown this, and others have shown this. Your igms to these oxidation specific epitopes go down as your coronary disease is going up with age. So that's one thing is, it's and the number of the cells go down. But also the types of antibodies they're making change. And they're not as protective. And we've shown that by adoptive transfer studies. So that's one mechanism and the other one which I'm I, as you probably know, or Ashley knows, and maybe many others do. There's these things called age associated B cells and so it's not a B one cell, though it's a B 2 cell that increases with age, and they're very pro inflammatory. And we have been studying those as well. And then actually, has a nice story. She's resubmitting the frontiers and immunology tomorrow. About the association of CD 11 C. Their CD 11 c. Positive. These

age associated B cells. So so they're on the bad guy side. The cells on the good guy side are kind of dwindling it. It's also link. Remember, I told you the same epitope is PC on strep pneumo. As people get older they don't do so well with the strep pneumo vaccine, right? So I think they're just not making the right igms to recognize these epitopes as you get older. So great question.

Colleen great talk, I mean, as always so we have seen some people who get entire red meat allergies after tick pipe, but then many of them recover over time, and we looked into those whether their Igm is Ig is going down? Or is there a another sub type of Ig say, is going up, which is yeah, known to be protective?

- Yeah, we haven't yet looked at. Ig, G, 4. But Jeff probably talked about it, and I know that we're gonna start doing it within our group. We all collaborate together, and Jeff Sterick's interested in this as well, but in terms of Ige it does go down it will, it will go. It will reduce with time for the most part, and most people, if you get bit again it spikes right up. It's a memory B cell response. And so it's it goes right back up, you know, just like your memory B cells will do when they're seeing Antigen for the second time. So that's the status with that so? And then you're, you know, right back to kind of where you started. But it'll come down again with time. Yeah maybe time for one more question from the chat. So Dan Tran, one of our third year residents, was, was wondering. So he said, Thank you for an amazing talk. Metabolic reprogramming of immune cells are known to change their immune phenotypes. Given. Lipid metabolism is dependent on mitochondrial oxidation. I wonder if there are any different changes in mitochondrial pathways in your site off data.
- I wonder if mitochondria targeting molecules could be a novel approach to modulate the atherosclerotic plaques? Yes. So another scientist in the crowd. That's a great question. And so it's not an area that we study. But you're exactly right. Metabolism regulates the function of immune cells. To be sure, we do have a a current project that that is somewhat related that is, we'll probably do the sharp tank at the at the school of Medicine retreat to kind of highlight this, but the role of G. Sixpd which is the most common enzyme defect in the world, right, and protects you from malaria. And it really regulates. If you guys go back and remember your pentose, phosphate pathway and generation of nadph and those processes. Well, we're finding that it has a significant impact on the biology of our B cells.
- So in in broad strokes stands like completely right now, I'm not smart enough to study mitochondria, so we might have to ask. You might have to ask Matt Wolfe, or somebody like that. I don't know much about the mitochondria. But I know they're pretty darn important. In metabolism and generating energy. So it's entirely possible targeting them would be a really great strategy.
- Okay, thank you.