EUROPEAN LIQUID BIOPSY SOCIETY

1st EV Technology Meeting of 2025: Minutes

WG Leads

An Hendrix Franz Ricklefs, Basant Kumar Thakur

Meeting Minutes / questions to the Speakers & Discussion

Welcome by WG lead: An Hendrix

An Hendrix

OK, since we have all speakers on board, I would assume that we kick off in time so that we have plenty of time as well to to discuss on our topic of today, which is EVs and personalised medicine so. On behalf of the the EV working group, including Basant and Franz, it's my pleasure to welcome all of you. And and we have a very exciting programme today covering different chapters around the world. So we will travel around the world just in the upcoming. Two hours, which is also fantastic, I think. But the main aim of today is to really move into depth a little bit more on how to implement extracellular vesicles in personalised. Medicine. So we really made a selection of three keynote speakers for today. Will bring different expertise to this topic and which we are really looking forward to discuss. So if if you have any questions that you would like to ask or discuss with the speakers, feel free to raise your hand after the presentations. You can also use the chat box if you don't have a speaker available so. The aim is really to discuss and interact and connect on the topic of today.

1st Presentation by Prof. Carlos Salomon (Director, UQ Centre for Extracellular Vesicle Nanomedicine, The University of Queensland, Australia)

Title: Translational Opportunities for Extracellular Vesicle Biomarkers: A Case Study in Ovarian Cancer

An Hendrix

So then it's my pleasure to introduce and welcome the first speaker of today and Professor Carlos Solomon. So he is director at the University Queensland Centre for Extracellular Physical NANOMEDICINES in Australia and he will tell us more about the translational opportunities of extracellular vesicle. Biomarkers focusing on ovarian cancer as a case study. So welcome, Carlos, thank you to accept our invitation and the screen is yours.

Carlos Salomon

Thank you. Thank you. And and thank you to all the organisers for you know to give me the opportunity to discuss a little bit what we have been doing in terms of our extracellular vesicle research for the last around 10 years and how we have been moving forward, hopefully for implementation of a new. As for ovarian cancer, able to detect the the the women with ovarian cancer, asymptomatic women with the Varian cancer, mostly early stages of the. This just to check, can you see the full screen right? I'm I'm I'm just putting the right one, yes.

An Hendrix

It's perfect, yes.

Carlos Salomon

Alright, OK. Fantastic. Good. Yeah. OK, so. So I'm gonna start actually just to introduce where I actually I'm based. I'm in Brisbane, Australia. I'm here in in the I wanna just put the light laser here. Probably that will help. There you go. Here. So I'm part of the. My group is the translational extracellular vesicle in the engine oncology group. We're focusing 2 main conditions. 1 is in ovarian cancer, which is gonna be a story that I gonna I'm gonna tell you today. But also we do a lot, a lot of biomarker discovery. In in in pregnancy, which is probably for for a for a different topic and based in the UK Centre for Clinical Research, which is in the Hurston camp of the University of Queensland in Brisbane and we are connected to the large host. Without knowing in Brisbane, but in the state of Queensland, which is the Royal Brisman, and Women Hospital, which is something that have been very, very useful for us in developing this particular programme, you're looking for biomarker and you're looking for implementation of bio market in general, not only in the biomarkers in the clinic. What you need to have is a direct contact with clinicians to understand actually what are the need and what is the gap that your potential buyer market need to address, right? The only thing I want to highlight with. This is I I have been in Australia for 12 years and one of the reasons I moved to Australia was because to establish a quality management managing system in in the UCR and obviously within my lab and and this is something that have been very useful for us for the last 10 years. Especially for our interaction with biotech companies, we all know that it's an issue about reproducibility of the research. So. To have a quality management system in place in your life actually

address specifically how you can actually reproduce your reserve funding, and we have not only comply with ISO 17 or 25, but also we have 35 for ISO 17 or 25. We have external entity which is the. National Association of Testing authorities in Australia, which certified that we have the expertise and capability. The to perform all the experiment within the scope of our accreditation and we focus in extracellular vesicles. So extract extracellular vesicle, isolation, characterization, proteomic analysis, macro, innate and lipidomics are part of our scope, which is super important and and invite everyone to try to look how you can be. 35 for this type of quality management system in your in your respective country, on your right here you can see a a general structure of what we're what we're doing in, in, in my lab. So we're focusing intracellular vesicle and we wanna mention some of the main characteristics of EB. Which are essentially present in all biofluids, so very attractive for biomarker discovery. Obviously the the main attractive characteristic of this is the capacity of the B to carrier biomolecules including proteins, micronation even though lipids. I'm not gonna mention much about lipid. Lipid is something that have been overly. We need be reserved for for a long time, so which is quite interesting because around 80% of the content of the EV is actually have liquid. So it's something that we also we are looking with very interest in the last few years as well. And our problem focus one of the half of the programme focus in ovarian cancer and. We're trying to address 3 main issues in ovarian cancer. One is obviously about detection, how we can identify asymptomatic women's early stage of ovarian cancer, which we know that surgery surgery, chemotherapy. Works and women's identified early stages or usually have a five year survival around over over 90% when the disease identified earlier stages. Obviously it is also attractive for other features in terms of the varying cancer identify. Potential therapeutic agents. We know that it is are participating in, for example, formation of the pre metastatic niche. So that is quite attractive to identify where this EV is from the tumour cell actually go. And also you can you are able to identify biomarkers that reflect the cancer, the present of the cancer of the volume of the cancer on the spread you can monitor how the patient are responding to a specific therapy as well. So I think you know all the the people present in this presentation. We are kind of heavy, enthusiastic, right? So we we like this. We think that are very attractive in and have a lot of potential and probably we all agree that if we are now part of important part of cell bio. But the translational part is still a little bit in infancy in terms of there are no many heavy based tests and biomarkers that actually are available in the in the market. So the translational part is something that we are moving forward hopefully in the future. Right. And and one of the reason for that is we need to really understand the biology behind the EV's and and some of the main characteristics of the transcellular vesicles is the diverse. And I'm gonna start with this story, which actually I think is very similar to the extracellular vesicle film. And and this is Ernest Starling, which was one of the first that coined the term hole and is very similar to extracellular vesicle. Hormone is actually a generic term. We know that we are very familiar with hormones obviously. And we know that there are different type of hormone. So it's very similar that we have also different type of extracellular vesicles that might have different functions as well. So Ernest Terning described the first hormone which actually was adrenaline, but the full benefit of adrenaline or management of surgical bleeding was not realised until adrenaline was isolated, characterised and synthesised right. So it's very important that we have to identify the nature of these signalling. The modalities to understand and and. Actually fully take advantage of the potential clinical application of EV's. So like all hormones, extracellular vesicles, stationary term. So we have to define what are the subpopulation of EB that actually are carrying your biomarker of interest or potentially or therapeutics. What are the the events that actually are cover covering your therapeutic

agent? Right. In terms of the translation potential of EV, there are 4 main areas. So because I don't have much time. Talk to talk about all of them. I wanna focus mostly in biomarkers of the disease, but obviously they are very attractive for target intervention carry of therapeutic agents and also delivery of of therapeutics, right. In terms of the biomarker, there are several advantages to use extracellular vesicle compared to other soluble bio. Market and one of the main characteristic is about the content, right? So we all and it's plenty of data suggesting that the content of the this is a reflection of the metabolic state of the originating cells, right? So get information about from where this come from actually is something that is an advanced stability and. Protection because the size and and the membrane of the bees will protect the content is something that also is an advantage as well. And the last part is about increased sensitivity and specificity. You are in the biomarker business. You need to make sure that you get enough. Sensitivity and specificity that is required for implementation of your biomarker test. Whatever biomarker you are looking at in whatever condition. So we focus and we get that into the ovarian cancer. So if you're interested in biomarkers why you need to identify why you want the biomarker for right. There is the clinical need and in the context of biomarkers for ovarian cancer, it's very clear that it's a specific clinical need about how we can identify women's at earlier stages of ovarian cancer. And you can see here for example in this graph on your right when here you have survival and then you survival in in blue and then you have the the the the percentage of new cases in in red. So you can see here that when we are able to identify women early stages of ovarian cancer especially stage. One the five year survival rate is 90%, which is fantastic. Unfortunately only a few percentage of the women actually are identified in this stage and the majority are identifying late stage of ovarian cancer with when these 90% actually dropped to 29% right. So. There is a clinical need and so you have to identify what is the gap that you want to address with your biomarker, what have been our approach to try to understand the role of is if it is in cell to cell communication especially within the tumour microenvironment in ovarian cancer. And I'm not going to go through all this sorry and and the reason for that. That is one of the main characteristics of ovarian cancer that compared to other type of cancer that ovarian cancer developed in the surface of the ovaries and the fallopian tube. So it's very, very easy to be actually spread to all the floating organs in your peritoneal cavity because they are in the surface, which is. Quite of the main characteristics compared to other cancers, and we're trying to understand the cell to cell communication between these tumour microenvironment and we this is several papers for for my group in in the last few years in which we identified that actually EV's have a role in the progression of the disease. So with that information, we focus is OK is if we have a role in the progression of ovarian cancer and if you are able to identify changes in, in the state of ovarian cancer based in the EV profile, perhaps you can focus in develop a biomarker in EV focus inhibits to identify women with. Early stage of ovarian cancer and this is the stuff that we have been doing for the last 8 to 9 years, right. So you can see that we start with. Project 8-9 years ago, fields available project to identify. Can we actually isolate these? Can we run the content of EV's and can identify women with ovarian cancer? Before to move forward to show you actually the data of our study, I want to clarify and and and and make some consideration about biomass right. The first thing that we need to understand is what is the definition of biomarkers, right. And this is something that I touched in the last I submitting in which I did the educational talk for the biomarker part. So you need to identify. Actually, what is a biomass? And biomarker is defined as a characteristic that we can measure right, which is super important and one of the main points from the MBA definition is need to be reflective of a normal or in in in our case focusing ovarian cancer of a pathological process, right. Also the different type of biomarker.

For what we're looking at, we're looking for diagnostics. So we're looking for a biomarker that can identify the disease or a specific disease or condition, right, which? Probably you can have different type of biomarker depending what is your area of research right monitoring also is quite attractive as well in ovarian cancer. But we know that for example CA-125 work quite well in in cancer in recurrence for ovarian cancer. So there are specific clinical need for early detection running to monitoring or respond to. Uh chemotherapy or? However, it is a very tough business because you can see in this particular slide that a few per person of the bio market that are proposing in the literature finally end up in the clinic and this is not even biomarker. This is in general biomarker, it's less to 1%. So it's a very tough business. And how we can improve that? And I think for our experience for the last 10 year, one of the main thing that we need to do is we need to define what is our goal when we are developing biomarkers, right? So are we developing a study just to publish the data or are we setting up to deliver something that is useful? And we can implement it in clinical practise and improve patient care because that goals, even though can be. Similar the design for your study might be different, right? And in this case, if you have several different advantages for biomarker and here you can see some of them, for example, reflecting the sale of origin access, there are plenty of that and the content that. Can be identified in in circular. Mentioned also the stability that I mentioned and also you can set up automatic workflow to with that are compatible with pathology lab which is quite interesting as well. So with that taking in mind we took the approach and the challenge to try to identify extracellular. Physical based biomarker. To be able to identify women at early stages of brain cancer, and this is our study, so we have been this is something that we haven't published yet. So hopefully we're gonna publish it this year and we we have around 1500 women involved in this particular study and then you can see that we have. Our training and verification study and then we interact and and partnership with our biotech company to try to actually implement and. Look how biomarker test that can reach to the clinic, right. So I'm going to focus in this particular data and which was our Discovery project. So you can see that we divide the cohort into one was our training and discovering trying to we open up the ABS and we check potent and we checked ernas. To determine if it is something this EV that can be informative to identify women with with ovarian cancer, and specifically in early. Stage from this study we select a set of biomarkers that then when they're verified in a different cohort of patients and then finally this set of biomarker we use in a workflow that is compatible with pathology lab to determine if we can that we can maintain. The performance in our previous stats, right? So we identify over 100,000 different variables in our sample. You can see we did a deep RNA sequencing. Different type of RNAs and and then also with the proteomic analysis pointed the deep analysis for for products right and and with this after that we put all this data in a in a modelling analysis in collaboration with the computer department here at YouTube but also with another. University in in in Australia to identify if there are something in this molecule that can give you all sensitivity and specificity and overall classification efficiency. So you can see that the only reason that you put the classification efficiency without any validation just to show you that you. Don't trust and data that is not cross validated, so you need to be able to have a good models when you are testing the model. Otherwise your your your pipeline is more like it that will overfit your data so you can see that actually our initial approach using this biomarker. Identify that a classification efficiency independent of the algorithm that you use. With our best performance around 93%. So the potential is there. Then we're focusing protein and microRNA because that where the bio market that are more responsible of the separation and you can see that when you have your benign condition versus cancer and also your your healthy versus cancer, you can see

different in. Terms of. So you can see here that the protein and microRNAs are different in in when you you separate your groups but different doesn't mean that they're a good biomarker. You still need to you you you need you, you still need to perform your proper biomarker analysis. The only way to do it is through rock core and activity. Right. So you can see right away with the record for protein and microRNA that we're able to separate mostly all the cases compared to the control and more importantly the rock curve that we obtain from protein and microRNA are much superior compared to C-125, especially when you separate. An eye versus cancer, which is something that. Is good so. Keep in mind that your biomarker that need to be perfect but need to be much better than is a Bible. In this case it's C-125, right? Then we move forward and what we observe if our our biomarker panel are able to identify and separate cancer versus versus control, but especially is quite good at early stages. So we focus in early stages of the disease and then you can see that we develop a signature that have a a very high sensitivity and specificity. Or ovarian cancer, right? The signature also was maintained in an independent set of samples. You can see that with almost 90% sensitivity at 99% specificity with which were fantastic for us. Then we validate this biomarker panel in an independent set of of patients and you can see. That was run in around 400 patients with ovarian cancer and in collaboration with Professor Annie Fasho from University of Sydney. And while we identified that the C-125 actually is no good in in the population, which actually consistent with all the data that is available and we if we run our biomarker panel, so you can see that it's much better 25 women with ovarian cancer and our overall classification frequency is around 98%, right. So. I'm I'm running a little bit behind, so I'm gonna go a little bit quick with this, but keep in mind what is your goal and for us it's hopefully deliver something, right? So Ryan to obviously we want to publish the data, but more important to publish the data we want to try to deliver something to the market, right. And for that we team up with that by the company in Melbourne. To have a a system in place that's compatible with pathology lab, all our previous training and Discovery project was that used inside fusion chromatography and then we move out using a system which is magnetic core with antibodies on on the surface to try to isolate our evil. Right. And that system is the exnet, which is is commercialised for this particular company. So we want to study with the company and one is the OC 97 and the OC 500 using this automatic workflow and you can see that the classification efficiency was over 90% and sensitivity and specificity. Was quite high and much better compared to C-125. More importantly, our last study showed that 99.6% specificity you can get a sensitivity of 77% which is the minimum required that you need for a screening especially for. Diseases that are low insulin, like ovarian cancer, right, and this is something I would like to also mention and and make this example because if you're working in cancer and you're looking for a biomarker for screening, obviously you need to have a very high specificity and you need to probably get at least. Positive predictive values of around 10%. So that means that in your test in in the screen in the screen population you have. 10100 women positive for the disease according to your test, you need at least to have ten of these women through positive to be able to work. And the reason for that is because ovarian cancers are low incident disease. So you have to, for example, screen over 100,000 women to get for for 40. The true positive of the disease. So and this is an example that can be applied to other condition with a low incident disease as well. Just to finalise to say that we have complete a discovery initial validation of our extracellular vesicle test for ovarian cancer. Our also analysis saw very consistent performance at different cohort of patients, which is fantastic for us and now we are moving to hopefully have a more prospective study to validate our signature right some of take take home message. Hopefully you are trying to get this challenge. To identify and validate value market, just clarify what is the the goal

of your of your of your study? Are you just doing the study to publish a paper or you want to actually deliver something to to to to the patient right that that that suffer from this particular? This uh also make sure that you recognise and and you have data on what is the advantage to use EV because you know to use EV's, you have to isolate DBS first. So one of the questions that I get all the time is can you run your signature in plasma running to isolate DBS right? So it's recognised and. I'm trying to determine what is the advantage to use EB. Right. Trying to ensure that also you have a proper validation and don't use the same cohort to do your validation, otherwise you are overfitting the model trying to use lock model for testing and validation as well and importantly trying to also have early conversation with your university. You are based on the university, the Commercialization office and trying to talk with. Companies that are biotech company because they know what type of the step need to be done to deliver something to Denmark, right? Just going to find this out with this we we were fortunate that we got funding last year for a trial to trying to get more information about the feasibility to run this test in Australia. And this is a is a programme run that will will run for five years. So I think it's one of the first clinical trials in biomarker. For evis. So we're very excited with this and hopefully we're gonna get good results in the next few years. Just a big thanks to my team. This is a multidisciplinary effort with a lot of people involved in this particular project. We are part, I'm the director of the new UQ Centre for Extracellular basing on amazing. So it's something that is a is a huge F4 from the university to focus in extracellular vesicle now. So we are very excited. We are over 100 different researchers that we have some kind of interest in the extracellular vessel. So we're very happy with that as well. Finally, obviously it's a multidisciplinary work and and there have been a lot of different funding agencies helping me for the last 10 year to reach to this particular point, right. Obviously I'm part of the ISS else Intersociety group and so we're very happy to be, you know to to to have this. Interaction and learn from the ELF group and and get the experience from them as well. Happy to answer any question if we have time and this is my contact here so thank you for your time.

An Hendrix

Thank you, Carlos, for this excellent presentation. Of course we have time for questions. So Klaus. Please go ahead.

Klaus Pantel

Yeah. Thank you very much, Carlos, also for this excellent study. It really shows how many samples you really need and and what kind of work is behind it. And thank you also for citing the nature papers showing that the chance that any of our publications make it to a product is really low. And the ELBS is really established to change that. OK. And So what I would really like is really your explanation also of what is needed for a low incidence cancer detection. You know all these slides that are not your original data but that are very educational. I mean, you could probably put such a little, you know, a slide set together for our website that would be, I mean, one idea, it might be even a little video if that is more attractive to people. People listen to these things, maybe. I mean the, I mean that would be very good. And the second thing that I would suggest. Discussing that also in the European Parliament last. Week that we need to set up probably a road map for diagnostic markers to be implemented into the clinic. I mean for for therapies, for drugs, it's clear you have a preclinical development Phase 123, right? And without that road map, nobody would develop drugs, right? Because it would be too unsure and for diagnostics it's a bit up in the

air, right? I mean, so I think it it would be also very good for the ELBS maybe to set up uh suggestion for such a road map for diagnostic implementation of tests.

Carlos Salomon

Yeah. Yeah. Absolutely. Yeah. Thank you, Paul, for the comment question. I I think you know, I'm gonna start with the with with your last comment, you know from my experience you know I have been working on this for the last 10 years. It's have been a learning experience, right? So you asked me what would be the more important thing when you develop biomarker.

Klaus Pantel

That was very helpful.

Carlos Salomon

I'm probably going. I'm going to point out two main things, right. One is identify the clinical need right? What is the? What is the intent of use of your biomarker which is probably super important because depending on the intent of use is depending if what type of your market you want to develop and you. You talked about the low incident condition, which is probably all the cancer. So you're looking. You have developed a biomarker which the intent of use is. You need to have a huge high specificity, right, because you don't want to. You know the the question is how many false positive are you willing to afford for each true positive that you identify with your your market and for the screening that is you know for buying cancers 999.6. Which is, you know, almost perfect. So I think that is important. If you're looking for differential diagnosis, which is also in ovarian cancer, a problem, right. You obviously you you can you, you can compromise your specificity and you can get more sensitivity which is essentially what you want, right? So I think would be fantastic especially earlier when when I I was starting this project 10 years ago to have a like a A you mentioned a website or something that.

Carlos Salomon

You know we are, we all know are familiar with phase one phase two, phase three for therapeutics, right. But for bio market it's a little bit in the limbo, right? It's a little bit like you don't know what at the step right. So I think it is a very, very important point that you yeah that you make here, yeah.

Klaus Pantel

Thank you.

An Hendrix

Are there any other questions? For the moment, I was wondering Carlos the pro I'm from. From the scientific point of view, the profile. So you focus on the proteins and the the micro RNA. So in the end it is one combined biomarker profile. Including both proteins and microRNAs, but for the well, first of all, how did you select? Can you elaborate a little bit more on that? And then second thing, what is then the readout because you have the diagnostic tests to capture?

An Hendrix

But then to analyse the downstream content is that targeted or is that still on mix based?

Carlos Salomon

Yeah. Thank you for. Yeah. So our Discovery programme was based on omics, right, because we did proteomics, we did RNA sequencing and then we identify all the variables for the test. It's a targeted approach. So we have for protein, we have a a Max speed approach which is targeting. So we target a specific peptide which are associated with the protein. And for the micro RNA we perform PCR. So it's very kind of straightforward for for clinical implementation. Right. How we select the proteins and the Macarena compared to other type of molecules that we identify. So part of our workflow and and I think I have a paper on that that I can I can circulate as well. So we we we collaborate with the Australian National University. School of Computing this computing department and which we use modelling, analysis and AI to determine what are the biomass. Curves are all the molecules in all our signature that contribute the most to the separation. Because you have 100,000 variables and we use all of them. So you put all these 100,000 variables and then you get a a perfect signature and then the you can see you can do a operation or you can do a. Values. To identify what are the molecules that contribute more to the separation and then you select 1020 whatever number you are able to to select for the final, for the for the final Test, and then what you do is you develop these biomarkers and then you develop in our case. To develop ovarian cancer. The score. So we have we we mentioned the bio market, we put everything in our algorithm and then you're gonna end up with a score. And then if the score is above .6 or whatever, depending on the threshold and the cut off that you identified with your previous study, you can see this is our positive or negative for ovarian cancer.

An Hendrix

OK, perfect. Thank you. And my my last question, if there is no question in between, I don't see any hands would be how do you control? The technical process because you evaluate 2000 patient samples, so what's what's? Type of controls that you implement to ensure that you are not looking to technological variability, but really to the disease related variability in the sense.

Carlos Salomon

Yeah, that's a very good question, especially for ovarian cancer, which is. You know, we can, I think I mentioned this at the start of the presentation that you know extracellular vesicle is an umbrella term, right that there are different type of events, right. So ovarian cancer is also an umbrella term, right? There are different type of ovarian cancer, different ESTO type, right? So so how we account for that is we include. Everything in our modelling analysis, one of the main variables that you have to take into account for for ovarian Cancer Research, is about the age of the women. UM, so this is something that all the clinical characteristic that you can, uh, you can have a bylaw from from your patient you need to include also to determine that the different that you observe is mostly because of your biomarker panel rather into different in the clinical characteristics of your patient. I think your your question was more about technical right. So how you know for example when isolate DB is how we know that that that that isolation is is successful right? So we have controls we have quality controls with our every run. So we we can measure for example we have a panel of the transplanting just to make sure. No, in the no in the sample, but in the control that we have for example we do we use 96 word play and we run random controls to

determine that the isolation works and then we have housekeeping. Proteins and microRNAs at the end of each run to determine if actually you have access amplification of the sample or not. But yeah you you have to account for the variability as well depending on the system depend on the sample. The other thing that we haven't because obviously this test is not available. We're still doing more research to identify potential source of variation and also the stability of the biomarker and this is one of the aim of the trial that we're gonna run because one of the things that we we are very interesting is implementation so. If you get your sample, for example, you know I'm in central Brisbane, I can get a sample that was collected in a hospital which is close to to our lab in two hours. But if I get a sample that was collected, for example in Kent, that sample will get probably 2 days. To get to to our love, so part of the aims of the trial is to determine the stability of the bio market based on variability of the collection of the sample and time to shipment to our. But that is one of the one of the aspects that we're gonna be looking with the trial and there are plenty more that potentially can can affect the, the the final signature as well.

An Hendrix

Yeah, indeed. Many, many steps to come through.

Carlos Salomon

Yeah, but the only the only way to answer this is is from the studies, right? Because you know, you people can make the argument or what about the truth, right, the you know, what about the I know you're gonna do everything fresh. Are you gonna store your TV? So all that kind of thing. Right. So the only way that you're gonna be able to answer this question. If you if you run the proper study so. Hopefully we're gonna have good data in the next few years to to to answer these particular questions.

An Hendrix

OK, then I saw one last question by Marcus or not.

Carlos Salomon

I think you have a I have another question. For mark, yeah.

Markus Sprenger-Haussels

Yeah, I had a question, but you almost answered it. Thanks Carlos. It was a great talk. I think I was thinking exactly the same direction. You you said that less than 1% makes it finally because the biomarker is not verified and I think. One reason is exactly the the uncontrolled preanalytical part of the of the workflow. Imagine if the blood sample is taken. There's oxygen stress, there is not. There's a different temperature, and so on it, and indeed could happen that people who take their patient cohort. From side A and then the positive control from a different side, they have a different logistics, different temperature, different timing and so on. You just touch the topic. So the question is.

Markus Sprenger-Haussels

You. Do you also investigate methods to to stabilise the exosome profile but at the time of collection do you look into different collection devices or is that something still of interest?

Carlos Salomon

Yeah. No, not at the moment. We we look the other thing that we have been learning and this is direct feedback from the company as well. Hopefully we're going to develop a workflow which is simply enough so they can run it in, so you have a test and the the only the only labs that can run that test is your lab. That that test will never reach to the clinic. You need to be able to develop a workflow. That can be implemented in all hospital if you want. For example, right. So at the moment our collection is ETA 2. Very very simple and the reason for that is because this is. This is essentially when you're going to, you know, donate your blood or you, you're gonna get your blood test done. Usually it's EDA two that is easily to be done there. So we're gonna do ETA. The question of stability, I guess, is going to depend what data come out about different collection size, different time of treatment and that that type of information to determine if that is a that is a variable that affect the performance of the test. If the answer is yes, it's more likely that we might need to go into. Ways to improve the stability of the biomarker the the other thing that I want to mention before I leave is the OR before I move to the next speaker is we have a signature of protein and Macarena. Which actually worked very well, but proteins and microRNA in a single test is a problem as well. So if you if you get information with biotech company, they prefer have a test for implementation which is either proteins or either whatever. Lipids or microRNA, another combination because it's an extra step which is extra cost. As well, which might affect the manufacturing of the this which is, you know as a scientist say well look we we we have to use the signal to give you the best separation which is not you know in in the reality that is not now might be the case we need to use the signature that give you a good performer but also is have a feasible way for implementation.

An Hendrix

OK. That's a perfect closing of the first lecture. Carlos. Thank you. Thank you for that. So excellent. We look forward to to the final results and thank you for joining us here today. Do you stay for the?

Carlos Salomon

I don't know. I'm gonna stay. Yeah, I'm gonna stay. I'm. I'm staying on the tops. Yeah.

An Hendrix

OK, perfect. Perfect. And so then I will hand over to present to introduce our next speaker.

2nd Presentation by Manda Venkata Sasidhar, PhD (Chief Scientific Officer, Apollo Hospital Educational and Research Foundation Health Street, Apollo Hospitals, India)

Title: Extracellular Vesicles as Diagnostic Powerhouses: Our Translational Journey in Biomarker Innovation

Basant Kumar Thakur

Thank you very much. Yeah. So I would like to introduce Manda from Apollo's research and hospital centre. And actually I know she's there now since like 2-3 years and. I can just say that he is one of the very promising scientists and emerging scientists in the field of personalised medicine and when it's related to brain tumours and neurodegenerative diseases. And he is also the President of founding President of Indian Extracellular Vesicle Society. OK. And and we are very much also involved in in the German relationship like we have also organised in in March into German workshop and he will give us now some insight into the development of personalised medicine and involve also in aspect of it. Solar vehicles, how it is influencing the the ideas in in countries like India, which are really, really emerging countries in terms of biomarker discovery and research. Yeah, so so that the stage belongs to you. Yeah. Thank you for joining.

Manda Venkata Sasidhar

Thank you, Basant, for your kind introduction. And are we good? With the screen.

Basant Kumar Thakur

Yeah. Yeah, it looks.

Manda Venkata Sasidhar

Perfect. So I thank Basant and ELBS to for giving us an opportunity to present our work here. And. And I thank actually Carlos for actually giving. For giving for giving a proper introduction to the exosomes so that I can skip all the introduction slides and everything and just concentrate on the actual flow. So what I'll do is I'll just introduce my the ecosystem, the Apollo Hospitals, Educational Research Foundation ecosystem which is available and what is the ecosystem. That is there, what are the EV capabilities that what we have, what we have acquired, how we are moving forward towards development of EV based lab LED's actually lab developed test. And what are the different translation pathways and different circles of support we are actually building here in Apollo Research Foundation so that the B field can properly grow so so this is our vision in the mission. So basically our why is to transform the healthcare? By translating the cutting edge science into accessible and personalised diagnostics. So how are we doing? We are integrating the EV research circulatory biomarkers. We are actively using the air bio powered analytics and we have very good access to the clinical collaborations. So you know, let me tell you that we are a Pan India hospital network with more than 76 hospitals in our. And and what we offer is we are delivering precision diagnostic platforms and as service models and CRO services for using the circulatory

biomarkers. So this is the ecosystem that is available for me, which is helpful in order to develop the circulating biomarker platform. So AHF that is what we are and for the diagnostics and the support services we have Apollo Diagnostics, the Apollo gene Centre, the pathology and the banks. And for the data and there we have the Apollo telehealth and the AI division. And for the clinical and the patient group, the Apollo Research Foundation, the documentation and the pharmacy. So we take, we take help from all of these different divisions. What we have and we call this the Apollo trial. Right. And this helps us actually in order to deliver what we are currently doing. So we are in the business right now of a novel developing the novel diagnostics therapeutics and drug delivery systems and I will limit myself towards that diagnostics in this present call. So we work with collaborative. Models with different organisations, with the pharma companies, universities and the things and we are developing biomarkers and drug delivery system. And our Research Foundation is also actually in house for different EV based startups, which are developing Alzheimer or neurodegenerative based diagnostics, salary based diagnostics, exosomal biogenerics. So these are the different things that are there. These are the spectrum of translation activities at our lab basically. So the research is basically in the circulator biomarker platform and the exosome biomarker plate. And how we do this is we have forming clinical consortia and we started with ophthalmology and we are now extending it to oncology neurology and then nephrology the CRA activity, we do the preclinical activity development of new diagnostics and the incubation, we physically we facilitate the clinical activity, clinical samples. For the startups and the education arm and the clinical consortium, arm basically helps in order to support our research. So. As we all know, these are the different circulatory biomarkers that are there in the current field like cell free DNA, M RNA, CTC's and the. Exosomes. We concentrate more on exosomes. The majority of our work and the cell free DNA and the CTC's we do with our collaborators, the. So the major reason actually is we got more success in terms of the reliability of the biomarkers with EV. And I assume that the the probable reason is the cargo in the EV's is actually protected in much better way. And when we compare it with the cell DNA or the CTC's. So prelude like as we all know the TV's are initially considered as cellular waste. But. With the in the recent years, we see the advent of. The different analytes in the EVs in terms of proteins or RNAs, or lipids, or metabolites, which actually has paved the way for development of various EV based applications, so let so this is briefly the support system. What we have what we have. We have developed in our Research Foundation. So we have the EV standardisation and the quality laboratory, the EV diagnostics and the CDX unit, the functional assay core group and the translation and the clinical development. So these are available in house in our Research Foundation and for the AI based thing and the GMP compliant TV manufacturing, we rely on our collaborators. For achieving the things, these are some of the instrumentation facilities what we have which we are delivering the the work in terms of isolation, characterization, profiling and the QC different standards. So and these are the we have developed the capabilities to access the different biofluids in terms of. Sorry so different biofluids in terms of plasma saliva, tears, urine and serum and the applications as we see regenerator diagnostics and drug delivery systems. This is our research portfolio where we work, so basically. Diagnostics, drug delivery and therapeutics. And the different areas are oncology, nephrology, diabetic, wound healing, hair growth and burn treatments, so. So this so we all know why we are working with biomarkers or diagnostics. So basically it is less invasive, more accessible to the samples, it helps in more longitudinal tracking and faster it facilitates faster clinical trials and also aids in treatment certification. Thing. So these are some of the studies which we have already published. So in the area of nephrology. So this is the work where we are

showing in the urinary EV's where we have established the housekeeping genes that are relevant for UV based research. So here in this slide, you can actually see how. The UV's are isolated, the different methods of characterization means of the size, shape and other things. And we established the housekeeping genes and for you unary evil. And basically this is the same mechanism we do for all of the biological fluids we establish, our own housekeeping genes before moving in actively into the research. So this study actually shows the early graph rejection, how we have come up with the different biomarkers. That can be used for early graft rejections and this and we have. Published 3 categories of things so that is the T cell markers, the B cell markers and the T cell markers that are actually response that are actually responsible for the rejection and you can detect this rejection much early and non invasively from the urinary. Is so everything right now is in the biomarker platform now. We are moving it into the diagnostic platform after the validation thing. So the salary is is another area where we are actually pursuing and there is also another company in our Research Foundation who is developing. HPV positive head and neck cancers from the celebrity evis. So this is an another study where we are established how we can able to detect the exosomal P10 and also the different markers that are associated with the EGFR variant 3 pathway downstream that are the app one. And the locks thing. So this is another study how we have established in gliomas actually how EV's can be used as a means of for companion diagnostics after further validation. So here this briefly, this is the way we have the brain tissue and we have the autologous serum from the brain tissue. And this there is a chemosensitivity assay which we do and we have come identified 2 sets of patients through this chemosensitivity assay that is known as the conscript assay with one of our collab. Letters and we have identified a score that is not M score, which actually is the response score towards the drug that is mitram isin actually. And once we have established them score we have established the exosomal process where we have identified 3 different markers. That is, the socks 2 only two and. Z1 which? Are one some of the most important transcription factors that are generally found in the cancer stem cells. And you can see here that the combination of the socks 2 requires zip one. It acquires a good sensitivity in the specificity and proper PPV and NPV so. And this companion diagnostics can actually be used. This companion diagnostic can actually be used for. For Mitra, mycin drug, so. And so until now we have seen how AVS can be used as a a proper biomarker. So let me go through how a tissue specific tissue specific events can actually be developed. And here I'm showing you the example of a case study of neuronal exosome. Now. Platform. So where this neural net exosomes gets transported from the brain and come to the periphery and we initially used L1 Cam for precipitation and now we are slowly adding different other markers like the Encamp 1, the lectin precipitation in order to improve the sensitivity of these neuronal exosomes. So basically you can cherry pick the type of exosomes you need, the organ specific exosomes you need for your work and this we have also supplementing with the genetic assays in order to come up with a proper essay where the protein and also the nucleic acid can be used. In order to go for the diagnosis. So this is basically the ATM staging tool that is generally used where we can actually identify that Eddy conundrum or the different stages of AD. And also you can identify ads from dementias and also you can identify. That other neurodegenerative diseases from the thing all this can be possible through a simple blood test and. To the ATM, we have added our neuroprotection markers actually, so this also gives a long longitudinally aspect to our panel and we are still improving the thing. So this is some of the preliminary data, how how amyloid pathway can be detected the top pathways. And we detected the neurodegeneration pathways can be detected and the neural protection pathways can be detected. So to sum it up, I remember somebody

has asked for the EV biomarker development pathway. How we actually do. So this is the brief steps that are there like the discovery and the feasibility, the analytical development stage, the assay validation validation, the clinical validation, the regulatory preparation. Maybe the digital AI integration and the pilot launch that is the LCD and you also have the ID conversion step after the Lt and this is a commercial. Scale up so. This is the. The key activity is the deliverables. What are the regulatory pathways that are there? So for this diabetic, for this Alzheimer's kit, we have actually passed the regulatory preparation. We are at the digital integration stage right now. So in our hands right now, the sensitivity for the different sports. They are in between this 89 to 93%. And also with the available data what we have, we are able to actually construct a digital twin in terms of the genetic score, the pathology, the, the, the, the biomarker score, the imaging score. And we also have a ATM score in the AT&P score. So we added. The digital twin aspects and we use different AML formats in order to get a much more sense to score along with the TV's because. Avis is it's only the biomarker score, so you can see a step wise increase of the sensitivity specificity as you add up the different components, the age, gender, the genotype as you. Go. And this is another paper. Another study what we have published recently, how we can detect. NFL in multiple sclerosis patients, actually. So the characterization how any any FL is support related in the Ms patients and how it actually changes with the therapy? EV's can be used in very different ways as diag, as biomarkers, and after validation it can pass into the diagnostic field. So these are some of the activities which we are properly trying to move from an offer through our Research Foundation. The bio country. So because we are a hospital community, we are also trying to see if we can have the EV biobanking as. And the regulatory support, because we have the Apollo Research and innovations which does clinical trials for. Us. So we are taking full advantage of the support and support system that is actually available here. So these are the we have a proper research incubator here. So these are some of the companies that are there. Which are working toward development of EV based diagnostics. So we are also the headquarters for the Indian Extravascular Society here. So these are the building blocks. What we are slowly trying to develop and offer to the our scientific community. So by accounting services and the clinical consortiums, the clinical consortia we started with ophthalmology and we are extending it to different things. Summarising this is what we are basically. So we we established in 2006. And we are focusing on translation and personalised medicine. And now. With we are moving ourselves as a centre of excellence in Navy. Studies. And in India and these are some of the collaborators, how we are actually executing our work actually and this is my team and yeah, thank ELBS for the opportunity and to discuss about the. TV activities at our Research Foundation. Thank you. I'll take any questions.

Basant Kumar Thakur

Thank you, Bessie, for your nice talk. So if there is a I see there are some hands up. From anyone. Until now. On this question. So I would like to ask one question about uh, what what, what are the regulatory limitations in ecosystem like in India if you want to bring something into the? Clinics, especially when you are talking about the therapeutics because we know that it's bitter kind of difficult in ecosystem like United States. I think there's only one study which has been now for mesenchymal stroma cells has been approved by FDA.

Basant Kumar Thakur

So what do you think about? That. That it's.

Manda Venkata Sasidhar

So yes, so?

Carlos Salomon

Independent.

Manda Venkata Sasidhar

So the EV ecosystem is even slowly maturing in India. So there are three organisations who are actually working in this. Directions 1 is the city school and that is the equivalent to the FDA in US. And second thing is the CMR, which is basically education. Or recommendation and purpose and the recently a regulatory body under the Bureau of Indian Standards has been formed. It is known as MH 20 actually. This will so these are the three different organisations and coming to the regulatory scenario, actually still EV's are treated as new drugs and they have to pass as full regulatory gamut as a new drug itself for the diagnostic purposes. You have different ISO aspects like you have the ISO 15181 for diagnostics for AI integrated LT's. You have the ISO 1518162304. So these are some of the ISO things. So basically what you can say is NPL liquidation and Cisco aligned that is the current state how in India it is. So the route basically everybody is it will change but basically it is in the LTD stage. So you'll enter into the market. As a lab developed test offer as a service model and then slowly acquire the data and then slowly develop the kit in order to make scale up into global markets. So that is the path. So LTD to device, is yeah.

Basant Kumar Thakur

Any questions from anyone? This. If not, then the another question would be that we are we are talking about several liquid biopsy biomarkers at present like and especially in personalised medicine like for therapeutic diseases or like CTCs we have we have EVs now. Circulating tumour DNA. How do you where you can place. At present, the EVs in this complete. Scenario like extracellular vesicles.

Manda Venkata Sasidhar

So the easiest route what we have saw see saw until now basically is say for example there is some, there is a marker that. Is already available. For example, the amyloid amyloid pet city or town pet city.

Manda Venkata Sasidhar

Uh. Take the markers which are already available which are. Expensive is one thing and time taking radioactive slash and all these things make an equivalent test that is possible through any of the circulatory biomarkers. So there are very less number of circulatory biomarker based tests that are currently there in. Yeah, mark it. So if something has to be placed, it is almost every the city says cities are already in the market. Actually also there are in the Indian market there are many Indian companies who are actually offering CTC based tests. So Evc's EVs are late entrance actually. So CTC's then. Selfie DNA. And then the babies. That could be the skin.

Basant Kumar Thakur

Thank you. So. No further questions. I think we will move then. Thank you very much, sister for your time and nice introduction of what's the current status in country like India like it's it's a it's a big developing country. And yeah, I think that it's just very interesting to. Trying to the to to introduce this into the club of European liquid biopsy society and also International Society of external vesicles. So with this I will I would give over to on the. And who's the next speaker? Yeah, thank you.

3rd Presentation by Johan Skog, PhD (Vice President and Chief Scientific Officer, ExosomeDx, a Bio-Techne Brand)

Title: Clinical Implementation and Impact of Exosome-Based Diagnostics

An Hendrix

Perfect. Thank you. So our last speaker for today and it's really my pleasure to introduce to be able to introduce Johan Skog. So he's the vice president and CEO from Exosome Diagnostics, which is a biotechnology. And and and actually in 2021 you want score for all the efforts he made towards realising the translation of an extracellular physical based biomarker into a clinical test was also awarded by ISAF with a special translation. Word. So it's really our honour to have you on school here today with us and he will learn us more about the clinical implementation and also the impact of exosome based diagnostic.

Johan Skog

Thank you so much and it's a pleasure to be here today and presenting to to all of you and I hope you can see my screen. Does this work?

An Hendrix

Yes, that's perfect.

Johan Skog

Great. Yeah. So thank you. So today, I'm going to talk about exosomes or EV's for for diagnostics as well as the next generation diagnostics. It's based on multi omic sort of features of liquid biopsies. So in this presentation. I will actually use exosomes and EVs interchangeably. But for our essays, it really means any Evie up to 800 nanometer in diameter. See if I can. See. OK, I'm I'm showing. The the slide sort of just to to. Highlight sort of the story. This started a very long time ago. That diagnostic journey is challenging as many of the speakers have have emphasised here. So this venture basically started more than 18 years ago after this publication. In 2008 that you could use exosomal RNA for diagnostics and prove that you have truly tumour derived transcripts in exosomes and circulation of of of cancer individuals. So it led to the start of exosome diagnostics back in 2000. 8. And I sort of grew that business over over the years and IN282016. So eight years after this publication, the world's first exosome based test was launched, which is our prostate cancer test. And we also got acquired 2 years later. In 2018 by Biotechnics. So for Full

disclosure. I'd like to just describe that we're part of the biotech company, which is a public S&P 500 company with more than 3000 people. Their main business is proteins, immunoassays, R&D systems, antibodies, etc. However, we form. Their liquid biopsy arm and diagnostic arm. And. Ethics zoom diagnostics. So we talked a lot about isolations. Needs to be. Reproducible needs to be able to be implemented in clinical laboratories. When you talk about. Exosomes as a diagnostics, it's multifaceted. Multi feature. Some would look at microRNA, some would look at proteins, some would look at long RNAs or mRNAs. Or even metabolomics, etc. And your isolation preparation needs to be tailored to the particular biomarker that that that you have. We've found over the years that there's no one-size-fits-all solution. So you have to optimise your process a little bit depending on if you're looking at. Urine sample versus saliva versus plasma. And are you looking at a protein or an RNA target? Those considerations will also affect sort of your minimum viable excess of isolation platform. So I'd like to highlight here. Yes, the different types of isolation platforms that we have developed and that we're working with on on clinical samples, so exsolution you prep is our urinary exosome isolation kit that is also used in the FP. Allstate cancer tests that I think we'll test probably close to 250,000 patients for this this test. The ADI platform is when you need an enrichment step or a depletion step from your sample to to look at subtractions of articles. X solution and X solution plus are the ones for RNA or exclusion plus exclusion or exosomal RNA plus cell free DNA as you know. A big field in the liquid biopsy space is looking for rare mutations and cell free tumour DNA has sort of gained a lot of traction in this space, although not as much as maybe it should. One of the problems with cell free tumour DNA when you're looking for mutations is that. They're often rare, and especially as you go in earlier stage diseases, you can have stochastic levels of these mutations on cell free tumour DNA or not even detectable. They're actually not there and. The funny part of. That is that most have thrown away more than half of limitations already in the sample preparation because they remove the exosomal RNA, the exsolution plus kit is a solution to this, where it Co isolates all cell free tumour DNA. Plus the exosomal RNA. So you get actually more copies of these rare mutations and therefore also increased clinical sensitivity. This is just a picture of what it looks like in in a high throughput scenario. All these methods need to be adapted so that you can process. Over 1000 samples in a single day and and and so we implement our clinical workflows accordingly with the right protocols, the right controls so that you can reach this kind of throughput and. Enable real diagnostics about these samples. We heard a lot of reproducibility and exosome isolation so that I always like to include this slide because I think it's a good representation of what you can actually achieve if you isolate the exosomes according to proper protocols. So this is actually not a reproducibility experiment per se, because. Those are often biassed because you tell your student, or your researcher or your employee to actually do a reproducibility experiment. They know that and they're pipetting super carefully to make sure that their result is going to be reproducible and robust. This is not that, actually, I. I called our clear laboratory and just asked them to send me our control urine batch, so every. Timely process 25 clinical samples. They include a control urine sample that we isolate the exosomes from and then we do qPCR on on those with the same genes that we look at in the clinical samples. So it's not the reproducibility experiment, but a control and what you can see in this. If you zoom in on, you're in batch 5. Here the variability of the exosome isolation RNA isolation, reverse transcription and qPCR is about .4 C values and for these two RNA targets and this is. Extractions done over years with dozens of different operators without them knowing that I would plot this as a reproducibility experiment. It can be illustrated also here in a different another example where we actually did a reproducibility experiment where I I asked Clear team to extract the same neuron sample

over 100 times and then we looked at different target genes in the. The urine, exosomes. And as you can see the standard deviation is very robust. So around .4 point 3.4 C values which is what you would expect from a standard qPCR experiment. So the exosome isolation part doesn't add variability to your, your, your, your assay. In the system. So when you're looking at exosome based liquid biopsies that one of the. Nice features is that these exosomes of course are released into every biofluid, although every biofluid has a very specific set of EVs or exosomes coming from different compartments where proximity is actually the key here. So when you're looking at. A urine sample these the the the the exosomes in urine are mainly from the the kidney, the bladder and the prostate. If the individual is male. A plasma sample is from sampling sort of everything in the body. CSF is enriched for for brain and saliva. We've seen works really well for a number of things, including autoimmune diseases and inflammatory responses. But if you do that, the exosome collection there can be urine, plasma or saliva. You can extract the RNA in a highly reproducible way and many of. Academic publications cite this very small RNA peak. Think making people believe that there's mostly small RNAs and microRNAs in EVs. However, and there's a lot of that. However, we've seen there's far more diversity and in our hands at least, better biomarker value in the long RNAs that's captured there and this is. Dependent on your exosome isolation methods you have to have a very good way of preserving the RNA integrity in your methods. So when you do that you do for example an RNA seek analysis. The long RNA is beneficial because it allows you to do. Pathway mapping so you can actually see what are the pathways that are regulated in this this patient in the different. Fluids. So this is something that we utilise a lot where but 50% of the activity that we do in the lab is working with pharmaceutical companies helping them develop the next generation therapeutic applications and and for them it's very important to understand if their. Drug is hitting the target. What are the pathways that are now being changed or that are being upregulated or downregulated in in, in these patients upon administration of the the, the the drug? So that the input that we use in our lab is any any biofluid basically plasma serums. You sat here and saliva and we've optimised the processes and the protocols for the diverse set of fluids we typically do. A lot of biomarker discovery where you have whole transcriptome, exome or exome plus link RNA analysis. And and then we go in into more targeted analysis for the actual diagnostics, but. The the the. The value here is that you get about the same diversity in detection of RNA as you get from a. Tissue sample. So if you sequence a tissue sample. RNA's to the same depth as as half a mile of plasma. You get the same number of genes that you're detecting, so we see around 30,000 of the 56,000 genes in gene code, or 17,000 of the 20,000 mRNAs in gencode. Every sample that we process is spiked with 9 to 2 exogenous RNAs of different sizes and concentrations so that every sample patient sample that we run also have a control system where we know that the extraction and the library prep and the. Analysis. Worked well with a high sensitivity and that broad dynamic range. So so over the years we've developed quite a few quite a few products, but I'm highlighting some here that also capture some of that diversity of what you can do with with exosome based testing. So the the first Test is the prostate cancer. Just that's now helped diagnose. I think close to 250,000 men in the United States to date. This test is included in the ensuing guidelines as well as the AUA guidelines for early prostate cancer detection. It's covered by insurance and Medicare and and and and private payers. And one of the good features for this task. This is a UN task is very moment massive. We talked about. Liquid biopsies and blood samples as non invasive, but they're not non invasive, they're less invasive. Than a biopsy. But they're not non invasive. You actually have to have the patient go to a phlebotomist and this is not. Trivial. For some patients, they have to travel for hours to actually do this. The A urine sample as well as saliva sample

enables certain empowerment of the patient to to to get the sample in without the intervention of a doctor. So we are actually an at home collection kit for our prostate cancer. Tests that we after the doctor orders the test, we send a box home to the patient where they can donate the sample at home, send it in in to us, and then the doctor will have the actual result. Before the patient even comes in through the door, so it speeds up the effective the effectivity, the effectiveness of the the the clinic as well as has has a lot of benefits for the patients. The actual true test is actually our kidney transplant projection assay that we've published on in the last couple of years. And we licenced that out to Thermo Fisher. So Thermo Fisher is doing the the commercialization of that one. And then we have a quantity ex qPCR, ESR, one mutation assay which is a breast cancer assay and this is a kitted solution where you actually have all the components in this kit to isolate the exosomes plus the cell free DNA and then do. As highly sensitive mutation as say for ESR 1 mutations for for monitoring of of of breast cancer patients. And the intent of this one, we sell it actually as an. Are you OK kit because it's quicker to clear labs that they internally validate and can offer? Which is different compared to the other, like the FBA prostate cancer test is not that that one is a centralised testing test that's run in our CLIA lab. So these are some of the examples of what the platform can do. What we're actually some of the examples of our internal pipeline and I'll give you a flavour of some of that work as we move forward, we have our next generation prostate cancer. Us in the work. We also just presented at ACR the some of the outcomes of our colorectal cancer screening assay that has been tailored to not only have good sensitivity for colorectal cancer but also advanced the nomas with cell free tumour DNA currently is unable to really detect. Kidney inflammation assays is another one, and sugar syndrome is actually an autoimmune disease that we we can monitor through our saliva sample, which is another completely non invasive process and we work in the neurogenetic disease space also mostly together with pharma. So to give you a little bit of a flavour of of the actual clinical diagnostic test that's being. Helping. 10s tens of thousands of patients every month. We have this urine collection kit that we can send home to our patient and that works for both the prostate assay as well as the transplant rejection assay, how however? That the prostate cancer test is actually designed for men. That have a slightly elevated PSA. So a PSA in the grey zone between 2:00 to 10:00. The reason this task was developed specifically for that intended use population is that if you don't have a PSA value, you're rarely going to a urologist. In any case, if you have a PSA. Now you're 50. You're gonna get a biopsy. And in in most cases, in another case, you don't need a second opinion here. However, PSA doesn't perform hardly at all in that grey zone between two to 10. Actually 2 to 20, so. That's where we focused the initial launch of this test, where you collect a standard urine sample, no digital rectal exam or prostate massage required. We get the Exo zooms out, we cracked them open. I mentioned we have over 35. 1000 genes in there. However, this test only looks at 3:00 and it's PCA PCA 3 organised and we do that by qPCR. Those the three, the outcome of those 3 genes goes into a score from zero to 100. So the higher the score is, the higher your chances of finding high grade prostate cancer upon a biopsy. So that test was launched in 2016. In 2020, we launched the App Home Collection and we've been following patients not only after the validation, but also through utility studies where we've done blinded double blinded. Studies where over 1000 men were on. Everyone got prostate cancer. The EPA test, however they were randomised and blinded and only half of the patients got the result back so that they could act on the result. And then the other half they didn't get the appeal result back, but they were free to do any other. Diagnostic testing that they they wanted to do and and then make a decision if they wanted to have our prostate biopsy. You're not, and it turned out that the patients who actually had the epic result back that population found

30% more high grade prostate cancer than the blinded standard of care are. Because it increases compliance to the doctor's doctor's recommendation, and you can also safely avoid unnecessary biopsies in the in the low risk patients. So when you do a urine exosome collection, it's very important to understand the plumbing system, how it actually looks because most people think that. The the prostate exosomes are actually in the the urine per se, but they're really not like as you see in this schematic picture, kidney exosomes are released this way into the bladder and the bladder. Exosomes are released into the bladder however. That the prostate exosomes are not they are actually. You get prostatic secretions that go into the urethra and they sit there. However, they never mix with the urine until you give a a donation, and that's when the prostate exosomes actually comes in out in the first catch, you're so if you continue to urinate. Back up, you will actually dilute your prostate signal, so we use this colopy device that collects only the first catch of your urine sample. So I don't know if you can see my cursor here, but there's a funnel here where you urinate into this funnel. That the urine goes in into this lower chamber where you see this green plastic and the green plastic is a float. So when the 1st 20 millilitres of urine has been collected, this float actually goes up and shuts off the the the the collection and the urine goes up this way. Into the toilet. And we built to to to understand what. Markers are in the different urine fractions. We actually took five of these colonies and we stacked them into each other and created this one metre long, ginormous colopy that we gave to some patients and they urinated. So we actually got every fraction of that, the urination. And then we looked at prostate. Markers as well as bladder markers and and as you can see in this table in the upper right, the prostate markers are all in the 1st 10 millilitres of urine. Whereas the bladder markers are steady, they are in every fraction of the urine. So over over the years, we've done a lot of publications on the both the validations and the utility of the EPA test. And this tells you a little bit about. An area that is rarely touched upon at these meetings, everyone talks about how to develop a biomarker. Actually developing A biomarker and validating A biomarker and launching it in a clinical lab is the easy part. That's about 10%, I would say maybe less than 10% of the road to actually a successful biomarker. And so all the hard work actually starts after commercialization. Of a test. And it goes into many different areas. However, you have to continue to to show utility of the study, the biomarker and and show better outcomes in in long term follow-up studies which is far beyond the the launch of the actual test. So so this one actually have now shown that it's useful for patients both that are doing first or repeat biopsies of their their their prostates. And in the blinded prospective studies that we've done, we show that there's actually a better outcome, both immediately at the time of the. Test but that. Outcome lasts for well over five years, where if you get the the this this test. You have a higher likelihood of you find 30% more high grade prostate cancers compared to doctors that use standard of care. And if you're negative with the test, you actually can safely avoid an unnecessary biopsy. So we saw in the control arm a lot of patients that were negative with our tests, but we didn't tell the doctor they received unnecessary biopsies and. So the the utility of this task hits both on the better outcome of finding cancer, as well as avoiding unnecessary procedures. So this is an. Example of of what the the. The test does basically that. The higher the FPS score is, the higher the chance of finding high grade prostate cancer upon a biopsy. So you can see that if you have an appy score of 30, you have roughly 30% chance of finding high grade prostate cancer. A score of 50, you have roughly 50% chance of finding high grade prostate cancer and. And. It plateaus after that. A lot of people ask me why is that? Well, we know that 12 core trust biopsies, which is the way you do most of the the, the the biopsies misses 50% of high grade prostate cancers. This has BeenVerified in study after study. So the gold standard biopsy or miss 50%, which means

in a real world scenario you can never reach higher than this unless you do outcome studies that go on for for many, many years. We've now followed these patients for over 5 years and I can say if you look at the risk of finding hydrate prostate cancer within the next five years, this graph continues to climb to over 80%. So right now the way this test is used in the clinic is that a patient comes to to see the urologist. There are similar ages, similar PSA values, who's going to get a biopsy here? Well, I mean, this is basically a random, this is random. It depends on the doctor. Depends on the. Patients fear prostate cancer versus fear of doing a biopsy. So here you can actually apply the test and better stratify the patient so you identify the low risk patients versus the high risk patients and. Which lead to them better outcomes. So now I'll just quickly go into the next generation exosome tests that is is leveraging some of the multi omic features of liquid biopsies where EVs are the perfect multi ohmic content carrier, right. And you can combine this with cell free tumour DNA. When when it's beneficial? We've shown this in in many studies in the past that. If you do. Both the exosomal RNA and the cell free DNA in a robust way. You can actually get up to 10 times more copies of the circulating mutations compared to looking at cell free tumour DNA alone. This was actually a blinded study that we did with Clovis oncology where. That pharmaceutical companies split the plasma in half, send half of the sample to us and half to selfie to Medina comps. And then we looked at samples blinded and we found about 10 times more copies of the the mutation, which increased the the clinical sensitivity in in these patients, especially in the patients with intrathoracic lung cancer. So basically when it's. It's it's, it's more confined and harder to detect on cell free tumour DNA. We saw a cell free tumour. DNA in this case had a 26% sensitivity in intrathoracic lung cancer patients also drove it up to 74%. And with the exosomal RNA and DNA and an overall sensitivity of 98% of the entire cohort, when you also included the the metastatic disease patients. I'm actually going to skip this one because this slide shows same same. Thing, but since we know that you can actually get benefit of looking at multiple omics like I showed you in the previous slide, we built this multi omic biofluid Discovery platform that can support any biofluid. So you can work plasma, urine, saliva or CSF. We isolate the cell free tumour DNA plus the exosome. So you can do epigenomics looking at DNA methylation where our current platform looks at about 4 million CPG islands or CPGS. Sorry, we look at the EV RNA. So both the exome and the link RNA, so we can do gene expression analysis. The infusion analysis and. Really importantly, splicing analysis, we're actually seeing that splicing is a heavily underutilised biomarker and having good quality RNA out of the biofluid enables you to actually look at over 70,000 onco splice variants. You also can look at small RNAs as well as proteomics. And since DNA, RNA and proteins are related, right? Yeah, it starts with the DNA, then RNA in the protein. There's actually value to be had throughout that workflow where you have DNA that's an amplified on the RNA level and then that RNA. Can be amplified also in some cases on the protein level. So you can integrate the analytics and see actually when especially in early stage diseases where that targets are rare, where can you complement the signal by different omics features. We've also shown we've tested this. The question came up before, do you need the exosome isolation can you? Can you just look at RA plus my in general can? You just add. Colour Tropic cells and precipitate everything. Well, you can. You can get RNA out. However, when you're actually doing the analysis, we're seeing less. Value when you're looking at neat plasma compared to exosomes. Because when you're looking at the RNA in plus. Most most of it is actually coming from platelet contributions. You get a tonne of globins and things that will actually mask your biomarker signal, whereas if you get a proper exosome isolation you actually get more of the reads that you're interested in and where you're more likely. To find actionable biomarkers. Same is true for proteins where we actually use the all linked platform for high

throughput analysis here and. We see very different profiles when you look at neat plasma compared to the exosomes. And some of them, of course, membrane bound proteins are enriched in the exosomes and. We're seeing some very interesting biomarker features come. Out of that. When it comes to cell free DNA methylation, it's also important how you do. It. We we we have tested bisulfite sequencing versus the more modern and somatic methylation sequencing and the EMC it actually enables you to do. Even urine cell free DNA analysis, which you. If you do buy sulphide. Sequencing. You can't see much cell free DNA in the in the urine, but EMC will actually enable you to use this as a biomarker in urine. I mentioned also that if you have good quality RNA you can get. Splicing analysis done. Here's an example where we looked at the cohort of 40 Parkinson's disease samples and resuming in on the alpha synuclein gene in in in particular here. Alpha Synuclein is a gene of importance in Parkinson's disease. However, it's also known that a lot of alpha synuclein is present in red blood cells, which is unrelenting, not are not relevant for the biomarker. So to really get to the biomarker value of alpha synuclein, you have to sequence in. And that that region very, very, very deeply so with whole transcriptome sequencing, you can only see. About 1000 different splice events in this gene, but if you do more targeted sequencing, which we do have a brain panel of about 1000 genes. In this case, you can actually see splicing events that you won't see with the whole transcriptome sequencing or exome sequencing and those splicing. And are known to actually be diseases associated. So we're actually seeing some really intriguing biomarker values when we zoom in on on on some of these diseases associated genes. The same thing is can be done in, in prostate cancer. So a lot of people also wonder what happens when you combine all of these multi omic features. Can you still do a proper biomarker discovery cohort? So so because you have so many variables and you have you add actually some. UM. Higher dimension problems when you're doing multi omic features, so I'll show you some examples of this in a prostate cancer study where we so far we have we're over 200 patients now enrolled, but we do a standard urine collection, we collect the. Exosomes and cell free DNA from the urine and then we also have multiparametric MRI's from all the patients. And we looked at the gene expression, the spliced transcripts, the splice events and the methylation. And throughout two different courts, we've shown that it's incredibly reproducible. So we know that the platform is very stable and and actually can be used for for biomarker discovery. And when we zoom in on the actual data to avoid some of the higher dimension problems we're looking at over 70,000 oncogenes. For the splicing, we're actually down selected to the top five features for splice transcript and splicing and cell free DNA. And we actually showed that even when we downsize it to 5 features from each omex, we outperform the standard of care including multiparametric. MRI. So the exosome RNA alone outperforms multiparametric MRI and standard of care with PSA and the risk. Letters. But you can also combine the features with RNA and cell free DNA and proteins and get even even higher performance. Most of these are some of these patients also have the commercial Episcopal test and we looked at the three genes with RNA seek and generated A pseudo epic score and showed that the. RNA seq data that we we actually generated correlates very nicely with the qPCR data from the commercial test that was done on a completely separate yeah urine sample. So that was really nice because that illustrates that we can easily translate the RNA SEQ data into a qPCR assay once. We're at that. Level. And yeah, in the interest of time, actually, I'm going to, I'm going to stop there and see if there's any any questions. Sorry for rushing through here at the end.

An Hendrix

You'll want to end here, you.

Johan Skog

I'm sorry.

An Hendrix

You want to end your presentation here.

Johan Skog

I I thought I was out of. I was just looking at the time. And do I do? I have a few more minutes. Do you wanna? Then I can add a couple more slides. But I didn't want ever want to sit around and go overtime.

Svenja Schneegans-Murano

An Hendrix

Yeah, maybe it's good to leave some time for for discussion. I think we have more or less 10 minutes left. So I think it would be nice for you to have some questions as well from from the participants. So thank you for the. For the presentation, I think it's very nice combined with the if you reflect also back on the presentation from Carlos, I don't know if he is still around, but maybe he was hoping to be almost there with the development of his biomarker test being in the last. The steps. But that is only at 5% of all the work that still needs to make it the commercial success. That's, I think one important key message because you went through the whole workflow from the academic side towards the development of such a test. And I'm really taking it to the. Commercialization. So I mainly remember that you mentioned the importance of really showing further the utility doing additional validation showing that it has additional benefits. So can you maybe. Elaborate a little bit more besides validation, because of course also the tests to be able to really get it commercially up and running. There are probably also some additional aspects and concerns towards regulation to consider there. Can you also highlight or reflect a little bit on that? Point.

Johan Skog

Yes. So I didn't say that that this would discourage any biomarker development. I didn't want want it to come up that way, but I want people to go in eyes open understanding that. Assay validation and launch is not it right? Look at diagnose other diagnostic companies right that are. It takes time to get to sort of a. A test needs to be profitable for you to actually get traction and and and run it. For that it needs to be paid for. You need to make sure that you have insurance that can cover and pay for the test and they don't want to do that unless you have. Proven utility of it right. And as you know, PSA has been used for prostate cancer for a very long time, right. And and it took. I think almost 20 years for us to understand if PSA testing was useful or not. We we thought we thought that that finding all of these cancers was a good thing, right? And then we have to, we realise we have to. Value sort of the the the cost of overtreatment versus overtreatment of indolent disease versus diagnosing new disease and and. So the utility studies and understanding if a biomarker truly adds clinical value is based on longer outcome studies that takes time and. And. Insurance and payers often require some of these utility studies before they are covering the tests mean everyone can go in and look at the the some of the public diagnostic companies like Garden Health for example. I mean, if if you have a billion dollars in revenue but you're not profit, right, you're

losing. Hundreds of millions of dollars every quarter. And. Same is true for many other tasks, so doing it in the right way is important. So I think we're one of the the unicorns in in, in that in that setting that we found the niche and the test that actually works really well. We've done the utility studies to prove that it actually leads to better outcome and has. Worked together with insurance companies to actually develop these utility studies to satisfy their requirements before getting paid.

An Hendrix

Perfect. We have a question for the participants, Kiani.

Qiaoli Wang

Hi, yes, it's Kelly here. Thank you for the great presentation. I'm just curious what is your, what's your opinion about like RNA, RNA? Exosome RNA versus exosome proteins, I see you present a lot of irony work. I'm just curious what's your, what's your thought in perspective of cancer diagnosis when comparing RNAs and with proteins in? Pros and cons?

Johan Skog

I think that's a great question and I am a little biassed because we started off with a lot of IP around the RNA RNA field, but one of the advantages I would say on the RNA is that there are very efficient screening mechanisms for the RNA. qPCR is very sensitive you can detect. Single copies of your target, right? You also have RNA sequencing that can screen very broadly. So in the protein space it's a little bit more challenging. It's been a little more challenging, it's getting much better now when we have platforms like Lync and app timers and ways to do more screening. Another challenge with proteins is understanding where the protein is is the protein inside the vesicle? Is it in the membrane? Is it bound to the corona of the vesicle? Is it bound? Tight or loose and depending on your washing stringency, how much of it will come off, etcetera. So understanding that biomarker sort of it adds a little bit more complexity versus the exosomal RNA that we know is protected in RMP's and inside. Hi. So that being said, I think there's going to be very valuable diagnostics on both proteins and and the RNA in the future. But we have we have started off I think with the RNA and now starting to sort of dabble a little bit with the proteins with Olin platforms and and others.

An Hendrix

Thank you. Yeah, I and indeed I think from uh Carlos's presentation there, it was also a combination of proteins and microRNAs that they included in their profile for the ovarian cancer testing. And it's basically, I think one of the advantages as well that we can exploit of analysing extracellular vesicles they are. They contain multiple components and allow for a complementary analysis of different types of molecules. So I think all information we can capture from them can be very relevant towards establishing A biomarker. It's just the trick of identifying the correct and most optimal combination. Of the biomarker sets, and this can depend based upon the disease States and the yeah the the the type of disease, the the stage of the disease, yeah.

Johan Skog

Yeah. Yeah. And I think I think it is one of the advantages, but we also have to go in with eyes wide open to us, also understand that it becomes a. A multiple feature problem, right? Because when you have an RNA sequencing where you can look at. 100,000 splice events and. 50,000 RNA targets and. You combine that with mass spec proteins and you have so many features that you will you will never be able to build a cohort that is powered for that right. So you have to be smart about it and the way you down, select the features combine also. Maybe biology in it, and I think AI will help us in the future with that to actually figure out how they are. The different processes are are related so that you can pick the right ones you can never. Power a cohort to actually. Get value of every marker that you're looking at, because you would need to do millions of patients.

An Hendrix

And besides that, there is also the power of combining the different types of liquid biopsy targets. So you also mentioned combining ctna with EV DNA. To make it more the essay more sensitive. So that's also another approach on how we can combine the different. Liquid biopsy targets too improved by the sensitivity of biomarker tests as well.

Johan Skog

Yes, it's been a. Interesting, when you when you hear people talk about the problems of the rare copies or limitations in ctna when they didn't even try to find limitations on the RNA they threw, it threw it away right. And and I think that's something we have to be. More careful about because we've shown in multiple indications that combining the RNA with the C DNA will give you a leg up on the the copy numbers.

An Hendrix

Perfect. And you and can I ask you to stop sharing your screen so that we can see all participants and maybe better to check whether there are some final questions here for you Han. No. Then thank you Yohan as well for attending our seminars today and. Presenting the process from establishing a test towards making it a commercial access, I think success. I think it was a very valuable information for all of. This and and then maybe to wrap up for today. So I want to thank all three speakers. So it was very nice to see how we are really moving from biomarker discovery going into the development of tests and then making these tests. A commercial success. So I think we had a perfect combination of the three different speakers here today on the team. Yet I think today we still have to consider that there are not too many successful EP based biomarker tests that really achieved the commercial success. So there is still many opportunities I think to further let this field grow and develop. And this is, I think, one of the aspects where elves can also play a a very important role. So. One main aim still remains to get access to the substantial amount of patient samples. So we have seen some tremendous amount of patient cohorting presentations today. And this is really great to be able to validate the biomarkers and move the field forward. And we look forward here to all the upcoming uh. Developments that are occurring from that site, so I don't know if there are any final questions or remarks to be made by some of the participants. Now is the time or the moment to do so. And if not, I think we can perfectly end our session of today and time. So thank you to all the speakers for being here for the participants to join in our discussion and then stay tuned for our next. EV working Group meeting, which is scheduled for October 7. You are correct.

Svenja Schneegans-Murano

Yes, exactly. I believe it was 28, but I need to check.

An Hendrix

OK, perfect. Yeah. And then we will focus on technology. So stay tuned for them. So hope to see you all soon and enjoy the rest of your day.

Markus Sprenger-Haussels

Thanks.

Basant Kumar Thakur

Thank you. Bye.

Johan Skog

Thank you.